THE EFFECT OF TEMPERATURE ON THE SURVIVAL AND REPRODUCTION OF *Daphnia magna* FOLLOWING EXPOSURE TO ULTRAVIOLET-B RADIATION

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ABSTRACT

In recent years there has been increasing concern regarding the damaging effects of ultraviolet radiation (UVR) on aquatic organisms. *Daphnia*, a well-studied genus of freshwater zooplankton, employs two mechanisms of deoxyribonucleic acid (DNA) repair that are temperature-dependent: photoenzymatic repair (PER, light-dependent repair), and nucleotide excision repair (NER, light-independent repair). In the presence of photorecovery radiation (PRR), *Daphnia* have the ability to undergo PER. This research focused on the effect of acute UV-B exposure, temperature and variable PRR on the survival and reproduction of *Daphnia magna*. Three day-old *D. magna* were exposed to a single six-hour dose of UV-B radiation followed by varying periods of PRR. Animals were maintained within several degrees of 32ºC throughout the experiment, and survival and reproduction of the parental and first filial (F1) generations were followed until the animals were twelve days old. It has been previously demonstrated at 26˚C that mortality of the parental generation following UV-B exposure is partially mitigated by PRR and absence of PRR results in 100 % mortality. Between 30˚C and 32ºC, it appears that in the absence of PRR, some survival occurred possibly as a result of temperature-related increased enzymatic repair. The UV treatment history of the parental generation had no effect on F1 survival; however, survival and reproduction of the F1 generation were compromised at 32 ºC.

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INTRODUCTION

CONCERN FOR AQUATIC LIFE

Stratospheric ozone functions to absorb ultraviolet radiation (UVR) from the sun, thus reducing the amount of harmful UV-B radiation reaching the earth's surface (Aucamp 2003). First observed in the late 1970s, the Antarctic ozone hole continues to increase in size and depth (Häder 1997). Physical factors and air pollutants combine to produce ozone depletion that creates the ozone hole, resulting in increased UV-B intensities each spring (Karentz 1991). In subarctic regions, springtime levels of UV-B radiation reaching the earth's surface have risen 10-20% from the late 1970s to 1995 (Rautio and Korhola 2002). Recognition of this atmospheric phenomenon has led to increased concern regarding the effects of elevated UV-B levels on aquatic ecosystems.

UVR is the shortest wavelength of radiation reaching the earth's surface (Rautio and Korhola 2002), and it can be subdivided into three bands: UV-A (320-400 nm), UV-B (280-320 nm), and UV-C (100-280 nm) (Karentz 1991). UVR reaching the earth's atmosphere is primarily comprised of UV-A and a small amount of UV-B; approximately 10% of UV-B radiation is transmitted through the atmosphere (Aucamp 2003). UV-B is a biological danger, and while most is normally absorbed by stratospheric ozone, there has been a downward trend of total column ozone of –6.1% per decade for April, and –3% per decade for June between 1979 and 1999, while UV-B is predicted to increase at a rate of 8% per decade (Häder *et al.* 2003).

It is now known that high intensities of UVR are detrimental to aquatic life (Rautio and Korhola 2002). While natural waters contain UV-absorbing substances, such as dissolved organic carbon (DOC) and dissolved organic matter (DOM), climate change

is predicted to alter the DOC concentration in many North American lakes (Persaud and Williamson 2005). Currently, many northern waters are poor in DOC. For example, the median DOC measured for 45 lakes across the treeline in Finnish Lapland was 3.0 mg L^{-1} (Rautio and Korhola 2002). When DOC concentrations fall below 2 mg L^{-1} in freshwater lakes, UV-B can penetrate to several metres depth (Rautio and Korhola 2002). Additionally, many organisms in aquatic ecosystems inhabit areas close to water surfaces where protection from UV-B is not easily found (Aucamp 2003). UV wavelengths, especially UV-B, can damage deoxyribonucleic acid (DNA) and other biological molecules, alter carbon distribution, and increase the development of highly reactive oxygen species (Persaud and Williamson 2005). Consequently, UVR can create environmental stress resulting in changes in net productivity, trophic dynamics and the taxonomic structure of communities (Karentz 1991).

STUDY ORGANISM

Zooplankton species are ideal model organisms to test the effects of UV-B radiation (Rautio and Korhola 2002). *Daphnia* spp., a circumpolar planktonic genus of freshwater crustacean zooplankton, is highly vulnerable to UVR (Zellmer *et al.* 2004). Daphnids play a vital role in aquatic food chains (Adema 1978). As primary consumers in the aquatic food web, they provide an essential link to populations that rely upon aquatic ecosystems for their food supply (Häder *et al.* 2003). UVR can cause changes to the basic biochemistry and productivity of the phytoplankton community, which may adversely affect zooplankton grazers such as *Daphnia* (Zellmer *et al.* 2004). Additionally, UVR has also been found to decrease survival of *Daphnia* during at least

one life stage and to reduce the number of broods and number of progeny per brood (Grad *et al.* 2001; Häder *et al.* 2003; Huebner *et al.* 2006).

Daphnia magna, the common water flea, was the experimental organism used in this study. It is the largest of all species of daphnids, measuring 2-5 mm at maturity (Appendix I) (De Gelas and De Meester 2005). It is characterized by a Holarctic distribution (De Gelas and De Meester 2005), where it is found in ditches, shallow ponds, and small eutrophic reservoirs (Hanski and Ranta 1983). *Daphnia* populations normally experience temperature ranges of 5ºC -25ºC in north temperate lakes (MacFadyen *et al.* 2004). As with most *Daphnia* species, *D. magna* is distinguished by a cyclical parthenogenetic reproductive cycle (De Gelas and De Meester 2005).

PHYSIOLOGICAL RESPONSES TO THE ENVIRONMENT

Daphnia and other aquatic organisms have developed several mechanisms by which to reduce the adverse effects of solar radiation: avoidance (moving away from the water surface); photoprotection (increasing the amount of pigment in the cuticle and body tissues); and, DNA repair (specifically photorepair) (Zagarese *et al.* 1997). This research examined the use of photorepair mechanisms to repair damaged DNA in *D. magna*.

