DOES TIME HEAL ALL WOUNDS? PHOTOREPAIR ACTIVITY IN *DAPHNIA MAGNA* FOLLOWING EXPOSURE TO ULTRAVIOLET-B RADIATION AT DIFFERENT AGES

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ABSTRACT

In recent decades, concern for aquatic ecosystems has grown due to the potentially harmful effects of increased ultraviolet (UV) radiation. While the adverse effects of UV radiation on mature zooplankton have been studied extensively, minimal research has examined the effects of UV radiation on juvenile zooplankton. *Daphnia*, a frequently studied genus of the freshwater zooplankton, have evolved several strategies to protect themselves from UV radiation. This research focused on two of these strategies in *Daphnia magna*: (i) the use of photorepair mechanisms to repair damaged DNA; and (ii) the development of photoprotective pigmentation to protect vital organs. One, two, three and four day-old *D. magna* were exposed to either (i) UV-B radiation followed by varying periods of photorepair radiation to determine if survival varied by length of photorepair exposure and by age; or (ii) UV-B, or UV-A and PAR radiation to determine if cuticular melanin accumulation varied by length of exposure and by age. Survival increased with both increasing length of photorepair exposure and increasing age, while melanin content increased only with increasing age. In addition, three day-old *D. magna* were exposed to UV-B radiation followed by varying periods of photorepair radiation to determine if reproductive output over a 12-day period varied with length of photorepair exposure. UV-B exposure without subsequent photorepair exposure significantly reduced reproductive output. Furthermore, exposure to a photorepair period beyond 30 minutes following UV-B irradiation did not significantly improve reproductive output.

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INTRODUCTION

ENVIRONMENTAL CONCERNS

Since the discovery of the Antarctic ozone hole in 1985, concern for aquatic ecosystems, including freshwater environments, has grown due to the potentially harmful effects of increased ultraviolet (UV) radiation (Farman *et al*. 1985). In the last two decades, the Antarctic ozone hole has continued to increase in both size and depth, and ozone concentrations over equatorial and mid-latitude regions have declined significantly as well (Häder 1997). The significant reductions in stratospheric ozone have resulted in an increase in UV radiation reaching the earth's surface (Madronich 1994), affecting aquatic organisms in a multitude of respects. Though water attenuates solar radiation, particularly UV-B radiation (Williamson 1995), aquatic organisms are highly sensitive to UV radiation, making the observed increases potentially devastating to both aquatic ecosystems, and via food web interactions, terrestrial ecosystems as well (Bothwell *et al*. 1994).

The earth is shielded from damaging UV radiation by a layer of stratospheric ozone located between 10 and 40 km above its surface (Häder and Worrest 1991). The production and degradation of ozone is cyclical in nature (Appendix I). Ozone is produced from oxygen by shortwave UV radiation from the sun and then broken back down to oxygen by longer wavelengths of UV radiation (Häder and Worrest 1991). Normally, the quantity of ozone is kept constant by an equilibrium between its rate of production and its rate of decomposition (Mayer 1992). The reduction in stratospheric ozone observed during the last several decades is a result of the irreversible destruction of ozone molecules, fuelled by the production and release of anthropogenic pollutants, notably chlorofluorocarbons (CFCs), into the atmosphere (Häder and Worrest 1991)

(Appendix II). CFCs were most commonly used in the production of certain industrial foams, for refrigeration, as cleaning agents, and as propellants for the delivery of aerosol products from pressurized containers (Madronich 1994). As early as 1974, it was suggested that continued use of CFCs would result in significant decreases in the amount of stratospheric ozone (Kerr and McElroy 1993). Though emitted in relatively small amounts (slightly in excess of 1 million tons per year), CFCs are extremely destructive because their inert quality allows them to build up in the lower atmosphere (the troposphere) over a long period of time (Häder and Worrest 1991). Eventually, CFCs make their way up to high altitudes (>40 km) where, when photolyzed by short-wavelength UV radiation, they release chlorine atoms. The chlorine atoms then travel down into the stratosphere, where they destroy ozone molecules, forming free oxygen and chlorine monoxide, allowing more harmful UV radiation to reach the earth's surface (Peter 1994). Considering that the total amount of ozone in the stratosphere has always been comparatively low (if ozone could be collected and compressed under atmospheric pressure, the layer's thickness would be a mere 3-4 mm) (Häder and Worrest 1991), it is not surprising that destruction of even small amounts of ozone can have a tremendous effect on the amount of damaging UV radiation reaching the earth. The worldwide production rate of CFCs remained constant from the mid-1970s until the late 1980s, and began to decrease only in the early 1990s. Nevertheless, significant quantities still remain in the troposphere and continue to destroy ozone molecules (Peter 1994).

Ultraviolet radiation can be divided into three sub-regions of wavelengths between 200 and 400 nm within the electromagnetic spectrum, based on measurements taken at ground level (Diaz *et al*. 2000). While only 5% of the solar radiation reaching the earth's surface is located within the UV spectrum, it is the most energetic and therefore biologically harmful (Rautio and Korhola 2002). UV-B radiation (280-320 nm)

is of greatest concern, and while most of it is normally absorbed by ozone, its penetration to the earth's surface has increased significantly as a result of ozone depletion. UV-A radiation (320-400 nm) is the least damaging type of radiation, and is present in high amounts because it is not strongly attenuated by the earth's atmosphere. UV-C radiation (200-280 nm) is biologically lethal, but is absorbed completely by oxygen and ozone in the upper atmosphere, and no measurable amount reaches the earth (Diaz *et al*. 2000).

Aquatic organisms, once thought to be better protected from damaging UV wavelengths compared to their terrestrial counterparts, are now considered as likely to be negatively affected by UV radiation. This is not only due to increased UV-B radiation reaching the earth's surface, but also to increasing UV-B penetration into the water column due to decreased dissolved organic carbon (DOC) content of the water. DOC absorbs damaging UV radiation, and a concentration of as little as several milligrams of DOC per litre prevents UV radiation from penetrating more than several decimeters into the water (Schindler *et al*. 1997). In marine environments, UV-B has been detected at depths of 60-70 m, and inhibition of biological processes due to UV-B radiation has been documented at depths of 20-30 m (Karentz and Lutze 1990). While it has been proposed by Kirk (1994) that UV-B is attenuated more rapidly in lakes than in oceans due to much higher DOC concentrations, freshwater organisms may still be highly susceptible to damage by UV-B radiation because the average depth of freshwater lakes worldwide is <10 m (Wetzel 1990). In addition, most freshwater organisms are usually confined to a depth of only a few meters below the water's surface, where the most favorable growth conditions exist (Williamson 1995). Schindler *et al.* (1997) have shown that major reductions in UV-absorbing DOC have occurred in North American boreal lakes as a result of two direct consequences of climate change, increased temperature and reduced precipitation, which have led to a strong increase in UV penetration.

EXPERIMENTAL ORGANISM

Daphnia spp. are ideal to test the effects of UV-B radiation on aquatic organisms. As primary consumers of algae, they are important components of the freshwater food web and are a food source for many species of both juvenile and adult fish (Adema 1978). They are easily cultured in a laboratory setting, reproduce quickly via parthenogenesis and have a high reproductive output. Most importantly, *Daphnia* inhabit shallow water where protection from high solar radiation intensities is not easily found, making them ideal candidates to test the effects of increased UV-B exposure (Zellmer 1996).

Daphnia spp. are microcrustaceans of the Family Daphniidae (superorder Cladocera, suborder Anomopoda) (Ruppert *et al*. 2004). Cladocerans, commonly known as water fleas, are filter feeders with a chitinous carapace that houses a dorsal brood pouch (Ruppert *et al*. 2004). At warm water temperatures (~20-25°C), most cladoceran species attain reproductive maturity at approximately 7 days of age and develop a brood sac shortly thereafter (Williamson *et al*. 2002). They produce their first brood between 8 and 10 days of age, releasing 8-12 neonates per brood. *Daphnia magna*, the largest species of *Daphnia*, measuring 2-5 mm at maturity, was the organism used in this series of experiments (Appendix IV). *D. magna* is found only in the western half of North America, most commonly in intermittent ponds, especially those which are either eutrophic or slightly saline, and coastal rock pools, as far south as Mexico and as far north as the Arctic Ocean coastline (Ruppert *et al*. 2004; Appendix IV).

ENVIRONMENTAL ADAPTATION STRATEGIES

Daphnia and other zooplankton have evolved several strategies to protect themselves from prevailing radiation conditions. This research focused on two strategies

in *D. magna*: (i) the use of photorepair mechnisms to repair damaged DNA; and (ii) the development of photoprotective pigmentation to protect vital organs. In addition, zooplankton also avoid damaging UV radiation by evasion or vertical (downward) migration (Gonçalves *et al*. 2002).

PHOTOREPAIR MECHANISMS

Studies on *Daphnia* and other related genera, such as *Ceriodaphnia* and *Simocephalus*, have shown that UV-B irradiated daphnids have significantly decreased survival rates compared to non-irradiated daphnids (DuGuay 2001; McClintock 2002). The higher mortality of irradiated daphnids can be attributed to lesions in the organism's DNA caused by UV-B radiation. Nucleic acids, which make up the DNA double helix, have high absorption coefficients for short UV wavelengths, peaking at 260 nm (UV-C) but also extending into the UV-B range (Vincent and Neale 2000). The lower UV wavelengths, which are high energy, cause breaks between the 5,6 double bonds of the pyrimidine bases of the helix, forming cyclobutane pyrimidine dimers (CPDs) between adjacent pyrimidines of one DNA chain (Fraikin *et al*. 2000). CPDs prevent DNA replication and transcription, resulting in cell death (Hearst 1995). While cell death may also be caused by another type of mutagenic DNA lesion, a (6-4) photoproduct, CPDs account for up to 75% of UV-induced DNA lesions (Fraikin *et al*. 2000).

Many organisms, including invertebrates such as *Daphnia*, possess the enzyme photolyase (Thoma 1999), which can be activated in the presence of photorecovery (or photoreactivating) radiation (PRR), consisting of long wavelength UV-A radiation (320-400 nm) and photosynthetically active radiation (PAR) (400-700 nm). Photolyase can repair UV-induced DNA damage using the energy of light (Thoma 1999; Fraikin *et al*.

2000). Photolyase is capable of recognizing CPDs and once bound, monomerizing the CPD to two pyrimidines (Karentz 1994).

Several studies have demonstrated that *Daphnia* have the capacity to undergo photoenzymatic repair in the presence of PRR to repair the damage induced by UV-B radiation (Grad *et al*. 2001; McClintock 2002; Grad *et al*. 2003). Grad *et al*. (2001) exposed *Daphnia pulicaria* to 6 hours of UV-B radiation and found that survival and reproductive output was significantly higher in daphnids exposed to PRR compared to daphnids placed in the dark following the UV-B exposure period. In addition, survival rates were dependent on the intensity and length of exposure; shorter, more intense exposure to UV-B radiation had more impact on survival than longer, less intense exposure (Grad *et al*. 2001). *D. magna* is also capable of photorepair in the presence of PRR (McClintock 2002). While many studies have examined the effects of UV-B radiation, with and without PRR, on *Daphnia* species, these studies have typically been confined to daphnids irradiated after reaching maturity. This research examined the effects of one 6 hour dose of UV-B radiation, followed by varying periods of PRR, in juvenile *D. magna*.

