# UV-B SENSITIVITY OF AQUATIC PLANTS: THE IMPACT OF ARTIFICIAL ULTRAVIOLET RADIATION ON THE SURVIVAL AND CHLOROPHYLL CONTENT OF *LEMNA MINOR* AND *SPIRODELA POLYRHIZA*

By

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## ABSTRACT

Ultraviolet-B (UV-B) radiation can have wide-ranging impacts on plants. UV-B exposure can decrease the rate of photosynthesis, alter pigment concentrations, and damage DNA, proteins, and lipids; however, not all species are equally sensitive. *Lemna minor* and *Spirodela polyrhiza*, two sympatric aquatic plants, were exposed to 5 or 11 days of artificial UV-B radiation for 6 hours per day, and monitored over a 29 hour recovery period. Following 5 and 11-days of UV-B irradiation, *L. minor* showed decreased survival and growth, compared to control plants, but chlorophyll concentrations were not affected. Five-day irradiated *S. polyrhiza* had lower daily growth rates compared to control plants, but survival and chlorophyll concentrations were not significantly influenced by UV-B exposure. The results of this study indicate that aquatic plant species respond differently to UV-B radiation, suggesting that community dynamics could be altered if the level of UV-B reaching the Earth's surface increases.

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# INTRODUCTION

Since the mid-1980's, there has been increasing concern about the depletion of the stratospheric ozone layer, a thin layer that protects the Earth from biologically damaging ultraviolet (UV) radiation. The sun emits solar radiation in the UV range. UV-A (315 – 400 nm), the least damaging of the UV wavelengths, is not readily absorbed by the ozone layer, and is therefore unaffected by changes in ozone concentration. UV-B (280 – 315 nm) can have wide-ranging negative impacts on living organisms. Approximately 90% of incoming UV-B is absorbed by the ozone layer, but this absorption varies significantly with ozone concentration; a relatively small decrease in ozone concentration can correspond to a large increase in UV-B transmittance. UV-C (100 – 280 nm) is extremely harmful to living organisms, but is completely absorbed by ozone and oxygen in the upper atmosphere (Diaz *et al.* 2000). As the ozone layer is depleted, UV-B wavelengths are of greatest concern to scientists.

In the stratosphere, ozone ($O_3$) molecules undergo a continuous cycle of UV induced degradation and restoration. Shortwave UV radiation breaks atmospheric oxygen ($O_2$) into singlet oxygen ($O^-$), which combines with $O_2$ molecules to form $O_3$. Longwave UV radiation then breaks down $O_3$ to form $O_2$. (Häder and Worrest 1991). This cycle effectively absorbs UV wavelengths, protecting the Earth from this harmful radiation. However, anthropogenic chlorine and bromine emissions, especially the chlorofluorocarbons (CFCs) once used in refrigeration and aerosols, have had a major impact on this cycle. CFCs photodegrade in the stratosphere, releasing chlorine atoms that then destroy ozone molecules (Day and Neale 2002). CFCs have led to a worldwide thinning in stratospheric ozone, most pronounced with the annual spring ozone "hole" over Antarctica. Since the implementation of the Montreal Protocol there has been a decline in CFC emissions

(McKenzie *et al.* 2003), but these molecules are persistent in the stratosphere and continue to degrade ozone.

Predictably, the decline in stratospheric ozone has resulted in an increase in UV transmittance. Madronich *et al.* (1998) reported a worldwide increase in surface erythemal (sun burning) UV radiation relative to the 1970's, including: a 7% (winter/spring) – 4% (summer/fall) increase in UV at northern mid latitudes, a 6% increase in UV at southern mid latitudes, and a 130% and 22% increase in Antarctic and Arctic spring levels respectively. This increase in surface UV may impact species and alter community dynamics (Häder and Worrest 1991). Evidence to this point is inconclusive, but UV-B may decrease primary productivity (Day and Neale 2002) and influence competitive interactions (Barnes *et al.* 1988). Many studies have been conducted on the possible biological effects of enhanced UV-B exposure on higher plants at the species level (Table 1). The results of these studies are varied, but the most commonly documented impacts of UV-B exposure are: damage to DNA, proteins, and lipids, alteration in growth and morphology, and decreased rate of photosynthesis.