PHOTOREPAIR MECHANISMS

One of the primary targets of UVR is a cell's nuclear DNA (Pakker *et al.* 2000). When DNA absorbs UV light, harmful photoproducts are produced (MacFadyen *et al.* 2004). Two adjacent pyrimidine nucleotide bases become covalently linked to form a dimer that bends the phosphate backbone of the DNA molecule (MacFadyen *et al.* 2004). Two types of pyrimidine dimers are commonly induced: cyclobutyl pyrimidine dimers

(CPDs), which typically account for about 80-90% of photoproducts formed (MacFadyen *et al.* 2004), and pyrimidine (6-4) pyrimidone photoproducts (Pakker *et al.* 2000). Consequently, this leads to a distortion of the DNA, culminating in an arrest of gene replication and transcription (Pakker *et al.* 2000). In addition to genetic damage, UV absorption can disrupt membrane structure and/or the chemical environment of cells, altering normal metabolic processes, resulting in decreased growth, impaired reproduction, or death (Karentz 1991).

Many organisms possess the ability to repair UVR-induced DNA damage, including *Daphnia* (Grad *et al.* 2001). One such mechanism is photoenzymatic repair (PER). In PER, the enzyme photolyase reverses the pyrimidine dimer to its normal monomeric form in the presence of photorecovery radiation (PRR), which consists of UV-A radiation (320-400 nm) and photosynthetically active radiation (PAR; 400-700 nm) (Pakker *et al.* 2000). Most organisms, from blue-green algae to marsupials, possess PER; however, the rate of repair varies greatly among species (Grad *et al.* 2001; MacFadyen *et al.* 2004). Previous studies have shown that daphnids have a significant amount of PER (Zagarese *et al*. 1997; Grad *et al.* 2001).

Nucleotide excision repair (NER), also known as light-independent/dark repair (Pakker *et al.* 2000), is another primary DNA repair mechanism (MacFadyen *et al.* 2004). NER is present in all taxa but is not specific to the repair of UV-induced DNA damage (MacFadyen *et al.* 2004). It is an intricate, multi-protein, multi-step pathway that excises the damaged oligonucleotide and replaces it with a newly synthesized fragment (Pakker *et al.* 2000). Organisms that demonstrate efficient PER seem to have less capability for NER (Pakker *et al.* 2000).

Aquatic organisms are faced with ozone-related increases in UV-B in their environment, and the possibility of increased average water temperatures as a result of climate change. Investigation into the effects of UV-B and temperature on aquatic organisms is of importance for two reasons. First, increased temperature can potentially reduce the negative effects of UV-B radiation, since DNA repair processes are enzymatic and hence temperature-dependent (MacFadyen *et al.* 2004). Second, survival, growth, and reproduction of many aquatic organisms seem to be greatly influenced by temperature (Pakker *et al.* 2000). It is likely that alterations in UV and temperature will inevitably affect the biological community (Persaud and Williamson 2005), and examining the effects of both increased temperature and UV-B may well reflect the future conditions of many aquatic species.

OBJECTIVE AND HYPOTHESIS

In the present study, *Daphnia magna* was used as an experimental organism to determine if temperature had an effect on survival and reproduction after one single, six-hour dose of UV-B radiation, followed by varying periods of PRR. Previous studies have demonstrated that exposure to PRR can increase survival of *D. magna* following UV-B irradiation (Grad *et al.* 2001; Williamson *et al.* 2002; Huebner *et al.* 2006). Williamson *et al.* (2002) found that in all their experiments, *Daphnia* does not survive exposure to UV-B without subsequent PRR, even at low doses. Due to the temperature dependence of DNA repair systems, I hypothesized that at elevated temperature there would be increased survival and reproduction of *D. magna*, especially for those animals that did not receive PRR following exposure to UV-B.

MATERIALS AND METHODS

STOCK CULTURES

D. magna stock cultures (Ward's Biological Supply Cp., Rochester, NY) were maintained in dechlorinated water at approximately 22ºC in two 50-gallon tanks. The tanks were half refreshed every five days, and fully cleaned every 15 days. Animals were exposed to a 16 hour light: 8 hour dark cycle. They were fed *Chlorella pyrenoidosa* suspended in WC' medium (Appendix II) three times a week.

C. pyrenoidosa (Ward's Natural Science Ltd., St. Catherine's, ON) were grown in WC' medium (Appendix II). Stock cultures were subcultured into 200 mL of medium every five to seven days and placed on a rotary shaker table in an environmental chamber. The algal cells were exposed to a 16 hour light: 8 hour dark cycle until they reached a concentration of approximately $3.5x10^6$ cells•mL^{-1}. The algal cells were then maintained as a semi-continuous cell culture (Calcott 1981) (Appendix III), and grown under continuous PAR light.

EXPERIMENTAL APPARATUS

Experiments were carried out in two exposure chambers measuring 150 cm wide, 65 cm high, and 70 cm deep (Figure 1). One UV-B bulb (Philips UV-B TL 40W/12RS) was suspended from the top of the UV-B chamber. Three UV-A bulbs (Philips F40BLB 40W T12) and four cool white fluorescent bulbs (GE F48T12 CW HO) were suspended from the UV-A and PAR chamber. The vials used in the irradiation experiments were

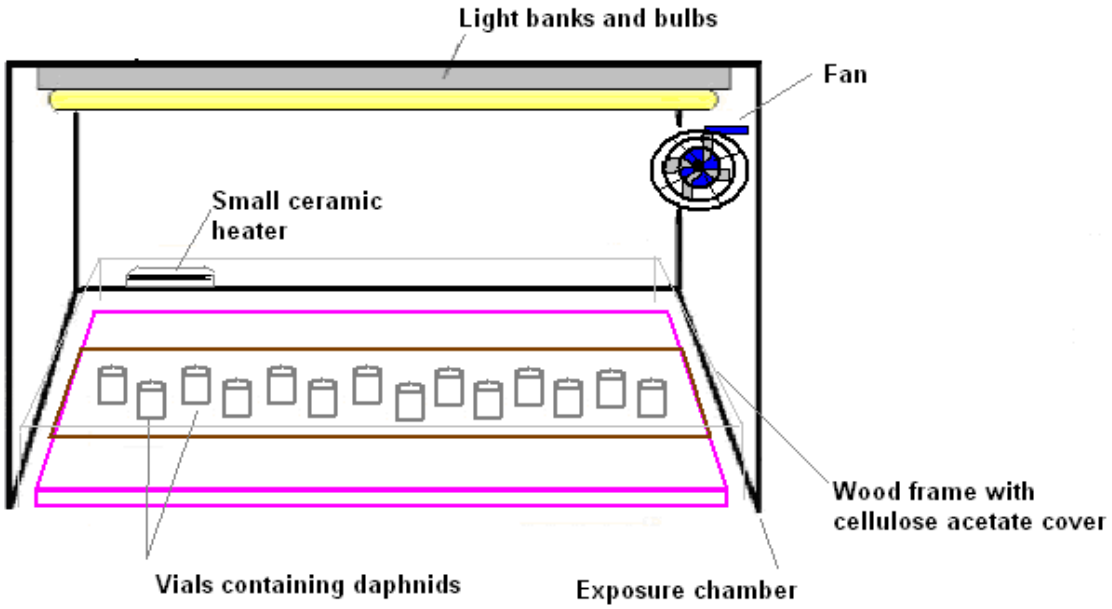


Figure 1: Diagram of a radiation chamber. Cellulose acetate was used only in the UV-B chamber. A fan and small space heater were placed in each chamber to maintain a temperature of ~32 °C. To help contain radiation within the chamber, a heavy black polyethylene tarp (not shown) was attached to the front of each chamber. Adapted from Gajda (2003).