PHOTOPROTECTION MECHANISMS

Photoprotection is an important protective strategy employed by cladocerans, including *Daphnia*. Two main types of photoprotective pigments have been identified in *Daphnia*: carotenoid pigments and cuticular melanin (Hessen and Sorensen 1990). Although carotenoids play an important role in protecting other crustaceans, such as copepods, from UV radiation (Hairston 1978), they are of little use as an indicator of UV stress in *Daphnia* and cladocerans in general (Hessen 1993). Instead, *Daphnia* populations which are highly light-exposed, such as those in arctic and alpine regions,

possess carapace melanization (Hebert and Emery 1990). While these populations are thought to be highly pigmented due in large part to increased UV-B exposure, there is evidence in other organisms that other wavelengths of light can also stimulate development of photoprotective compounds. Both PAR and UV-A have been shown to induce synthesis of photoprotective compounds in plants and lichens, respectively (Adamse *et al*. 1994; Solhaug *et al*. 2003). *D. magna* are known to have four peaks in spectral sensitivity, with one of these peaks occurring at 348 nm, within the range of UV-A wavelengths (Smith and Macagno 1990). Studies have shown that *D. magna* move vertically downward in the water column upon exposure to UV-A radiation, and less so in response to UV-B, suggesting that this species is more sensitive to longer wavelengths of light (Rhode *et al*. 2001, Thuen 2002). Thus, it is possible that exposure to UV-A could also stimulate melanin synthesis in *D. magna*.

Melanins are widely distributed in both the animal and plant kingdoms (Blois 1988). They are insoluble, which distinguishes them from most other biopolymers and makes their chemical analysis difficult (Blois 1988). They are the most visible of the biological pigments and can be classified into two groups: the eumelanins, which are brown to black in colour, and the pheomelanins, which are yellow to red (Britton 1983). Both types of melanins are synthesized in melanocytes from a common precursor, dopaquinone, which is produced from tyrosine by the action of tyrosinase. Dopaquinone can undergo several redox reactions leading to the formation of eumelanins or alternatively, in the presence of a sulfhydryl compound such as cysteine, can form pheomelanins by a similar process (Ozeki *et al*. 1995).

Studies have shown that melanin's primary function is direct protection from light (Hobaek and Wolf 1991), by acting as a general screen against all UV wavelengths (Hessen 1993). Melanin, though comprising only ~0.03% of *Daphnia* body weight, has

been shown to prevent transmission of more than 90% of incident solar radiation and is therefore considered a key evolutionary adaptation in many *Daphnia* species (Hebert and Emery 1990). However, since the majority of melanin is stored in the carapace, which is shed during molting, the repeated synthesis of melanin after each molt is energetically costly (Rautio and Korhola 2002). Not surprisingly, highly pigmented cladocerans, such as *Daphnia pulex*, tend to be restricted to habitats where exposure to intense UV radiation is high, such as in arctic and alpine regions (Hebert and Emery 1990). Some highly melanin pigmented cladocerans have colonized shallow ponds and lakes at lower latitudes and lower altitudes, likely a result of increased UV-B radiation observed in these regions as well (Hebert and Emery 1990). There is another trade-off for highly pigmented cladocerans because, while highly pigmented organisms are more tolerant of UV radiation, their increased visibility increases the risk of capture by visual predators such as fish (Hansson 2000). Studies have been completed on other photoprotective pigments such as carotenoids in *Daphnia* (Green 1957; Hallegraeff *et al*. 1978; McClintock 2002), but little research has examined melanin synthesis in *Daphnia* in response to UV-B radiation, especially in non-arctic dwelling species. This research examined the effects of one or two 6 hour doses of UV-B, or UV-A & PAR radiation on the production of cuticular melanin in juvenile *D. magna*.

OBJECTIVES

1. To assess the effect of photorepair exposure in *Daphnia magna* of different ages by exposing the organisms to varying periods of photorecovery radiation (PRR) following exposure to UV-B radiation to determine if survival varied by length of time of photorepair exposure and by age at time of UV exposure.

2. To determine if the reproductive output of *D. magna* irradiated at a particular age (which was determined based on the results of Experiment I) varied with the length of time of photorepair exposure.

3. To determine if the length of time of short-term exposure to UV-B radiation, or UV-A and PAR radiation, influenced survival and the production of cuticular melanin in *D. magna* exposed at different ages.

MATERIALS AND METHODS

MAINTENANCE OF STOCK CULTURES

Daphnia magna stock cultures were obtained from Ward's Biological Supply Company (Rochester, New York) and maintained in one 50 gallon tank and two 5 gallon tanks. The tanks were filled with dechlorinated water (1 mL of 10% sodium thiosulfate $Na_2S_2O_3 \bullet 5H_2O$/gallon), and kept aerated at room temperature (~22°C). The water was half refreshed every 5 days and the tanks were cleaned with Javex™ every 15 days. Every second day, the cultures were fed *Chlorella vulgaris* suspended in WC' medium (Appendix III); on each of these days, each 5 gallon tank was fed 50 mL *C. vulgaris* and the 50 gallon tank was fed 500 mL *C. vulgaris*. To mimic outdoor summer light conditions as closely as possible, the *Daphnia* were exposed to a photoperiod of 16 L: 8D daily.

C. vulgaris were previously collected from Delta Marsh, Manitoba, and were grown in WC' medium (Appendix III). The algal cells were subcultured into either 200 mL or 500 mL of medium and placed on a rotary shaker table in a controlled environment chamber at 19°C ± 1°C. The algal cells were grown under continuous PAR light for approximately 5 days until they reached a concentration of ~7.6 x 10^5 cells•mL^{-1}, previously determined by McClintock (2002), at which time the cells were fed to the *Daphnia*.

EXPERIMENTAL APPARATUS

Two exposure chambers, wood-frame boxes measuring 150 cm wide, 65 cm high and 70 cm deep, were used for the irradiation experiments (one chamber for UV-B exposure, the other for UV-A and PAR exposure) (Figure 1). One UV-B bulb

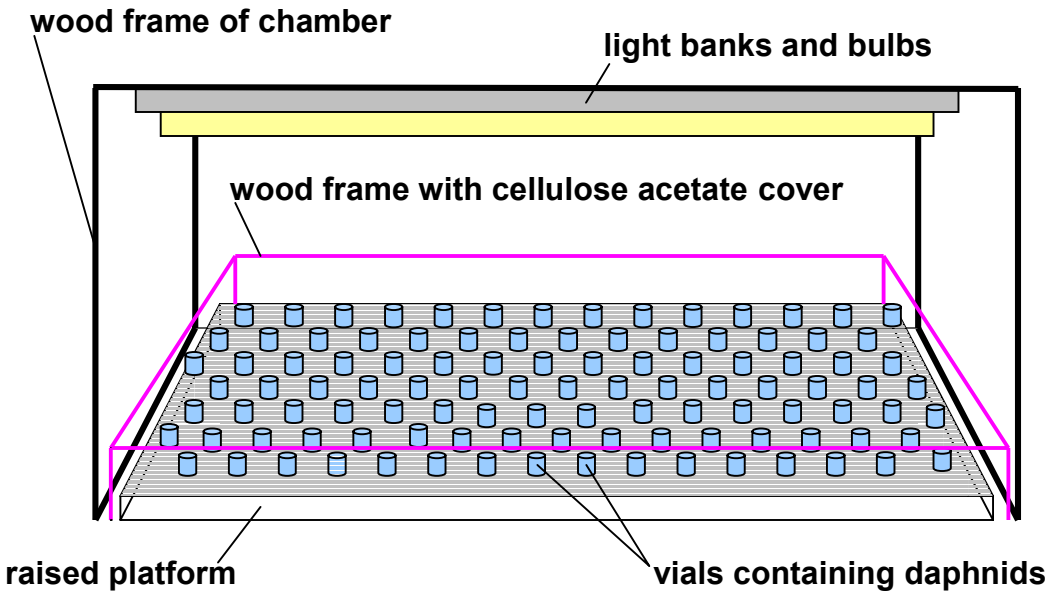

Figure 1: Schematic representation of the irradiation chambers used for Experiments I, II & III. The chamber measured 150 cm x 70 cm x 65 cm. Two fans (missing from diagram) were attached to the front of the raised platform to maintain the temperature of the chamber at 25°C. Modified from Gajda (2003).

(Philips UV-B TL 40W/12 RS), four UV-A bulbs (Philips F40BLB 40W T12), and two high output cool white fluorescent bulbs (GE F48T12 CW HO) were suspended from the top of each chamber. The vials used in the irradiation experiments were placed upon a raised platform that elevated the water level to 33 cm below the light source. Cellulose acetate sheets (0.003 inch thickness, Dick Blick Art Materials, Galesburg, Illinois) measuring 130 cm x 65 cm were pre-burned for 72 hours under UV-B light and then attached to frames that were placed on top of the raised platforms in each chamber, such that the cellulose acetate was positioned 5 cm above the water level. Filtering the light with cellulose acetate eliminates UV-C radiation and the shortest-wavelength UV-B radiation which are not present in incident solar radiation (Grad *et al*. 2003). To help contain the radiation within each chamber and deflect the ambient room light, heavy black polyethylene was attached to the front of each chamber and taped into place, minimizing exposure from one chamber to the other. To help maintain the temperature at 25°C, two fans, attached to the front of each chamber, were turned on during irradiation experiments. Following exposure to the appropriate amount of photorecovery radiation (PRR) (Experiments I & II), or UV radiation or PRR (Experiment III), *Daphnia* were transferred to an inoperative incubator (~25°C), which served as a dark chamber, and protected them from exposure to all wavelengths of light.

measurements for each irradiation period were taken at three different positions along the length of the bulbs, from which an average emission intensity was calculated. Light intensities detected during the lab experiments were compared to natural mid-summer light intensities, measured at water level on 26 July 2004 in Grand Beach, Manitoba, between 1100h and 1500h (Table 1).

Table 1: Comparison of average UV-B, UV-A and PAR intensity levels between lab exposure during Experiments I, II & III and natural mid-summer mid-day light intensities measured in Southern Manitoba on 26 July 2004.

	Lab Exposure	**Solar Radiation**
UV-B ($\mu W/cm^2$)	15.7	13.4
UV-A (W/m^2)	10.9	39.8
PAR (W/m^2)	16.8	403

The nature of PAR and UV-A bulbs is such that it is impossible to reproduce natural light intensities in a laboratory setting. However, it is possible to attain natural UV-B intensities in the laboratory. The cellulose acetate used in these experiments filtered out the shorter wavelengths of UV-B radiation emitted by UV-B bulbs. It also reduced the UV-B intensities reaching the *Daphnia* to natural levels.