placed upon a raised platform that elevated the water level to 37 cm below the light source. A cellulose acetate sheet (0.003 inches thick, Dick Blick Art Materials, Galesburg, IL) measuring 130 cm x 65 cm was pre-burned for 72 hours in the UV-B chamber and then attached to a wood frame, resulting in the sheet being positioned 12 cm above the water level. The cellulose acetate sheet was used to filter the shortest wavelengths of UV-B radiation (Grad *et. al* 2001). A ceramic heater (Braun; The Heat Force Ceramic Heater) and small fan were placed in each chamber to maintain an experimental temperature of approximately 32°C. Temperature was recorded every 15 minutes with a model PMA2100 meter with an integrated sensor for temperature (PMA2163K) (Solar Light Company).

IRRADIATION INTENSITIES

Irradiation levels were measured at water level using a PMA2100 light meter with integrated sensors for UV-B (PMA2104), UV-A (PMA2111), and PAR (PMA2132). Light intensities were adjusted to match levels used by Huebner *et al*. (2006). Intensities were measured along the length of the bulbs and the average emission was calculated.

***D. MAGNA* NEOTATE COLLECTION PROTOCOL**

Approximately 20 large daphnids with developing brood sacs were isolated from stock cultures and placed in separate 150 mL vials filled with 125 mL of dechlorinated water. This "maternity ward" was exposed to 24 hours of PAR light, and each daphnid was fed 2 mL of *C. pyrenoidosa* daily. Any neonates produced during this light period were returned to the stock cultures and the "maternity ward" was placed in the dark for approximately 24 hours. Neonates collected the following day 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 their parents' vials under red light. Approximately 50 neonates were randomly selected and placed into individual 150 mL vials filled with 125 mL of dechlorinated water. The animals were kept in the dark and fed 2 mL of *C. pyrenoidosa* until the morning of experimental day three, their scheduled day of irradiation. At this time, the vials were labeled and placed in the appropriate chamber.

UV-B Exposure and Photorepair on Day 3 of Development

Three day-old *D. magna* were exposed to UV-B radiation and PRR according to one of the following treatment regimes described in Table 1. Each treatment group contained ten vials and each vial housed a single three day-old daphnid. Experimental treatments B-0, B-60 and B-600 were placed in the UV-B chamber, while the light control (LC) vials were placed directly into the UV-A and PAR chamber. Dark control (DC) vials were kept in the dark by placing them under a box covered with a polyethylene tarp in the light chamber.

Following the UV-B period, animals in each vial were fed 2 mL of *C. pyrenoidosa*. With the exception of the B-0 vials, which did not receive any PRR, all vials in the UV-B chamber were transferred to the UV-A and PAR chamber to commence their photorecovery period. The B-60 and B-600 groups received 60 and 600 minutes of PRR, respectively. At the termination of the appropriate PRR period, the vials were transferred to the dark where the animals remained until 48 hours after the conclusion of the UV-B period. Organisms in each vial were fed 2 mL of *C. pyrenoidosa* daily, and survival was monitored at 24 and 48 hours post UV-B irradiation.

Following the dark period, vials were placed in the UV-A and PAR chamber where they were exposed to a 16 hour light: 8 hour dark cycle for the remainder of the experiment. The vials were randomly rotated each day to ensure all vials received the same level of UV-A and PAR radiation. Each day when first filial (F1) generation offspring were produced, neonates were removed from their parents' vials, counted, and pooled for each treatment group. Five F1s were then randomly selected from each parental treatment group and placed into individual vials and exposed to the 16 hour light: 8 hour dark cycle. Survival and reproduction of the parental and F1 generations

Table 1: UV-B and UV-A/PAR irradiation schedule for each treatment group in this study. All treatments received varying combinations of UV-B, PRR and dark, totaling 54 hours. Survival was monitored under red light at 24 and 48 hours post-UV-B irradiation. Modified from Lichtman (2003).

DC	Dark				
LC	UV-A+PAR			Dark	
B-0	UV-B	Dark			
B-60	UV-B	UV-A+PAR	Dark		
B-600	UV-B	UV-A+PAR		Dark	
	54 hours				
	6 hours	60 minutes	9 hours	14 hours	24 hours

were followed until the animals were twelve days old.

Three trials were performed at or near 32˚C. A fourth trial performed at 25˚C by Donna Neufeld and Laura-Anne Warszycki allowed for a comparison of the effect of temperature on survival and reproduction following UV-B exposure and variable PRR.

DATA COLLECTION AND STATISTICAL ANALYSIS

For each treatment group within each trial, survival and reproduction were determined as follows: percent survival of the parental generation on day twelve was determined by dividing the total number of surviving daphnids on day twelve by ten, and multiplying by 100. Total reproductive output by parent daphnids over a twelve-day period was determined by summing the total number of F1 offspring produced over that time. To determine F1 production per surviving parent daphnid, the sum was divided by the number of parent daphnids that survived to reproductive age.

F1 animals produced on each day of parental reproduction were followed to twelve days of age. Dividing the total number of surviving F1 daphnids on day twelve by

the number of original F1 daphnids and multiplying by 100 determined percent survival of F1 daphnids on day twelve. Percent survival was averaged over the number of days in the parental reproduction period. Total reproductive output by F1 animals over a twelve-day period was determined by summing the total number of F2 offspring produced over that time. To determine F2 production per surviving F1 daphnid, the sum was divided by the number of F1 daphnids that survived to reproductive age.

A modified arcsine square root transformation was applied to all percent survival data prior to analysis.

Data containing two or more replicates for each treatment condition were analyzed by two-way ANOVA with a significance level of $\alpha \leq 0.05$ using SPSS software (SPSS Inc., Cary, NC). In instances where the ANOVA results were significant, the Bonferonni post-hoc test of multiple comparisons was performed. An independent-samples t-test was performed on transformed percent survival data of the light control groups of the parental and F1 generations to determine the temperature effects on survival of pre- and post-natal (F1 generation) versus post-natal only (parental generation) exposure to heat.