D. MAGNA NEONATE COLLECTION PROTOCOL

Prior to beginning the irradiation experiments, approximately 100 large daphnids with developing brood sacs were isolated from the stock cultures and placed into 100 mL vials filled with dechlorinated water (1 daphnid per vial). The "maternity ward" received continuous PAR light and each daphnid was fed 2 mL of *Scenedesmus* spp. per day since a feeding experiment completed by Thuen (unpublished research) showed that reproductive output was significantly greater in *D. magna* fed *Scenedesmus* spp.

compared to *C. vulgaris*. *Scenedesmus* spp. were obtained from Ward's Biological Supply Company. Culturing protocol for *Scenedesmus* spp. was identical to that used for *C. vulgaris*. During the evening on the day immediately prior to the first day of an experimental trial, the maternity ward was placed in the dark. Neonates were removed from the maternity ward immediately prior to being placed in the dark to ensure that all daphnids collected the following morning were less than 24 hours old and had not been exposed to light.

EXPERIMENTAL PROTOCOL

Pre-irradiation Protocol

In the morning on the first day of the experimental trial, neonates were removed from the maternity ward under red light. If a trial with one day-olds was to be performed that day, the neonates were immediately placed into 150 mL vials filled with 125 mL dechlorinated water and labeled according to the treatments described below. If a trial with two, three or four day-olds was scheduled, the neonates were placed into a Carolina™ culture dish measuring 25 cm in diameter and filled with 500 mL of dechlorinated water. These *D. magna* were kept in the dark and were fed 50 mL of *C. vulgaris* per day until their scheduled day of irradiation (Day 2, 3 or 4), at which time they were also placed into vials as described for the one day-olds above. On their scheduled day of irradiation, the vials were placed either on the raised platform in the appropriate positions in the appropriate irradiation chamber or in the dark chamber. As the emission of UV radiation varied along the length of the bulbs, it was necessary to ensure that all vials were randomly distributed in the irradiation chamber. For each experiment (or each experimental trial), the position of the vials was determined using a computer randomizer program designed for Microsoft Windows® (Thuen, personal

communication), from which a template was designed and mounted on top of the raised platforms in each chamber.

Experiment I:
UV-B Exposure & Photorepair on Days 1, 2, 3 & 4 of Development

One, two, three and four day-old *D. magna* were exposed to UV-B radiation and PRR according to one of eight light regimes, resulting in 32 different test combinations. Each experimental treatment included a dark period, followed by a 6 hour period of UV-B, followed by varying periods of PAR and UV-A and concluding with a dark period (Table 2). A dose response experiment completed by Thuen (unpublished research) demonstrated that, at the intensity used in this series of experiments, a 6 hour period of UV-B exposure resulted in approximately 50% mortality in one day-old *D. magna*. Based on these findings, this length of time was used for this experiment. The light regimes were as follows:

1. **LT** Light control
2. **DK** Dark control
3. **B-0** UV-B 6 hours
4. **B-30** UV-B 6 hours, UV-A & PAR 30 minutes
5. **B-60** UV-B 6 hours, UV-A & PAR 60 minutes
6. **B-90** UV-B 6 hours, UV-A & PAR 90 minutes
7. **B-120** UV-B 6 hours, UV-A & PAR 120 minutes
8. **B-600** UV-B 6 hours, UV-A & PAR 600 minutes

Each vial contained five *D. magna*. The vials to be irradiated with UV-B (B-0, B-30, B-60, B-90, B-120 and B-600) were placed in the UV-B chamber, while the LT vials were placed in the photorecovery irradiation chamber. The DK vials were placed directly into the dark chamber. At the conclusion of the 6 hour period of UV-B irradiation, organisms in each vial were fed 10 mL of *C. vulgaris*. At this time, all vials in the UV-B irradiation chamber, with the exception of the B-0 vials, were transferred to the photorecovery irradiation chamber to begin their period of photorecovery irradiation.

Table 2: UV and PAR irradiation schedule for each of the treatment groups in Experiments I & II. Each treatment, with the exception of the LT and DK groups, began with a period of darkness, followed by a 6 hour period of UV-B, followed by a period of photorecovery radiation (PRR). All treatments concluded with a dark period lasting a minimum of 38 hours. Survival was monitored under red light at 24 and 48 hours post UV-B irradiation. Modified from Lichtman (2003).

DK	Dark	Dark							
LT	Dark	UV-A+PAR						Dark	
B-0	Dark	UV-B	Dark						
B-30	Dark	UV-B	UV-A+PAR	Dark					
B-60	Dark	UV-B	UV-A+PAR		Dark				
B-90	Dark	UV-B	UV-A+PAR			Dark			
B-120	Dark	UV-B	UV-A+PAR				Dark		
B-600	Dark	UV-B	UV-A+PAR					Dark	
	12 hours	6 hours	30 min.	30 min.	30 min	30 min.	8 hours	14 hours	24 hours
			24 hours						
								24 hr survival check	48 hr survival check

B-0 groups were not exposed to any PRR after exposure to UV-B. B-30, B-60, B-90, B-120 and B-600 groups were exposed to 30, 60, 90, 120 and 600 minutes of PRR, respectively. At the conclusion of the appropriate number of minutes of PRR, the vials were transferred to the dark chamber, where they remained until 48 hours after the conclusion of the period of UV-B irradiation. Organisms in each vial were fed 10 mL of *C. vulgaris* at 24 hours post UV-B irradiation. Survival was monitored under red light at 24 and 48 hours post UV-B irradiation. As each of the six vials per treatment was considered a replicate, only one trial per age was completed for this experiment.

Experiment II:
UV-B Exposure & Photorepair on Day 3 of Development

Experiment II was based on the results of Experiment I. To further expand on the age-dependent effects of PRR, three day-old *D. magna* were irradiated according to the same light regimes as Experiment I, but were followed over a 12-day period so that reproductive output for each treatment could be assessed (Table 2). In order to facilitate the analysis of reproductive output, each vial contained only one daphnid. The methods of Experiment II were identical to those of Experiment I, with the exception that at the conclusion of the post irradiation dark period, all vials were returned to the irradiation chamber where they were exposed to UV-A and PAR on a 16L: 8D cycle for the remainder of the experiment in order to ensure reproduction. Each organism was fed 2 mL of *C. vulgaris* per day. Survival and reproductive output were monitored daily over the course of the 12-day period. During the 48 hour post UV-B irradiation dark period, survival was monitored under red light. Values expressed are the averages of two trials with 16 vials/treatment in each trial.

Experiment III:
UV Exposure & Synthesis of UV-induced Photoprotective Pigmentation on Days 1, 2, 3 & 4 of Development

One, two, three and four day-old *D. magna* were exposed to either UV-B or UV-A and PAR radiation according to one of five light regimes, resulting in 20 different test combinations. Each experimental treatment included a dark period, followed by a 6 hour or 12 hour period of UV-B or UV-A & PAR (Table 3). The daphnids exposed to the 12 hour period of UV were exposed on two consecutive days (i.e. 6 hours the first day, followed by 18 hours of dark, followed by 6 hours the second day). The light regimes were as follows:

1. **DK** Dark control
2. **LT-6** UV-A & PAR 6 hours
3. **LT-12** UV-A & PAR 12 hours (6 hours per day for two consecutive days)
4. **B-6** UV-B 6 hours
5. **B-12** UV-B 12 hours (6 hours per day for two consecutive days)

Each vial contained five *D. magna*. The vials to be irradiated with UV-B (B-6 and B-12) were placed in the UV-B chamber, while those to be exposed to UV-A & PAR (LT-6 and LT-12) were placed in the UV-A and PAR irradiation chamber. The DK vials were placed directly into the dark chamber. At the conclusion of the appropriate light period, the UV-B and UV-A and PAR exposed daphnids were placed in the dark chamber for 48 hours. All vials were fed 10 mL of *C. vulgaris* at 24 hours post UV and light exposure. Survival was monitored under red light at 24 and 48 hours post UV and light exposure. As each of the six vials per treatment was considered a replicate, only one trial per age was completed for this experiment.

After monitoring survival at 48 hours post UV and light exposure, surviving *D. magna* were analyzed for accumulated melanin content using a spectrophotometric assay for total melanin. The melanin extraction method was modified from that described by Ozeki *et al*. (1995). Between one and ten surviving *D. magna* (depending on the number

Table 3: UV and PAR irradiation schedule for each of the treatment groups in Experiment III. Each treatment, with the exception of the DK group, began with a period of darkness, followed by a 6 hour period of either UV-B or UV-A & PAR, concluding with an 18 hour dark period. Treatment groups LT-12 and B-12 received an additional 6 hour period of UV-B and UV-A & PAR, respectively, on the second day, which was also followed by an 18 hour dark period. Survival was monitored under red light at 24 and 48 hours post UV-B irradiation.

DK	Dark	Dark					
LT-6	Dark	UV-A + PAR	Dark				
LT-12	Dark	UV-A + PAR	Dark		UV-A+ PAR	Dark	
B-6	Dark	UV-B	Dark				
B-12	Dark	UV-B	Dark		UV-B	Dark	
	12 hours	6 hours	18 hours		6 hours	18 hours	6 hours
			24 hours			24 hours	
					24 hr survival check		48 hr survival check

of survivors per treatment) from each test combination were allowed to air-dry for 24 hours on bibulous paper. The *D. magna* were then weighed as a group, placed in 1.5 mL microfuge tubes and crushed with a micropestle. To solubilize the tissue, 1.5 mL of 9:1 Solvable™ (Perkin-Elmer, Inc., Boston, Massachusetts): distilled water (v/v) was added to each microfuge tube in 0.5 mL aliquots. After vortexing, the microfuge tubes were placed in a boiling water bath for 30 minutes and then cooled to room temperature. After cooling, the microfuge tubes were returned to the boiling water bath for an additional 15 minutes and again cooled to room temperature. The microfuge tubes were then centrifuged in an Eppendorf 5415 C centrifuge (Brinkmann Instruments, Inc., Hamburg, Germany) at 10,000 rpm for 10 minutes. The absorbance of the supernatants at 500 nm (A_{500}) was measured using 1.5 mL spectrophotometric cuvettes in a Pharmacia Ultrospec III™ UV spectrophotometer (Biochrom Ltd., Cambridge, England). The spectrophotometer was zeroed with 9:1 Solvable: water. A standard curve of the absorbance of a dilution series of synthetic (sepia) melanin (No. M-8631, Sigma Chemical Co., St. Louis, Missouri) dissolved in 9:1 Solvable: water was prepared, from which the melanin content of *D. magna* from each test combination was derived (Appendix V). Although melanin does not have a distinct absorption coefficient at 500 nm, shorter wavelengths are not used as a result of considerable background noise due to proteins (Ozeki *et al*. 1995).

DATA COLLECTION AND ANALYSIS

For Experiments I and III, percent survival was determined by dividing the number of surviving daphnids in each vial by the total number of daphnids originally in each vial for each treatment group. All percent survival data were subjected to a modified arcsine square root transformation prior to analysis. For Experiment III,

accumulated melanin content data were subjected to a modified square root transformation. All transformed data were analyzed using a two-way analysis of variance (ANOVA) with Type III sum of squares (Dytham 2003; Babb, personal communication).