RESULTS

AVERAGE TEMPERATURE

The average temperature for trials 1 and 2 was 32ºC ± 0.05. Trial 3 individuals experienced a slightly cooler temperature of 30ºC ± 0.05 (Table 2).

Table 2: Average recorded temperature (± standard error) of the four trials performed in this study.

Trial	Average (˚C)	± Standard Error
1	32.34	0.05
2	32.29	0.05
3	29.97	0.05
4	25.68	0.21

SURVIVAL OF PARENTAL GENERATION

As each treatment group only contained one data point, no statistical tests were performed on the survival of the parental generation on day twelve. In all trials, percent survival of *D. magna* not exposed to UV-B radiation (LC and DC treatments) was higher than all exposed groups (Figure 2). Mortality following exposure to UV-B was partially mitigated by PRR (B-60 and B-600). *D. magna* not exposed to PRR following UV-B exposure (B-0) had the lowest overall survival of all treatments, and experienced 100% mortality in trial 4 (26˚C).

REPRODUCTION BY PARENTAL GENERATION

As each parental generation treatment group only contained one data point, no statistical tests were performed on total F1 production by the parental generation. Total

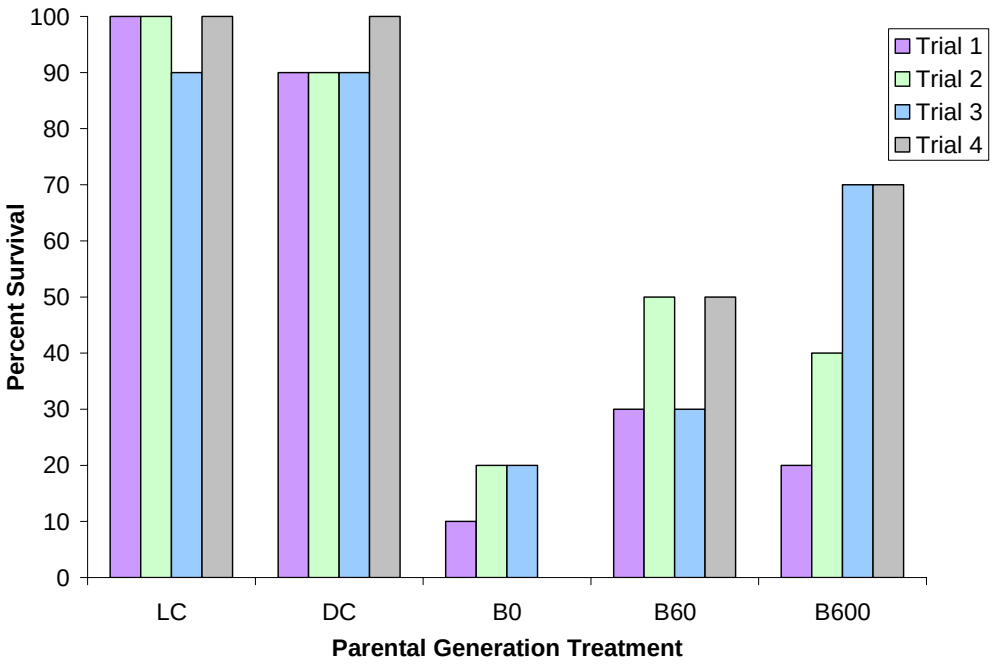


Figure 2: Percent survival of *D. magna* parental generation on day twelve.

F1 production by the parental generation was higher for *D. magna* not exposed to UV-B radiation (LC and DC treatments) than exposed groups, except LC trial 1 (Figure 3). There was no significant difference among treatment groups for F1 production per parent daphnid that survived to reproductive age ($p>0.05$), while temperature had an overall significant effect on the production of F1s per surviving parent daphnid ($p<0.01$) (Figure 4 and Appendix IV).

SURVIVAL OF F1 GENERATION

Temperature had a significant effect on F1 survival ($p<0.01$), while the UV treatment history of the parental generation had no effect on F1 survival ($p>0.05$) (Table 3). Trials 1 and 2 experienced high levels of mortality, while those animals exposed to cooler temperatures (trials 3 and 4) had the highest survival (Figure 5).

EFFECT OF PRE- AND POST-NATAL VERSUS POST-NATAL EXPOSURE TO HEAT

To determine if pre- and post-natal versus post-natal only exposure to elevated temperature affected survival, survival rates of the LC groups of the parental and F1 generations were compared by an independent samples t-test. The parental generation developed and was born at room temperature, and transferred to elevated temperature at three days of age, whereas the F1 generation was exposed to elevated temperature during development and from birth. Animals exposed to 32ºC starting at three days of age (parental generation) had significantly higher survival than animals exposed to 32ºC continuously throughout pre- and post-natal development (F1 generation) ($p<0.01$) (Table 4). There was no significant difference in survival between parental and F1 animals at 30ºC (trial 3) or at 26ºC (trial 4) ($p>0.05$) (Table 4).

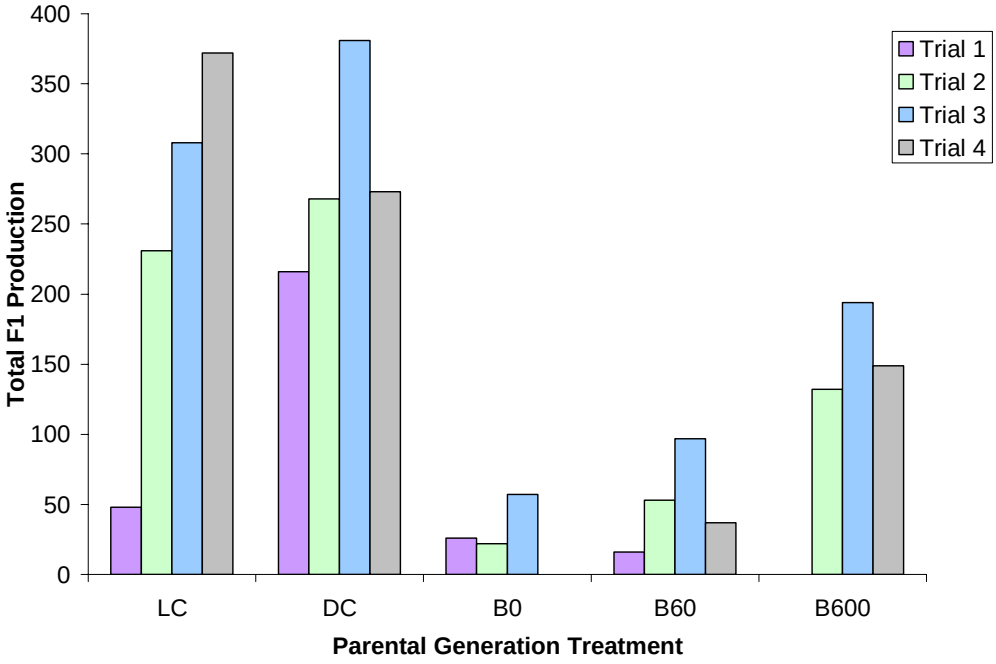


Figure 3: Total F1 production by parental *D. magna* over twelve days.