For Experiment II, percent survival was determined by summing the total number of surviving daphnids and dividing by the total number of daphnids originally in all vials for each treatment group for each trial. All percent survival data were subjected to a modified arcsine square root transformation prior to analysis. To assess reproductive output, neonates were counted and removed from the vials. Total reproductive output over a 12-day period for each treatment group was determined by adding the number of offspring for each vial for each treatment group over the 12-day period. Results of the two trials of Experiment II were combined and all statistical tests were performed on the average of the two trials. All data were analyzed using a one-way ANOVA (Dytham 2003; Babb, personal communication).

For all statistical tests, the significance level was set at α=0.05. If ANOVA results were significant, Fisher's Least Significance Difference (LSD) post-hoc test of multiple comparisons was performed (Babb, personal communication). All data analysis was performed using SPSS software (SPSS Inc., Cary, North Carolina).

RESULTS

Experiment I:
UV-B Exposure & Photorepair on Days 1, 2, 3 & 4 of Development

An increase in photorecovery radiation resulted in a corresponding increase in percent survival of *Daphnia magna* in all treatments at both 24 and 48 hours post UV-B irradiation. In addition, percent survival in each treatment increased with increasing age, also at both 24 and 48 hours (Figures 2 & 3). At both 24 and 48 hours, percent survival of *D. magna* not exposed to UV-B radiation (DK and LT treatments) was significantly higher ($p<0.001$) than all exposed groups (Tables 4 & 5). Within the exposed groups, *D. magna* not subjected to any PRR (B-0 treatment) had significantly lower survival than the groups subjected to 120 minutes and 600 minutes of PRR following UV-B radiation ($p<0.05$). Statistical analysis indicated an interaction effect ($p<0.05$) between age and length of photorecovery exposure for percent survival at 24 hours post UV-B irradiation (Table 4). Multiple regression analysis using transformed percent survival as the dependent variable and age and length of photorecovery exposure as the independent variables indicated a significant positive effect of both age and length of photorecovery exposure on survival ($p<0.001$).

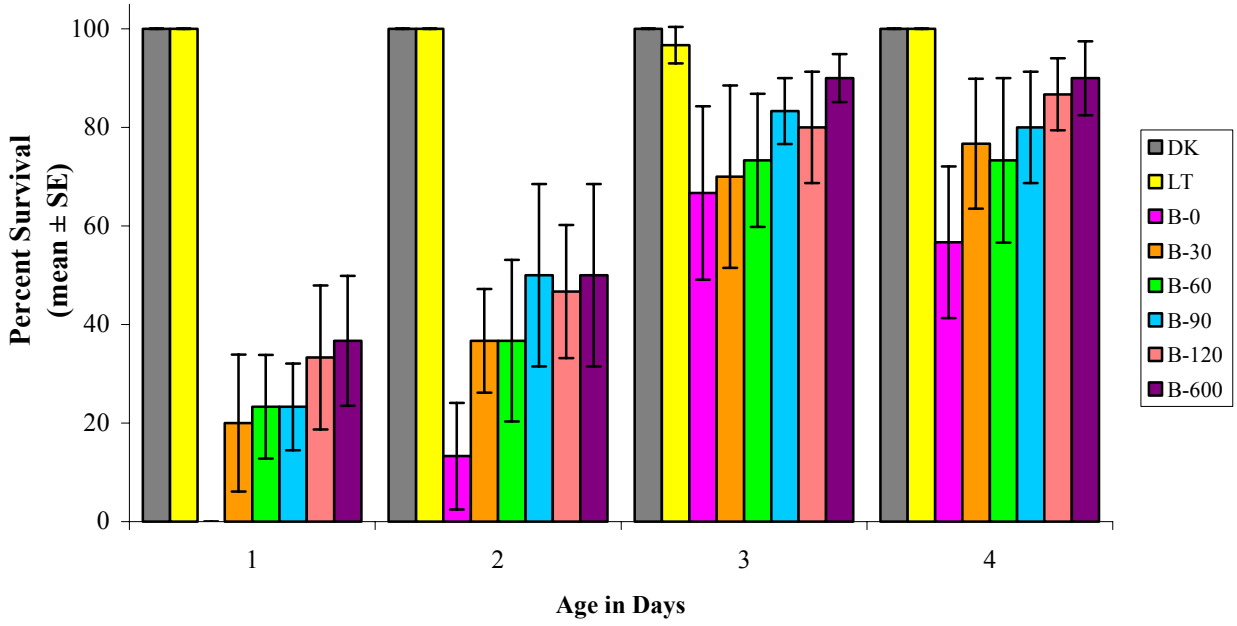

Figure 2: Percent survival (± standard error) of *Daphnia magna* at 24 hours post UV-B irradiation after exposure to 6 hours of UV-B radiation followed by exposure to varying periods (in minutes) of photorecovery radiation (PRR) on the first, second, third or fourth day of life (n = 6).

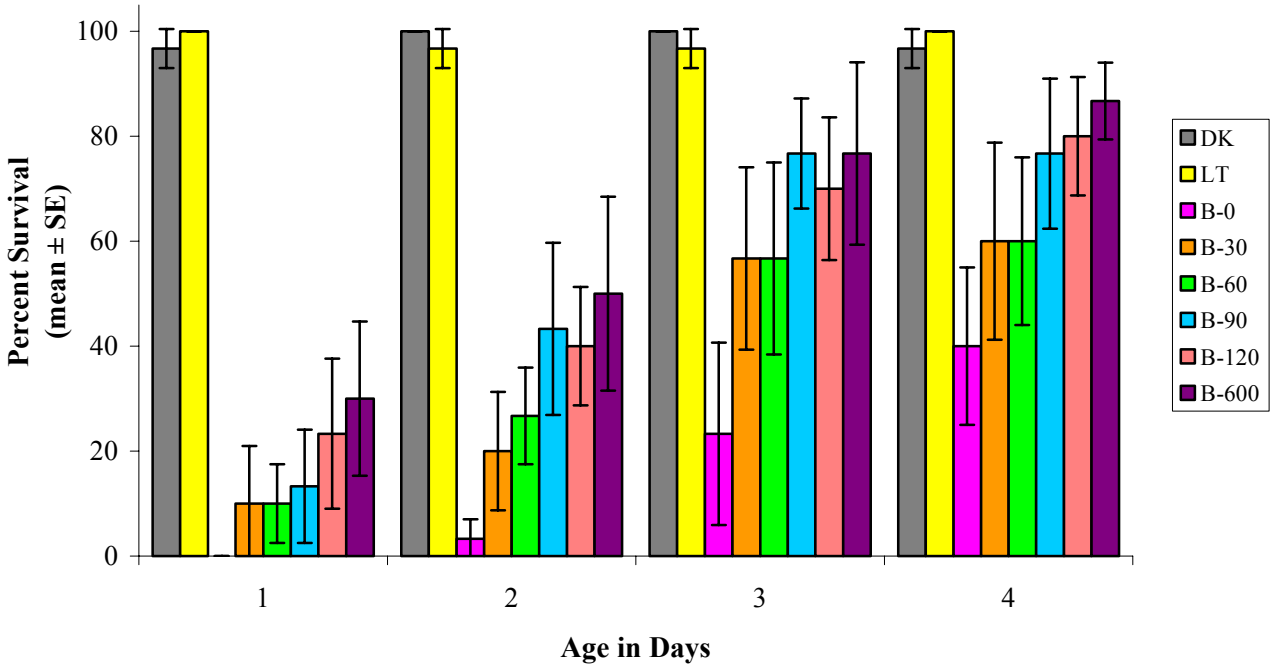

Figure 3: Percent survival (± standard error) of *Daphnia magna* at 48 hours post UV-B irradiation after exposure to 6 hours of UV-B radiation followed by exposure to varying periods (in minutes) of photorecovery radiation (PRR) on the first, second, third or fourth day of life (n = 6).

Table 4: Two-way ANOVA and Fisher's Least Significant Difference pairwise comparison test results for percent survival of *Daphnia magna* at 24 and 48 hours post UV-B irradiation after exposure to 6 hours of UV-B radiation followed by exposure to varying periods of photorecovery radiation (PRR) on the first, second, third or fourth day of life.

Source of variation	ANOVA *p*-value	Pairwise comparison	*p*-value	
			24 hours	48 hours
Age	**0.000**	Day 1 and Day 2	**0.020**	**0.024**
		Day 1 and Day 3	**0.000**	**0.000**
		Day 1 and Day 4	**0.000**	**0.000**
		Day 2 and Day 3	**0.000**	**0.000**
		Day 2 and Day 4	**0.000**	**0.000**
		Day 3 and Day 4	0.926	0.334
Light treatment	**0.000**	Dark control/Light control	0.903	1.000
		Dark control/B-0	**0.000**	**0.000**
		Dark control/B-30	**0.000**	**0.000**
		Dark control/B-60	**0.000**	**0.000**
		Dark control/B-90	**0.000**	**0.000**
		Dark control/B-120	**0.000**	**0.000**
		Dark control/B-600	**0.000**	**0.000**
		Light control/B-0	**0.000**	**0.000**
		Light control/B-30	**0.000**	**0.000**
		Light control/B-60	**0.000**	**0.000**
		Light control/B-90	**0.000**	**0.000**
		Light control/B-120	**0.000**	**0.000**
		Light control/B-600	**0.000**	**0.000**
		B-0/B-30	**0.022**	**0.009**
		B-0/B-60	**0.016**	**0.004**
		B-0/B-90	**0.001**	**0.000**
		B-0/B-120	**0.000**	**0.000**
		B-0/B-600	**0.000**	**0.000**
		B-30/B-60	0.911	0.801
		B-30/B-90	0.263	**0.041**
		B-30/B-120	0.143	**0.029**
		B-30/B-600	**0.035**	**0.002**
		B-60/B-90	0.314	0.073
		B-60/B-120	0.176	0.053
		B-60/B-600	**0.046**	**0.005**
		B-90/B-120	0.727	0.886
		B-90/B-600	0.318	0.289
		B-120/B-600	0.516	0.359
Interaction Effect				
24 hours	**0.034**			
48 hours	0.155			

Bold red probability values indicate a statistically significant difference at the 5% level.

Table 5: Results of Fisher's Least Significance Difference test showing pairwise comparisons of percent survival between treatment groups at 24 and 48 hours post UV-B irradiation for *Daphnia magna* exposed to 6 hours of UV-B radiation followed by exposure to varying periods of photorecovery radiation (PRR) on the first, second, third or fourth day of life.