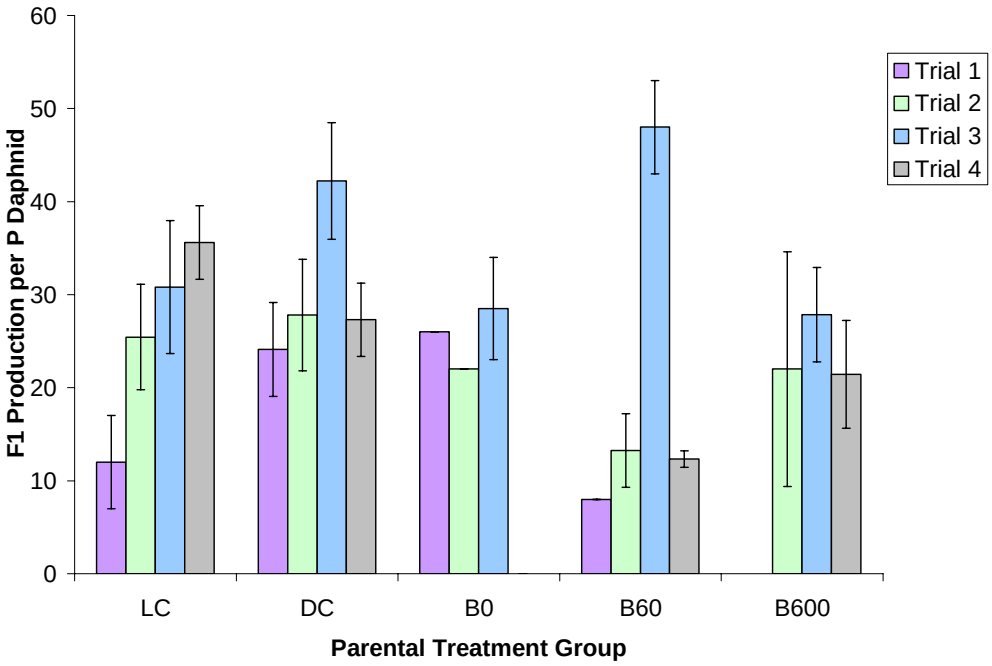


Figure 4: F1 production (± standard error) per parent *D. magna* that survived to reproductive age. Standard error bars were not present in instances where only one animal reproduced, or when two or more animals produced the same number of offspring.

Table 3: Two-way ANOVA and Bonferonni multiple comparison test results for average percent survival of F1 generation *D. magna* on day twelve.

Source of Variation	**ANOVA *p*-value**	**Pairwise Comparison**	***p*-value**
Temperature	**<0.010**	Trial 1:2	1.000
		Trial 1:3	**<0.010**
		Trial 1:4	**<0.010**
		Trial 2:3	**<0.010**
		Trial 2:4	**<0.010**
		Trial 3:4	1.000
Parental generation treatment	0.746		

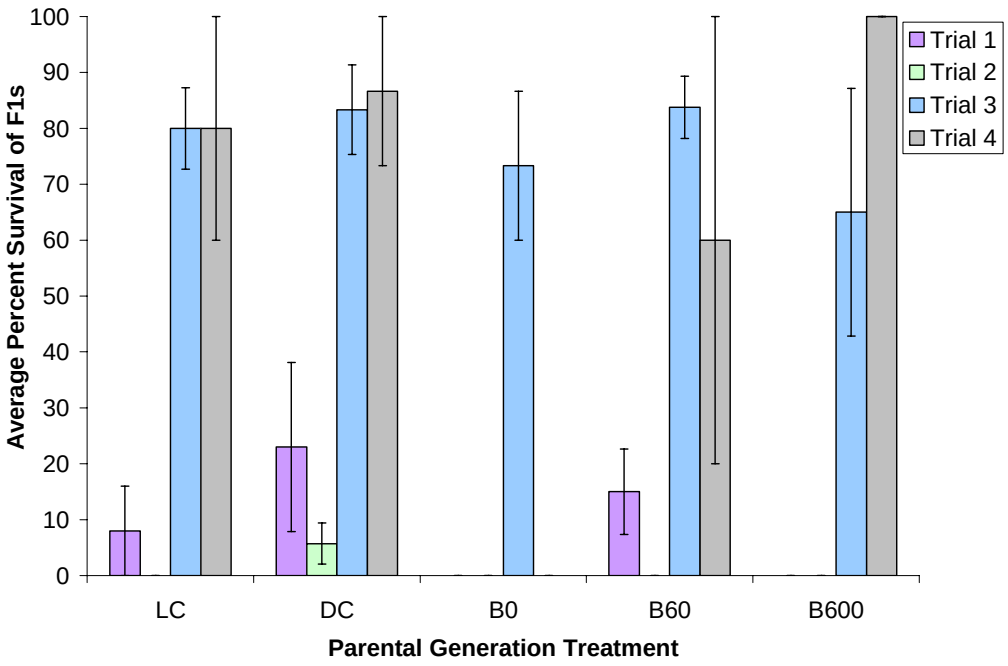


Figure 5: Average percent survival (± standard error) of F1 generation *D. magna* on day twelve.

Table 4: Independent samples t-test results for pre- and post-natal versus post-natal only exposure to heat.

Trial(s)	**Two-tailed *p*-value**
1 and 2	**<0.010**
3	0.864
4	0.685

REPRODUCTION BY F1 GENERATION

Although the survival of F1 animals was greatly reduced in trials 1 and 2 (32ºC), most F1 animals that survived reproduced in trials 3 and 4 (Figures 6 and 7). Animals at 30ºC (trial 3) produced significantly more offspring than animals at 26 ºC (trial 4) ($p<0.01$) (Table 5).