Treatment Comparison	Day 1		Day 2		Day 3		Day 4	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
Dark control/Light control	1.000	0.773	1.000	0.803	0.811	0.843	1.000	0.825
Dark control/B-0	**0.000**	**0.000**	**0.000**	**0.000**	**0.028**	**0.000**	**0.003**	**0.001**
Dark control/B-30	**0.000**	**0.000**	**0.000**	**0.000**	**0.048**	**0.020**	0.101	**0.026**
Dark control/B-60	**0.000**	**0.000**	**0.001**	**0.000**	0.073	**0.021**	0.055	**0.025**
Dark control/B-90	**0.000**	**0.000**	**0.004**	**0.000**	0.242	0.190	0.152	0.217
Dark control/B-120	**0.000**	**0.000**	**0.003**	**0.000**	0.177	0.096	0.320	0.301
Dark control/B-600	**0.000**	**0.000**	**0.004**	**0.001**	0.474	0.190	0 .458	0.517
Light control/B-0	**0.000**	**0.000**	**0.000**	**0.000**	**0.048**	**0.000**	**0.003**	**0.000**
Light control/B-30	**0.000**	**0.000**	**0.000**	**0.000**	0.079	**0.032**	0.101	**0.016**
Light control/B-60	**0.000**	**0.000**	**0.001**	**0.000**	0.118	**0.033**	0.055	**0.015**
Light control/B-90	**0.000**	**0.000**	**0.004**	**0.000**	0.349	0.263	0.152	0.147
Light control/B-120	**0.000**	**0.000**	**0.003**	**0.000**	0.264	0.140	0.320	0.211
Light control/B-600	**0.000**	**0.000**	**0.004**	**0.002**	0.632	0.263	0.458	0.386
B-0/B-30	0.121	0.425	0.165	0.244	0.811	0.071	0.146	0.211
B-0/B-60	0.063	0.400	0.161	0.100	0.659	0.069	0.243	0.217
B-0/B-90	0.060	0.279	**0.036**	**0.008**	0.280	**0.005**	0.097	**0.025**
B-0/B-120	**0.011**	0.061	0.052	**0.013**	0.369	**0.014**	**0.037**	**0.016**
B-0/B-600	**0.006**	**0.020**	**0.036**	**0.002**	0.126	**0.005**	**0.021**	**0.006**
B-30/B-60	0.743	0.964	0.988	0.618	0.840	0.988	0.769	0.987
B-30/B-90	0.728	0.773	0.454	0.115	0.398	0.282	0.830	0.301
B-30/B-120	0.277	0.269	0.558	0.160	0.508	0.476	0.506	0.217
B-30/B-600	0.187	0.115	0.454	**0.045**	0.194	0.282	0.359	0.107
B-60/B-90	0.984	0.807	0.463	0.275	0.519	0.289	0.611	0.293
B-60/B-120	0.445	0.289	0.568	0.358	0.645	0.484	0.339	0.211
B-60/B-600	0.317	0.125	0.463	0.124	0.271	0.289	0.228	0.103
B-90/B-120	0.457	0.412	0.870	0.861	0.853	0.714	0.652	0.837
B-90/B-600	0.327	0.194	1.000	0.645	0.645	1.000	0.481	0.550
B-120/B-600	0.810	0.626	0.870	0.526	0.519	0.714	0.799	0.695

Bold red probability values indicate a statistically significant difference at the 5% level.

Experiment II:
UV-B Exposure & Photorepair on Day 3 of Development

Average percent survival of *D. magna* over a 12-day period increased with a corresponding increase in length of photorecovery exposure in all treatments (Figure 4). After twelve days, average percent survival of *D. magna* subjected to the DK treatment was significantly higher ($p<0.05$) than all exposed groups (Table 6). There was a significant difference ($p<0.05$) between *D. magna* subjected to 0 minutes of photorecovery radiation following UV-B radiation and all other groups (Table 6). There were no significant differences ($p>0.05$) in average percent survival among the LT and the groups subjected to 30, 60, 90, 120 or 600 minutes of PRR (Table 6). There were no changes in average percent survival for all treatments after Day 7 (Figure 4).

The average number of total offspring produced per surviving *D. magna* (Figure 5) was significantly greater ($p<0.001$) in the LT treatment compared to all other groups (Table 6). *D. magna* subjected to the DK treatment produced a significantly greater ($p<0.05$) average number of total offspring than daphnids subjected to 0 minutes and 30 minutes of PRR following UV-B exposure (Table 6). There were no significant differences ($p>0.05$) in the average number of total offspring produced among the B-30, B-60, B-90, B-120 and B-600 treatments (Table 6).

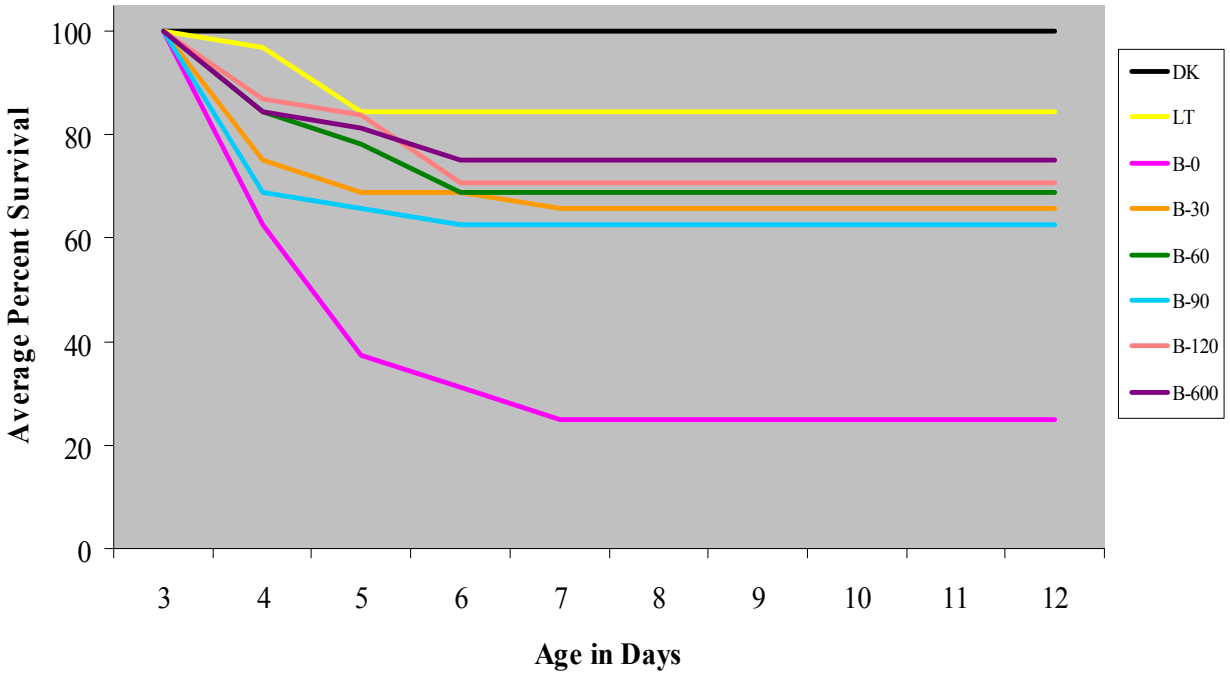

Figure 4: Average percent survival of *Daphnia magna*, for two twelve-day trials, after exposure to 6 hours of UV-B radiation followed by exposure to varying periods of photorecovery radiation (PRR) (in minutes) on the third day of life (n = 16).

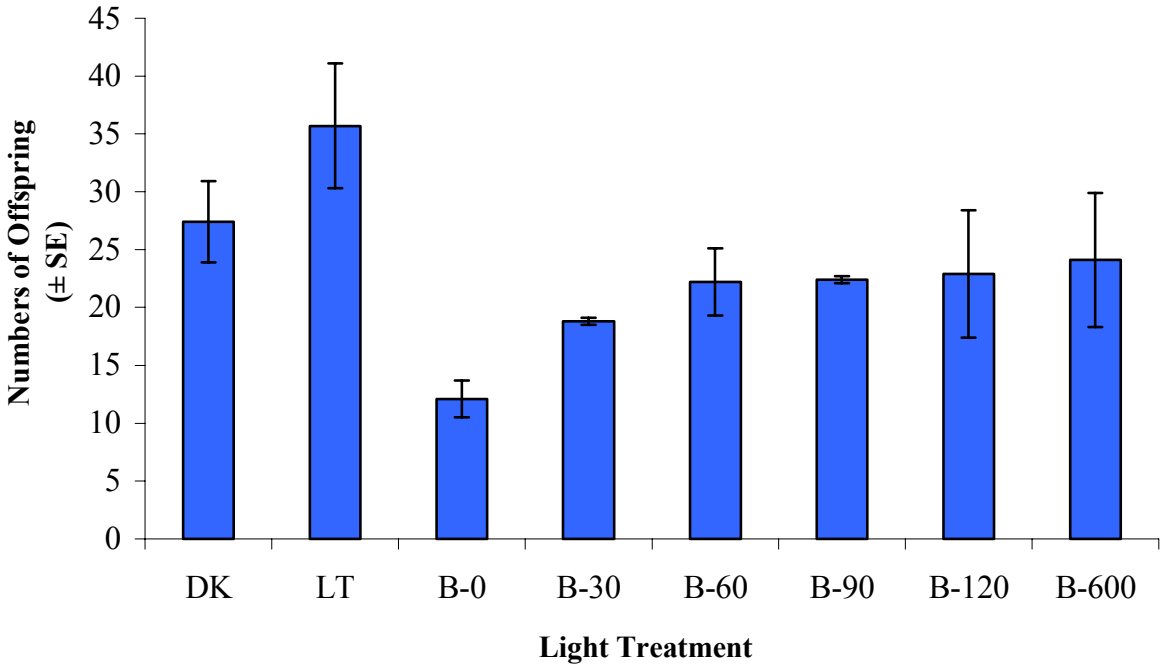

Figure 5: Average total offspring produced (± standard error) per surviving daphnid for *Daphnia magna*, for two twelve-day trials, after exposure to 6 hours of UV-B radiation followed by exposure to varying periods of photorecovery radiation (PRR) (in minutes) on the third day of life (n = 16).

Table 6: Results of Fisher's Least Significance Difference test showing pairwise comparisons of percent survival and average total offspring produced per surviving daphnid between treatment groups, for two twelve-day trials, for *Daphnia magna* exposed to 6 hours of UV-B radiation followed by exposure to varying periods of photorecovery radiation (PRR) on the third day of life.

Treatment Comparison	Percent Survival *p*-values	Total Offspring Produced *p*-values
Dark control/Light control	0.267	**0.004**
Dark control/B-0	**0.000**	**0.002**
Dark control/B-30	**0.019**	**0.008**
Dark control/B-60	**0.028**	0.125
Dark control/B-90	**0.014**	0.144
Dark control/B-120	**0.036**	0.102
Dark control/B-600	0.060	0.357
Light control/B-0	**0.001**	**0.000**
Light control/B-30	0.123	**0.000**
Light control/B-60	0.174	**0.000**
Light control/B-90	0.086	**0.000**
Light control/B-120	0.220	**0.000**
Light control/B-600	0.349	**0.000**
B-0/B-30	**0.012**	0.225
B-0/B-60	**0.009**	**0.047**
B-0/B-90	**0.018**	0.051
B-0/B-120	**0.007**	0.058
B-0/B-600	**0.004**	**0.015**
B-30/B-60	0.821	0.294
B-30/B-90	0.824	0.308
B-30/B-120	0.704	0.355
B-30/B-600	0.486	0.097
B-60/B-90	0.655	0.994
B-60/B-120	0.877	0.908
B-60/B-600	0.632	0.548
B-90/B-120	0.550	0.906
B-90/B-600	0.365	0.568
B-120/B-600	0.745	0.477

Bold red probability values indicate a statistically significant difference at the 5% level.