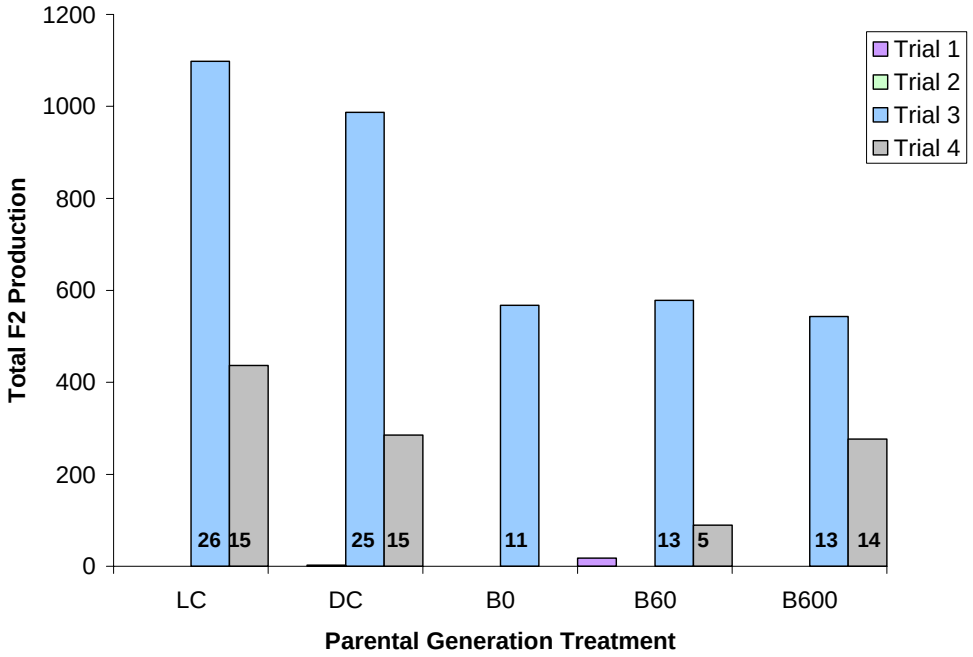


Figure 6: Total F2 production by F1 *D. magna* over twelve days. Numbers on the bars indicate the number of F1s from which the F2s were produced.

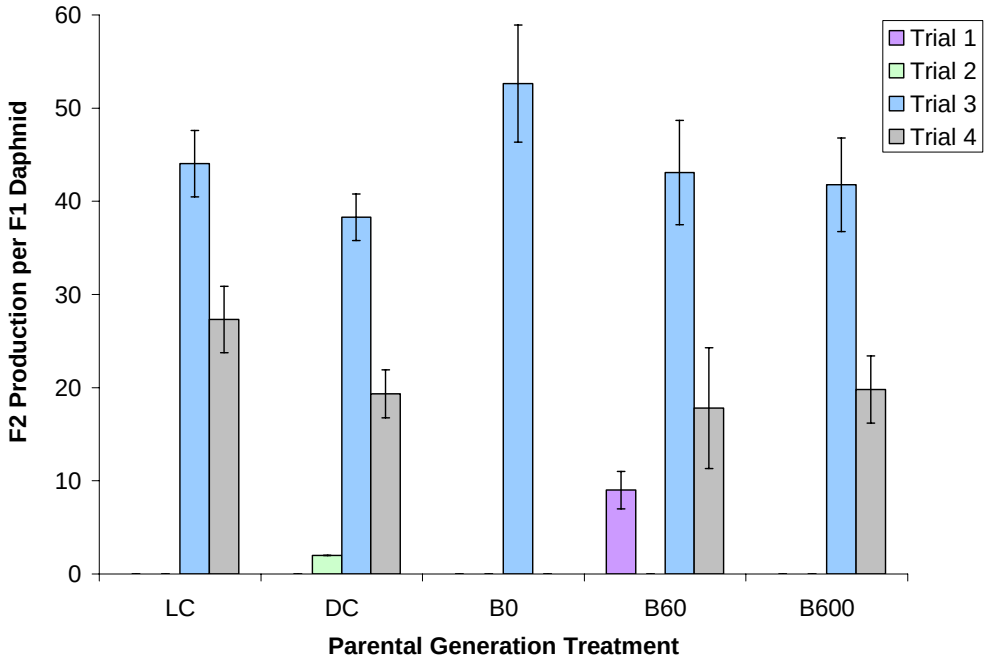


Figure 7: F2 offspring production (± standard error) per F1 *D. magna* that survived to reproductive age.

Table 5: Two-way ANOVA test results for F2 production per F1 *D. magna* that survived to reproductive age.

Source of Variation	**ANOVA *p*-value**
Temperature	<**0.010**
Parental generation treatment	0.091

DISCUSSION

There is considerable evidence that UV-B exposure is harmful to a variety of aquatic organisms (Zagarese *et al.* 1997; Pakker *et al.* 2000; Grad *et al.* 2001; Williamson *et al.* 2002; Aucamp 2003; Häder *et al.* 2003; MacFadyen *et al.* 2004; Zellmer *et al.* 2004; Persaud and Williamson 2005; Huebner *et al.* 2006; Loadman *et al.* unpublished data). When UV-B exposure is combined with other aspects of global climate change such as increasing temperature, this may pose additional stress on aquatic organisms. This study is one of the first to investigate the effects of temperature and UV-B exposure on the survival and reproduction of *D. magna* across two generations.

SURVIVAL OF PARENTAL GENERATION

Daphnia magna, like other *Daphnia* spp., exhibits sensitivity to UVR (MacFadyen *et al.* 2004). The results of this investigation are consistent with other studies that have demonstrated that UV-B radiation reduces survival in *Daphnia* (Grad *et al.* 2001; Huebner *et al.* 2006). PER accounts for a large proportion of UV tolerance in *Daphnia* spp. (Williamson *et al.* 2002; MacFadyen *et al.* 2004), and like all enzyme-mediated processes, it has been shown to be positively associated with temperature (Persaud and Williamson 2005). UV tolerance has been shown to increase in *Daphnia* spp. with increasing temperature, up to 25˚C, in the presence of PRR; however, in the absence of PRR, animals do not survive (Williamson *et al.* 2002; Häder *et al.* 2003; Loadman *et al*. unpublished data). Likewise, in this study, at 26 ˚C (trial 4) animals did not survive in the absence of PRR following exposure to UV-B radiation (B-0 animals).

At temperatures above 26 ˚C (trials 1, 2 and 3), however, a small number of animals in the B-0 group survived, suggesting that elevated temperature may have increased the rate of NER.

Although *Daphnia* populations generally experience temperature ranges between 5˚C and 25˚C in north temperature lakes (MacFadyen *et al.* 2004), this finding suggests that temperature increases associated with climate change could benefit *D. magna* populations exposed to UV-B in the absence of light. While this finding has important implications, it is unlikely that *Daphnia* would have the ability to escape prevailing daylight conditions in their natural habitat. In fact, many zooplankton including *Daphnia* maximize their time in warmer surface waters during daylight hours, leading to exposure to high UV levels, which can have potentially lethal effects (Persaud and Williamson 2005).

REPRODUCTION BY PARENTAL GENERATION

Total F1 production was higher in control groups than UV-B treated groups, which could have an impact on population sizes of *Daphnia* spp. in the natural environment. Contrary to results found by Huebner *et al.* (2006) at 22º-25ºC, in the current study, the number of offspring an individual parent daphnid produced was not reduced by exposure to UV-B radiation. Persaud and Williamson (2005) found that temperature and UV can interact to affect the abundance and reproduction of zooplankton. Therefore, it may be that elevated temperatures, as suggested by this study, mitigate the detrimental effects of UV-B radiation on offspring production.

Temperature had an overall significant effect on offspring production per parent daphnid, and although no significant pairwise differences between temperatures were

found, offspring production approached significance at p=0.069 and p=0.078 for Trials 1:3, and Trials 2:3, respectively (Appendix IV). These results suggest that elevated temperature (32˚C, trials 1 and 2) may reduce an animal's ability to produce offspring.

SURVIVAL OF F1 GENERATION

Survival of the F1 generation was severely compromised at 32˚C (trials 1 and 2) compared to trial 3 (30˚C) and trial 4 (26˚C). This indicates that even a small difference in temperature can have a significant effect on survival. In a similar study, Loadman *et al.* (unpublished data) observed 100% survival of F1 generation *D. magna* between 22˚C and 25˚C, suggesting that the UV exposure history of the parents did not affect survival. Likewise, in this study, the UV exposure history of the parents did not affect survival of the F1 animals, and therefore decreased survival rates (trials 1 and 2) are probably attributable to elevated temperature.

EFFECT OF PRE- AND POST-NATAL VERSUS POST-NATAL EXPOSURE TO HEAT

There was a significant effect of temperature on the survival of pre- and post-natal versus post-natal animals. Parental LC animals exposed to elevated temperatures of 32ºC at three days of age experienced higher survival rates compared to F1 animals born and maintained at 32ºC. F1 animals were exposed to elevated temperatures throughout the developmental (pre-natal) period, so not only may younger (one day-old) *Daphnia* be more vulnerable than three day-olds, higher temperatures probably have a detrimental effect on the development process itself.

REPRODUCTION BY F1 GENERATION

Although survival of F1 animals was compromised at 32˚C (trials 1 and 2), most surviving animals reproduced (trials 3 and 4). There were more animals that reproduced in trial 3 than trial 4, which may account for the disproportionate numbers of total offspring produced. As survival of the F1 generation was severely compromised at 32˚C (trials 1 and 2), the effect of parental UV treatment history on the production of F2 offspring could not be determined.

The results of this study demonstrate a complex interaction of the effects of UV-B exposure and temperature on the survival and reproduction of *D. magna*. The interactive effects of UVR and temperature may be more pronounced in nature, and therefore results from laboratory studies cannot be accurately extrapolated (Williamson *et al.* 2002). One of the most important concepts, however, for assessing the impact of UV-B radiation exposure and temperature on aquatic ecosystems is that complex, rather than simple responses, are likely to be the outcome (Häder *et al.* 2003).

CONCLUSIONS

1. UV-B exposure decreased survival of *D. magna*, and mortality was partially mitigated by PRR.
2. At 26˚C, there was 100% mortality of parental B-0 animals, while at elevated temperature (30ºC and 32ºC) survival increased, suggesting that the rate of NER increased enough to allow for the survival of a small number of animals.
3. F1 production per surviving parent daphnid was not affected by parental UV-B treatment history.
4. F1 survival was not affected by parental UV-B treatment history.
5. Survival and reproduction of F1 animals was compromised at 32˚C. Therefore, 32˚C may be approaching the upper limit of survival and reproduction for F1 generation *D. magna*.
6. No conclusions regarding the effect of parental UV-B treatment history on the production of F2 animals could be made, as F1s did not survive in trials 1 and 2.

REFERENCES

Adema, D.M. 1978. *Daphnia magna* as a test animal in acute and chronic toxicity tests. Hydrobiologia **59(2)**: 125-134.

Aucamp, P. 2003. Eighteen questions and answers about the effects of the depletion of the ozone layer on humans and the environment. Photochemical and Photobiological Sciences **2**: 4-24.

Calcott, P.H. 1981. *Continuous cultures of cells, Vol.I*. CRC Press, Boca Raton, Florida.

De Gelas, K. and L. De Meester. 2005. Phylogeography of *Daphnia magna* in Europe. Molecular Ecology **14**: 753-764.

Gajda, B.M. 2003. Shedding light on the photorepair of ultraviolet radiation induced DNA damage in goldfish (*Carassius auratus*) embryos. Honours Thesis in Biology, Department of Biology, University of Winnipeg, Winnipeg, Manitoba.

Grad, G., Williamson, C.E., and D.M. Karapelou. 2001. Zooplankton survival and reproduction responses to damaging UV radiation: a test of reciprocity and photoenzymatic repair. Limnology and Oceanography **46(3)**: 584-591.

Guillard, R.R. and C.J. Lorenzen. 1972. Yellow-green algae with chlorophyllide c. Journal of Phycology **8(1)**: 10-14.

Häder, D. 1997. Stratospheric ozone depletion and increase in ultraviolet radiation. *In* The effects of ozone depletion on aquatic ecosystems. Edited by D.Häder. R.G. Landes Company, Austin, Texas.

Häder, D., Kumar, H.D., Smith, R.C., and R.C. Worrest. 2003. Aquatic ecosystems: effects of solar ultraviolet radiation and interactions with other climatic change factors. Photochemical and Photobiological Sciences **2**: 39-50.

Hanski, I. and E. Ranta. 1972. Coexistence in a patchy environment: three species of *Daphnia* in rock pools. Journal of Animal Ecology **52(1)**: 263-279.

Huebner, J.D., Young, D.L.W., Loadman, N.L., Lentz, V.J., and M.D. Wiegand. 2006. Age-dependent survival, reproduction and photorepair activity in *Daphnia magna* (Straus, 1820) after exposure to artificial radiation. Photochemistry and Photobiology **82**: 1656-1661.