Experiment III:
UV Exposure & Synthesis of UV-induced Photoprotective Pigmentation on Days 1, 2, 3 & 4 of Development

Percent survival of *D. magna* exposed to either 6 or 12 hours of UV-B or UV-A and PAR radiation was consistent with previous experiments (personal observation). No mortality was observed in the Dark control, 6 hour and 12 hour UV-A and PAR treatments for *D. magna* irradiated on each of the four days of development. Percent survival in the 6 hour UV-B treatment increased with increasing age at time of UV-B exposure.

Cuticular melanin accumulation in *D. magna* increased with increasing age in the Dark control, 12 hour UV-A and PAR and 6 hour UV-B treatments (Figure 6). *D. magna* subjected to either the Dark control treatment or any of the light treatments on the fourth day of life had significantly greater ($p<0.05$) melanin accumulation than *D. magna* exposed on the first day of life (Table 7). *D. magna* exposed to either the Dark control treatment or any of the light treatments on the fourth day of life had greater melanin accumulation at any age than *D. magna* exposed on the second day of life; this difference was nearly significant ($p=0.073$) (Table 7). There was no significant difference ($p>0.05$) in melanin accumulation between any of the treatments (Dark control, UV-A and PAR, or UV-B) for either the 6 hour or 12 hour exposure period (Table 7). There was no survival for *D. magna* exposed to 6 hours of UV-B radiation on the first or second day of life and for *D. magna* exposed to 12 hours of UV-B radiation on all four days of development and thus, a melanin extraction could not be performed.

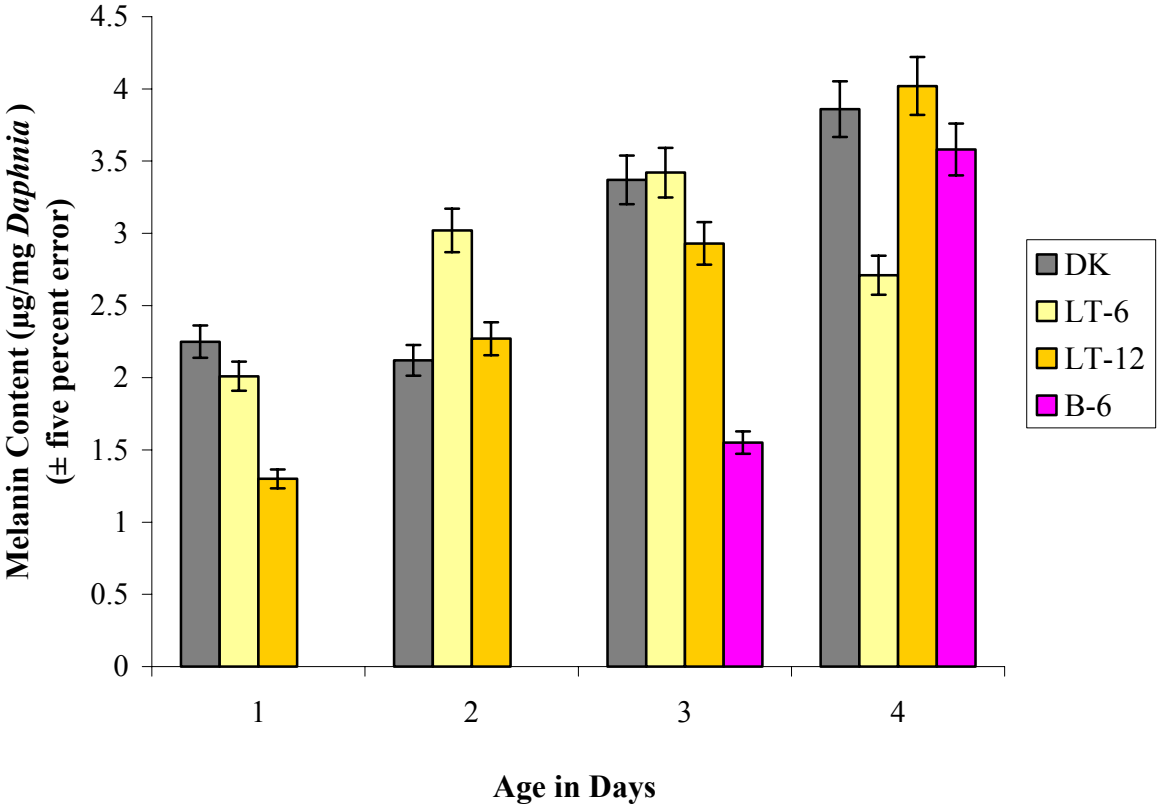

Figure 6: Cuticular melanin accumulation (± five percent error) of *Daphnia magna* after exposure to a dark treatment, 6 hours of UV-B radiation, or 6 or 12 hours of UV-A and PAR radiation on the first, second, third or fourth day of life. *D. magna* irradiated for a 12 hour period were irradiated for 6 hours on each of two consecutive days (n = 1).

Table 7: Two-way ANOVA and Fisher's Least Significant Difference pairwise comparison test results for cuticular melanin accumulation in *Daphnia magna* after exposure to a dark treatment, 6 hours of UV-B radiation, or 6 or 12 hours of photorecovery radiation (PRR) on the first, second, third or fourth day of life.

Source of variation	ANOVA *p*-value	Pairwise comparison	*p*-value
Age	**0.044**	Day 1 and Day 2	0.294
		Day 1 and Day 3	0.100
		Day 1 and Day 4	**0.013**
		Day 2 and Day 3	0.517
		Day 2 and Day 4	**0.073**
		Day 3 and Day 4	0.168
Light treatment	0.501	N/A*	N/A

Bold red probability values indicate a statistically significant difference at the 5% level.
Bold probability value indicates a significant difference at the 10% level.
* Post-hoc test not performed as *p*-value was not significant.

DISCUSSION

In recent decades, organisms living in aquatic environments have been subjected to an increasing number of environmental threats, necessitating rapid adaptation to enhance survival of future generations. The short life cycles of *Daphnia* permit them to adapt more quickly to environmental stresses than many other organisms. *Daphnia* populations in freshwater environments will comprise individuals of all ages, not all of which will show these adaptations. Although only one study has investigated the age-related susceptibility of cladocerans to UV-B radiation (Ramos-Jiliberto *et al*. 2004), age-specific sensitivity has been documented in several other aquatic organisms, including copepods (Lacuna and Uye 2000 & 2001), the rotifer *Asplanchna girodi* (Grad *et al*. 2003) and goldfish embryos (Wiegand *et al*. 2004).

PHOTOREPAIR ABILITY OF *D. MAGNA*

In the presence of UV-A and PAR, *D. magna* are able to reverse some of the damage created by UV-B exposure. The combination of UV-A and PAR can stimulate photoenzymatic repair to reverse the DNA damage created by UV-B exposure (Mitchell and Karentz 1993). As a general observation, survival increased as the length of photorecovery radiation (PRR) increased for *D. magna* irradiated on either the first, second, third or fourth day of life. This suggests that the photoenzymatic repair mechanism was operational during the photorecovery periods; exposure to longer periods of PRR allowed the repair mechanism to repair a larger portion of the DNA chain, resulting in higher survival. One day-old *D. magna* which were not exposed to any PRR following UV-B radiation (B-0 treatment) were no longer alive by 48 hours post UV-B irradiation, and only a very small proportion of two day-olds exposed to the same light regime remained alive. Comparable results were obtained by Grad *et al*. (2001) who

demonstrated that *D. pulicaria* exposed to 6 hours of UV-B followed by no photorecovery radiation did not survive past 48 hours post UV-B irradiation. It should be noted, however, that while Grad *et al*. (2001) used *D. pulicaria* which had not yet attained maturity, the daphnids used in their experiments were older than the one and two day-old *D. magna* discussed here. Interestingly, the older *D. magna* used in this experiment, which are comparable in age to the daphnids used in Grad *et al*.'s (2001) experiment, showed significantly higher survival than the *D. pulicaria* used by Grad *et al*. (2001). This may be attributable to the much larger size of *D. magna* compared to *D. pulicaria* (Rowe and Hebert 2002), which may increase its tolerance for UV-B radiation. Previous research has shown a size effect on survival of *Daphnia* exposed to UV-B radiation (Prosick 2002).

It appears that the length of photorepair exposure is crucial to the survival of one, two, three and four day-old *D. magna*. Only *D. magna* exposed to 120 or 600 minutes of PRR had significantly greater percent survival after both 24 and 48 hours post UV-B irradiation compared to all other exposed groups. These results are also very similar to those of Grad *et al*. (2001), who found higher survival rates in *D. pulicaria* in treatments with higher doses of PRR. Interestingly, the results of Grad *et al*. (2001) showed that survival in the treatment with the highest combined dose of UV-A and PAR was actually lower than in the treatment with the second highest combined dose of UV-A and PAR. This is perhaps not as surprising as it seems; in *D. magna* of all four age groups, there was no significant difference in survival between the B-120 and B-600 treatments, despite the sizeable difference in number of minutes of PRR. This may suggest that there is a limit to the amount of photoenzymatic repair that a juvenile daphnid is able to undergo after an intense 6 hour UV-B irradiation period, and that this limit is attained by the end of the 120 minute photorecovery period. In a preliminary trial of Experiment I,

Lichtman (2003) found slightly higher survival at 48 hours post UV-B irradiation in the B-600 treatment compared to the B-120 treatment for one day-old *D. magna*, and equal survival (100%) for both treatments in three day-old *D. magna*, however, these differences were not statistically significant.

One and two day-old *D. magna* appear to be considerably more susceptible to UV-B radiation than three and four-day olds. Considering that a substantial difference in size was observed between two and three day-old *D. magna* during the experimental period (personal observation), it follows that one and two day-olds should be less UV-tolerant, as smaller daphnids have a lower surface area: volume ratio. Similar results were obtained by Ramos-Jiliberto *et al*. (2004), who showed that the sensitivity of newborn (<48 hours of age) *Ceriodaphnia dubia* was significantly different from adults of the same species, when both were exposed to either UV-B, or UV-B with simultaneous exposure to UV-A and PAR. Interestingly, in the photorepair treatment, survival of *C. dubia* newborns, though significantly lower than adult *C. dubia*, did not differ with respect to adult *Daphnia chilense* and *Daphnia ambigua* (Ramos-Jiliberto *et al*. 2004). This suggests that different *Daphnia* species may have different UV tolerance levels, which may be a result of size, as mentioned above, or alternatively, degree of pigmentation (Hebert and Emery 1990). *C. dubia* newborns average only 1.3 mm in length, which is comparable to the size of both adult *D. chilense* and *D. ambigua*, the latter actually being the smallest of the *Daphnia* (Rowe and Hebert 2002). This may explain the similarities in UV tolerance of these three species, despite belonging to different genera.