Karentz, D. Ecological considerations of Antarctic ozone depletion. 1991. Antarctic Science **3(1)**: 3-11.

Lichtman, S. 2003. Age-dependent effects of ultraviolet radiation and photorecovery radiation on *Daphnia magna* neonates. Directed Studies in Biology, Department of Biology, University of Winnipeg, Winnipeg, Manitoba.

Loadman, N.L., Huebner, J.D., Young, D.L.W., and M.D. Wiegand. The effects of parental exposure to UV-B radiation on offspring survival and reproduction in *Daphnia magna* (unpublished data).

MacFadyen, E.J., Williamson, C.E., Grad. G., Lowery, M., Jeffrey, W.H., and D.L. Mitchell. 2004. Molecular responses to climate change: temperature dependence of UV-induced DNA damage and repair in the freshwater crustacean *Daphnia pulicaria*. Global Climate Change Biology **10**: 408-416.

Pakker, H., Martins, R.S.T., Boelen, P., and A.G.J. Buma. Effects of temperature on the photoreactivation of ultraviolet-B-induced damage in *Palmaria palmate* (Rhodophyta). 2000. Journal of Phycology **36**: 334-341.

Persaud, A.D. and C.E. Williamson. 2005. Ultraviolet and temperature effects on planktonic rotifers and crustaceans in northern temperate lakes. Freshwater Biology **50**: 467-476.

Rautio, M. and A. Korhola. 2002. Effects of ultraviolet radiation and dissolved organic carbon on the survival of subarctic zooplankton. Polar Biology **25**: 460-468.

Williamson, C.E., Grad, G., de Lange, H.J., Gilroy, S., and D.M. Karapelou. 2002. Temperature-dependent ultraviolet responses in zooplankton: implications of climate change. Limnology and Oceanography **47(6)**: 1844-1848.

Zagarese, H.E., Feldman, M., and C.E. Williamson. 1997. UV-B induced damage and photoreactivation in three species of *Boeckella* (Copepoda, Calanoida). Journal of Plankton Research **19(3)**: 357-367.

Zellmer, I.D., Arts, M.T., Abele, D., and K. Humbeck. 2004. Evidence of sublethal damage in *Daphnia* (Cladocera) during exposure to solar UV radiation in subarctic ponds. Arctic, Antarctic, and Alpine Research **36(6)**: 370-377.

APPENDIX I: STUDY ORGANISM

Daphnia magna

APPENDIX II: WC' MEDIUM

Formula and protocol for WC' algal growth medium originally formulated as WC medium by Guillard and Lorenzen (1972). The final pH of the medium should be adjusted to between 7.48 and 7.52 using HCl and NaOH.

	Compound	Final Concentration
[*]**Major Elements**	$NaNO_3$	17.0 (mg/L)
	KH_2PO_4	1.4
	KCl	3.0
	$MgSO_4 \cdot 7H_2O$	37.0
	$CaCl_2 \cdot 2H_2O$	36.8
	$NaHCO_3$	12.6
	$NaSiO_3 \cdot 9H_2O$	56.8
[†]**Trace Elements**	Na_2EDTA	2.185 (mg/L)
	$FeCl_3 \cdot 6H_2O$	1.575
	H_3BO_3	0.500
	$MnCl_2 \cdot 4H_2O$	0.090
	$Na_2MoO_4 \cdot 2H_2O$	0.003
	$ZnSO_4 \cdot 7H_2O$	0.001
	$CoCl_2 \cdot 6H_2O$	0.005
	$CuSO_4 \cdot 5H_2O$	0.005
[‡]**Buffer**	Tricine	179.2 (mg/L)
[§]**Vitamins**	A: Cyanocobolamine (B_{12})	0.50 (µg/L)
	B: Biotin	0.50
	C: Thiamine	100.00

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

[†]A concentration of 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.

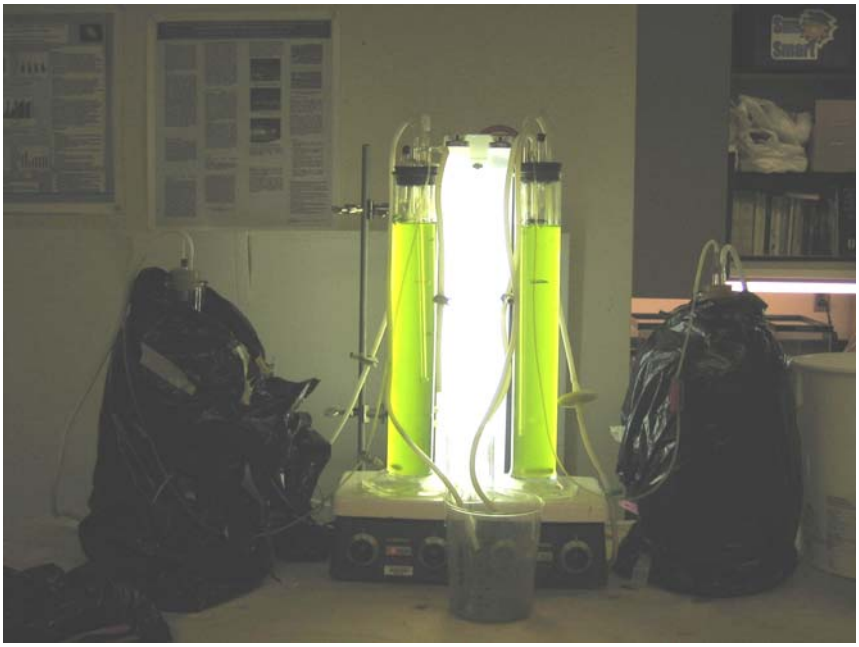
[‡]A concentration stock solution of buffer should be prepared by adding 17.92 g of tricine to 1L of distilled water, then adjusting the pH of the stock solution to 7.0. Add 10 mL of stock solution to 1 L of medium.

[§]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 1L medium.

APPENDIX III: SEMI-CONTINUOUS CELL CULTURE

Semi-continuous cell culture set-up



APPENDIX IV

Two-way ANOVA and Bonferonni multiple comparison test results for F1 production per parental *D. magna* that survived to reproductive age.

Source of Variation	**ANOVA *p*-value**	**Pairwise Comparison**	***p*-value**
Temperature	**0.033**	Trial 1:2	1.000
		Trial 1:3	0.069
		Trial 1:4	1.000
		Trial 2:3	0.078
		Trial 2:4	1.000
		Trial 3:4	0.626
Parental treatment group	0.392		