Survival over a 12-day period in three day-old *D. magna* also appears to be correlated with length of PRR, with longer exposure to photorecovery radiation resulting in higher survival. However, while percent survival in most treatment groups had

stabilized by Day 5 (i.e. 48 hours post UV-B exposure), percent survival of *D. magna* exposed to the B-0 treatment continued to decrease until Day 7, at which point it too stabilized. This likely indicates that the amount of damage inflicted upon these *D. magna* was considerable, and that they would have benefited greatly from even a short period of PRR. This relates to the principle of reciprocity, an important consideration in photobiology. This principle states that the response to a short but intense UV-B exposure should result in the same effect as exposure to a long but less intense UV-B exposure (de Gruijl *et al*. 1986). However, Grad *et al*. (2001) postulate that reciprocity fails when significant photorepair capability is exhibited by an organism. If an organism is exposed to a high dose of UV-B irradiance for a short period of time in the presence of, or immediately prior to, PRR, the damage done by the high irradiance may be too great for the cells to repair, resulting in lower survival. Alternatively, an organism exposed to a lower dose of UV-B for a longer period of time in combination with PRR will likely have higher survival because cellular damage will be minimized as repair keeps up with damage (Grad *et al*. 2001). The validity of this suggestion becomes apparent when the results of this research are compared with those of McClintock (2002). McClintock's (2002) experiment differed from this research in that one day-old *D. magna* were exposed to only 30 minutes of UV-B at a time (total exposure time = 6 h), and both UV-B and UV-A and PAR were provided daily for a 12-day period. In addition, the average PAR intensity was only 1/3 of that obtained in this series of experiments because McClintock (2002) did not use high output fluorescent bulbs. Although McClintock (2002) also observed a significant decrease in percent survival of *D. magna* exposed to shorter periods of PRR, survival decreased at a slower rate, indicating that exposure to a shorter duration of UV-B, followed by PRR, daily, as opposed to a longer exposure period of

UV-B, with subsequent photorecovery exposure on only one occasion, does in fact increase survival significantly.

Trends in reproductive output paralleled trends in survival with greater reproductive output in three day-old *D. magna* when exposed to longer periods of PRR. Similar results were obtained by McClintock (2002), who demonstrated that reproductive output of *D. magna*, though lower than expected, did increase with increasing length of photorecovery exposure. Similarly, the reproductive output of *D. pulicaria* has also been shown to increase with increasing photorecovery exposure (Grad *et al*. 2001). It follows that *D. magna* exposed to longer periods of PRR should have higher reproductive output, as a greater portion of the DNA chain has been repaired, thus allowing the processes of DNA transcription and cell replication to occur, both of which are necessary for the production of offspring. The number of offspring produced by the UV-B exposed groups, even if exposed to PRR, was significantly lower than the LT treatment. Several studies have shown that UV-B exposure reduces the number of broods and the number of viable offspring in *Daphnia* (Jüttner 1989; Grad *et al*. 2001). Additionally, *D. magna* kept in the dark until the fifth day of life (DK treatment) produced fewer offspring than the LT treatment, indicating that earlier exposure to light appears to increase reproductive ability, assuming, of course, that the first exposure to light is not UV-B radiation.

INDUCTIVE PHOTOPROTECTION ABILITY OF *D. MAGNA*

Experiments involving several *Daphnia* species have shown that the extent to which daphnids avoid UV radiation is inversely proportional to their degree of pigmentation (Rhode *et al*. 2001). *D. magna*, a species with a distribution covering an extended range (Appendix IV), and therefore exposed to a variety of climatic conditions, has little cuticular pigmentation compared with other cladocerans (Rowe and Hebert

2002). However, Hessen (1993) suggested that zooplankton which evolve under less UV exposure, such as *D. magna*, should be more susceptible to UV, and consequently be more sensitive indicators of UV stress. While the results of this research indicate that cuticular melanin synthesis increases in *D. magna* with increasing age, melanin synthesis does not appear to be UV-induced on any of the four days of development studied. The absence of any differences in melanin pigmentation between the various light treatments suggests that *D. magna* may not be able to increase melanin synthesis. It is possible however, that the irradiation chamber intensities of both UV-A and PAR were not high enough to elicit a photoprotective pigment induction response. Only a fraction of natural PAR and UV-A intensities were obtained in the lab (4% and 27%, respectively), and while the intensity of PAR can be increased considerably with the use of high output bulbs, as was the case in these experiments, both likely remained too low to stimulate an observable change in melanin pigmentation. Nevertheless, the presence of highly pigmented cladocerans in areas where exposure to intense UV radiation occurs repeatedly over time suggests that some species have an innate ability to increase melanin synthesis, should environmental conditions necessitate it.

The relative ease with which normally highly UV-stressed *Daphnia* populations produce melanin suggests that arctic and alpine species have evolved a genetic make-up that easily allows them to do so (Hessen 1993). This, combined with the high energetic cost, offers one explanation as to why *D. magna* do not appear to increase melanin production upon UV stimulation. Previous studies have demonstrated that detecting such an effect, despite exposure to even ambient UV levels, is by no means guaranteed. Both Hessen (1996) and Häkkinen *et al*. (2003) found no evidence of the existence of a relationship between ambient levels of UV radiation and degree of melanin pigmentation in *D. pulex* and coregonid larvae, respectively, in regions with less intense UV exposure.

Alternatively, while it is possible that *D. magna* may be able to synthesize melanin upon UV light stimulation in the natural environment, the nature of stock cultures is such that *D. magna* from these cultures may have lost this ability, as a result of being maintained in the lab under artificial light for at least six years. Though the stock cultures are refreshed periodically, this may not be sufficient to ensure that the population remains capable of melanization.

Neonate cladocerans hatch as miniature adults, growing larger at each molt without any significant morphological changes (Rose *et al*. 2004). At ~22-24°C, molting occurs an average of every 36 hours during juvenile developmental stages, increasing to once every 45 hours once maturity is attained (Pavlova 1959). This is an important factor to take into consideration when studying cuticular melanin accumulation. For obvious reasons, it is imperative to perform the melanin extraction on the carapace which was stimulated by the UV light. While the external carapace of *Daphnia* is composed of a tough cuticle, a new softer chitinous carapace is formed *de novo* beneath the external carapace, and hardens upon molt of the external carapace (Espie and Roff 1995). Any UV light irradiating the daphnid would stimulate melanin production in the soft internal carapace, as this is the only one which would be capable of incorporating new melanophores, or increasing the size of current ones, in the cuticular tissue. Though not designed for this purpose, the 48 hour post UV-B irradiation period allowed for shedding of the external carapace after UV irradiation, assuming that juvenile *D. magna* do indeed molt every ~36 hours. Thus, when the daphnid was sacrificed at the end of the 48 hour dark period, the melanin extraction was performed on the carapace in which increased melanin synthesis occurred, if in fact it occurred.

Though UV tolerance experiment results have always been difficult to translate from simple laboratory experiments to highly complex ecosystems (Ramos-Jiliberto *et al*.

2004), the additional consideration of age, or life-history stage, makes this an even more daunting task. When studying *D. magna* in aquatic ecosystems, the additional consideration of the population's age structure must be taken into account, as the age structure of the population will affect its susceptibility to UV radiation. This has the potential to result in both intraspecies and interspecies population shifts, thus affecting population dynamics. Certain species, or zooplankton of certain ages, which are more UV tolerant should exhibit better growth rates, thus increasing survival. Such species would be able to profit most from the favorable growth conditions found immediately below the surface, thereby increasing reproductive output to counterbalance the mortality exerted by both visual predators, and especially, UV-B radiation (Stich and Lampert 1984). Future work could thus examine the effects of UV-B radiation on *D. magna* of different ages in the natural environment. Additionally, it would be interesting to repeat this experiment with a more highly pigmented species such as *D. pulex*, to determine if it is more resistant to UV radiation at the same juvenile life-history stages, and if it is capable of synthesizing melanin to a greater extent than *D. magna*.

It is important to examine the effects of UV radiation on zooplankton of different ages to become aware of the age-specific susceptibility of aquatic organisms to increased UV exposure. The relative vulnerability of zooplankton to UV radiation, especially at juvenile stages, increases the probability of population shifts, which could have a dramatic impact on food web dynamics, resulting in a multitude of new environmental stresses, in addition to direct UV exposure, in aquatic ecosystems. Though many more possible ramifications of increased UV exposure on these environments likely remain unknown, the nature of trophic level interactions is such that the detrimental effects of increased UV radiation will come to be felt not only within lake ecosystems, in which *Daphnia* are found, but also within surrounding terrestrial ecosystems.

CONCLUSIONS

1. Survival increases with increasing age and length of photorecovery exposure in *Daphnia magna* exposed to photorecovery radiation following UV-B radiation on the first, second, third or fourth day of life.

2. UV-B exposure without subsequent photorecovery exposure significantly reduces survival and reproductive output in *D. magna* irradiated on the third day of life.

3. Exposing *D. magna* to a photorecovery period beyond 30 minutes following UV-B irradiation on the third day of life does not significantly improve survival or reproductive output.

4. Cuticular melanin content increases with increasing age in *D. magna* when exposed to UV-B radiation or UV-A and PAR radiation on the first, second, third or fourth day of life.

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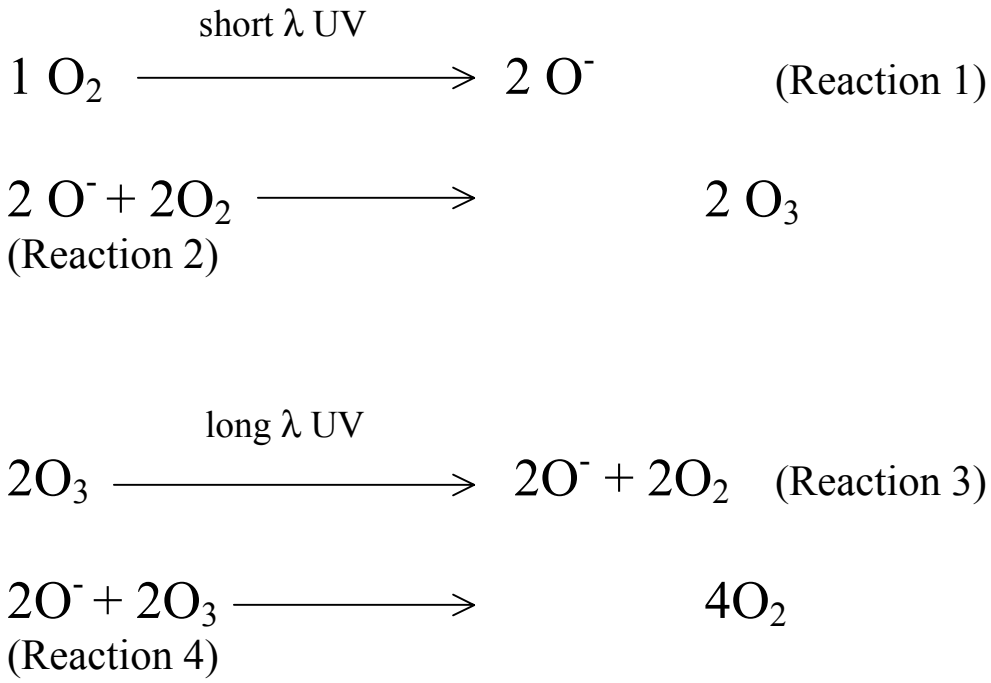
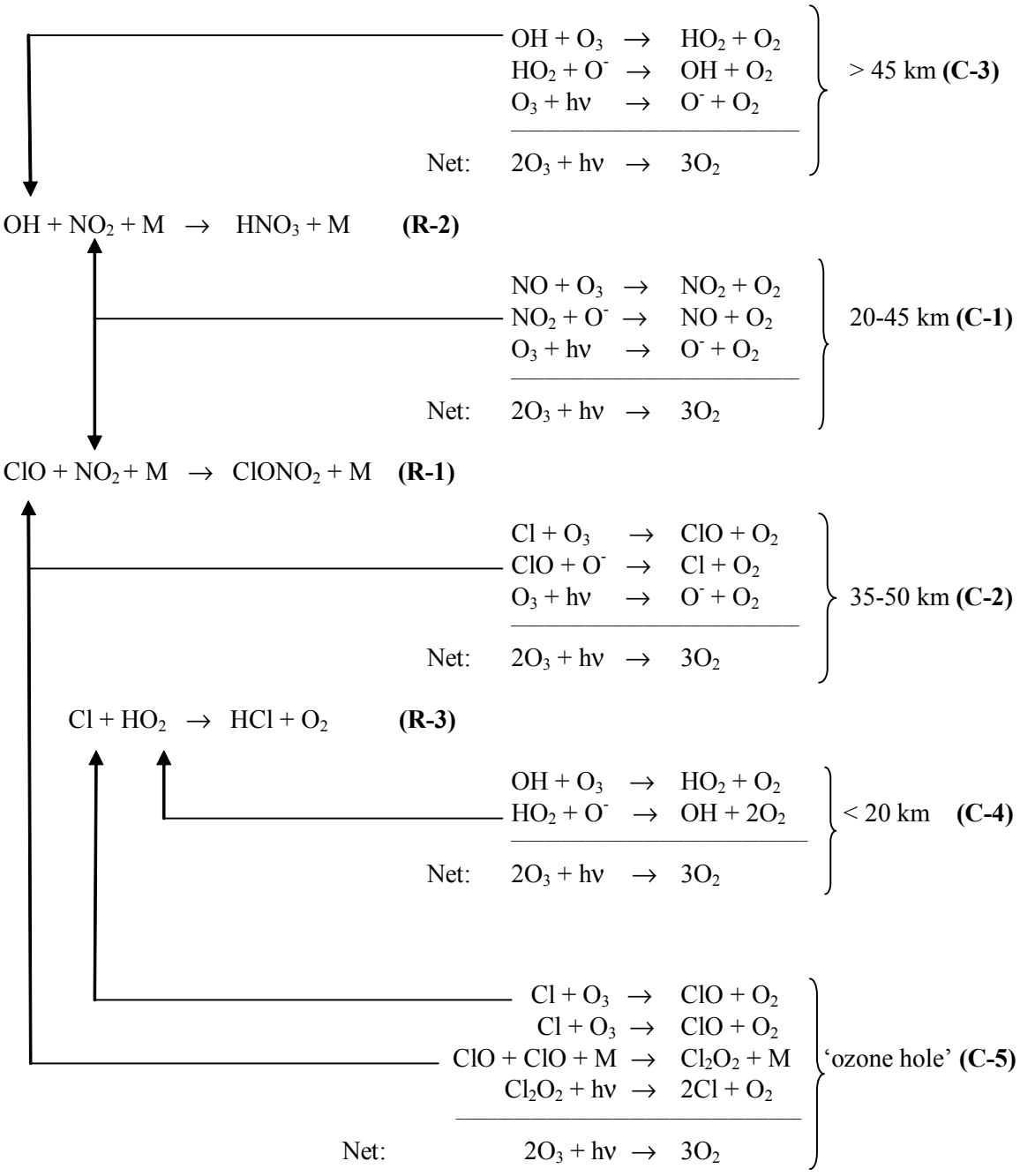
APPENDIX I: The Complete Ozone Cycle



The complete ozone cycle: Each oxygen molecule is broken down by shortwave UV radiation ($\lambda < 242$ nm) to two oxygen radicals (Reaction 1), which in turn combine with two oxygen molecules to form two ozone molecules (Reaction 2). Upon absorption of longer wavelengths of UV radiation ($\lambda \approx 200$-320 nm), the two ozone molecules are broken down to two oxygen radicals and two oxygen molecules (Reaction 3). The two oxygen radicals combine with two molecules of ozone to form four molecules of oxygen (Reaction 4). The cycle can then begin once more (Häder and Worrest 1991; Peter 1994).

APPENDIX II: Ozone Degradation Cycles



Catalytic ozone destruction cycles (C-1)-(C-3) and coupling of cycles (R-1)-(R-3) due to the formation of reservoir gases ($ClONO_2$, HCl, HNO_3): The HO_x, NO_x and ClO_x cycles (C-1)-(C-3) depend on the formation of the O radical and are active in the middle and upper stratosphere. Cycle (C-4) does not depend on the O radical and works at lower altitudes. Cycle (C-5) is believed to be mainly responsible for the Antarctic ozone hole. M represents a collision partner (Peter 1994).

APPENDIX III: WC' Medium

Formula for WC' algal growth medium originally formulated as WC medium by Guillard and Lorenzen (1972) and later modified by Healey and Hendzel (1979) for the purpose of creating a growth medium suitable for phosphate limited algal cultures. The final pH of the medium should be adjusted to between 7.49 and 7.51 using HCl and NaOH.

	Compound	Final Concentration
***MAJOR ELEMENTS**	$NaNO_3$	42.5 (mg/L)
	KH_2PO_4	1.4
	KCl	3.0
	$MgSO_4 \bullet 7H_2O$	37.0
	$CaCl_2 \bullet 2H_2O$	36.8
	$NaHCO_3$	12.6
	$Na_2SiO_3 \bullet 9H_2O$	56.8
†TRACE ELEMENTS	Na_2EDTA	2.185 (mg/L)
	$FeCl_3 \bullet 6H_2O$	1.575
	H_3BO_3	0.500
	$MnCl_2 \bullet 4H_2O$	0.090
	$Na_2MoO_4 \bullet 2H_2O$	0.003
	$ZnSO_4 \bullet 7H_2O$	0.011
	$CoCl_2 \bullet 6H_2O$	0.005
	$CuSO_4 \bullet 5H_2O$	0.005
‡BUFFER	Tricine	179.2 (mg/L)
§VITAMINS	A: Cyanocobolamine (B_{12})	0.50 (µg/L)
	B: Biotin	0.50
	C: Thiamine	101.00

* Aqueous stocks of the major elements should be added to distilled water in the order given.

† A concentration stock solution of trace elements should be first prepared by adding the trace elements in the order listed to 1L of distilled water, then adding 2.5 mL of the stock solution to 1L of medium.

‡ A concentration stock solution of buffer should be prepared by adding 17.92 g of tricine to 1L of distilled water, then adjusting the pH of the stock solution to 7.0. Add 10 mL of stock solution to 1L of medium.

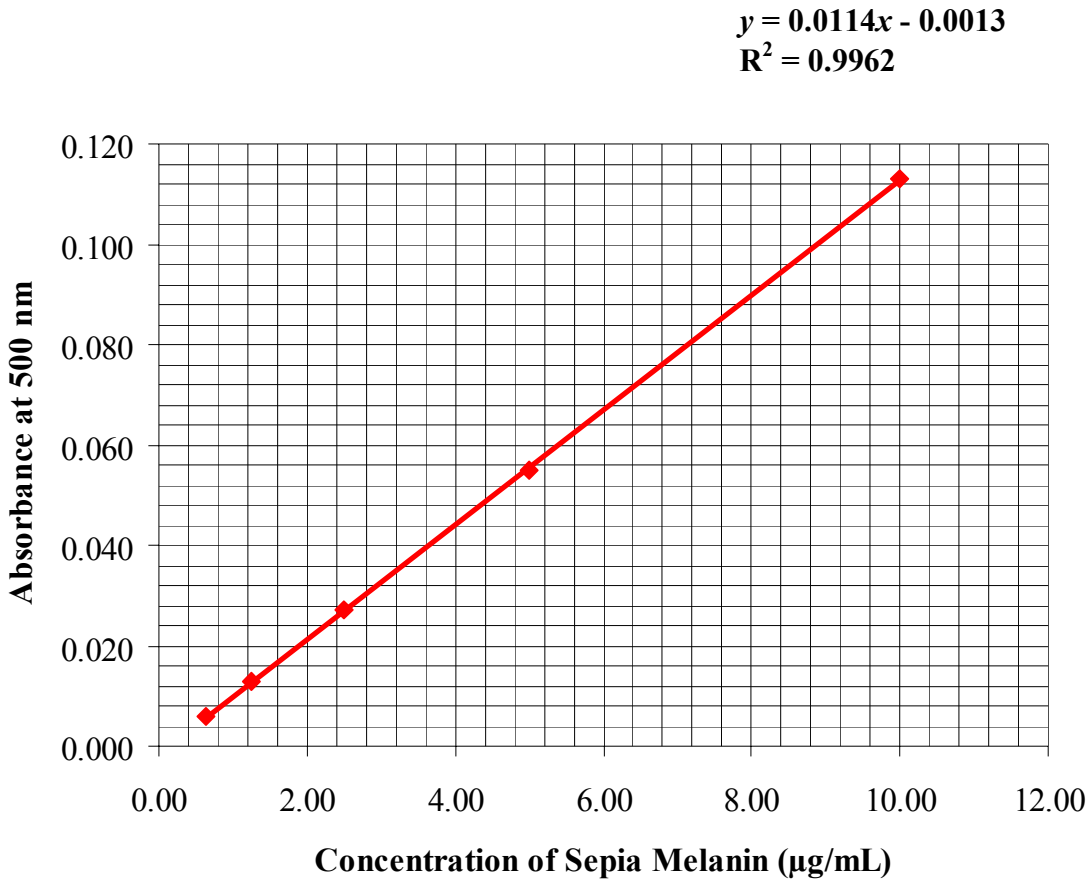
§ Three separate solutions should be prepared.
A: 10.0 mg/100 mL of distilled water
B: 10.0 mg/100 mL of distilled water
C: 10.0 mg + 0.5 mL solution A + 0.5 mL solution B + 99.0 mL of distilled water
Add 1.0 mL of solution C to 1 L of medium.

APPENDIX IV: The Experimental Organism

A

B

A. *Daphnia magna* (2-5 mm)
B. Distribution of *D. magna* in North America (Rowe and Hebert 2002)

APPENDIX V:

Standard Concentration Curve for a Dilution Series of Sepia Melanin Dissolved in 9:1 Solvable™:Water