**DNA**

UV-B exposure can damage DNA by inducing the formation of cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidinone dimers, which block transcription and replication, and may be a significant factor in the reduction of plant growth with UV-B exposure (Britt 1996). Damage to DNA may be repaired via photoreactivation from UV-A and photosynthetically active radiation (PAR).

**Table 1**: Impacts of UV-B exposure on various higher plants

| Species | Effect | Reference |
|---|---|---|
| *Acer pseudoplatanus* L. (sycamore maple), *Betula pendula* Roth (European white birch), *Fraxinus excelsior* L. (European ash), *Quercus robur* L. (English oak), *Tilia cordata* P. Mill (little-leaf linden) | Reduced light-saturated photosynthesis, water use efficiencies, leaf area and stomatal density. No change in light saturated $O_2$ production. | Keiller and Holmes 2001 |
| *Arabidopsis thaliana* (L.) Heynh (mouseear cress) | Induced peroxidase-related enzymes and increased NADPH-oxidase activity. | Rao *et al.* 1996 |
| *Azolla microphylla* Kaulf (water fern) | Reduced biomass, growth rate, PSII activity, chlorophyll and carotenoid content. | Jayakumar *et al.* 2002 |
| *Brassica napus* L. cv. Topas (canola) | Decreased Rubisco activity, UV-B resistance unaltered by simultaneous exposure to UV-A. | Savitch *et al.* 2001 |
| *Calamagrostis purpurea* (Trin.) Trin. (Scandinavian small reed) | No effect on plant dry weight, leaf and shoot morphology. Decreased root carbohydrates. Increased the soluble sugar: starch ratio. | Gwynn-Jones 2001 |
| *Glycine max* (L.) Merr. (soybean) | Increased leaf thickness, decreased photosynthetic capacity, decreased leaf area and seed yield with high exposure. | Sullivan *et al.* 1994 |
| *Lemna minor* L. (lesser duckweed) | Decreased growth rate and chlorophyll content, no impact on PSII efficiency at high levels of UV-B. | Germ and Gaberščik 1999 |
| *Phaseolus vulgaris* L. (cv. Stella) (bean plant) | Resistant to UV-B in the presence of high levels of PAR. With low PAR, UV-B induced changes in leaf structure, inhibited PSII, and decreased leaf area and dry weight. | Cen and Bornman 1990 |
| *Pisum sativum* L. (pea plant) | Increase concentration of UV-B absorbing compounds. Decrease in foliage area, plant dry weight, and plant height. | Gonzalez *et al.* 1996 |
| *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco (Douglas-fir) | Initially showed a reduction in growth, but recovered. Stimulated lateral branch production. No impact on chlorophyll and UV absorbing compounds. | Bassman *et al.* 2002 |
| *Spirodela oligorrhiza* (Kurtz) Helgm. (greater duckweed) | Accelerated degradation of PSII reaction center proteins | Jansen *et al.* 1996 |
| *Spirodela polyrhiza* (L.) Schleid. (greater duckweed) Note: referred to by a previous name (*Lemna major*). | Decreased chlorophyll, starch, protein, and free sugar content. | Farooq *et al.* 2000 |

**Photosynthesis**

UV-B radiation has been shown to decrease the rate of photosynthesis, primarily through a reduction in photosystem II (PSII) efficiency. The D1 polypeptide in the PSII reaction center undergoes a continual cycle of light induced synthesis and degradation. However, UV-B radiation decreases the stability of the protein, leading to inactivation. Non-functional PSII reaction centers accumulate in the thylakoid membrane because degradation is more rapid than new protein synthesis, resulting in an overall decline in PSII efficiency (Mackerness *et al.* 1997). Additionally, UV-B exposure can result in a decline of Rubisco (ribulose 1,5-bisphosphate) content and activity (Keiller *et al.* 2003), as well as a reduction in stomatal conductance (Middleton and Teramura 1993).

UV-B radiation can reduce photosynthesis by damaging photosynthetic pigments (Hollósy 2002). Mackerness *et al.* (1997) found a dramatic decrease in the level of chlorophyll *a*/*b*-binding protein mRNA in UV-B exposed pea leaves. Cen and Bornman (1990), found a 20% decrease in the chlorophyll content of *Phaseolus vulgaris* exposed to UV-B and low light conditions. Marwood and Greenberg (1996) found that UV-B irradiated *Spirodela oligorrhiza* had lower chlorophyll concentrations than control plants, and exposure periods as short as one hour could negatively impact chloroplast development. Some studies suggest that chlorophyll *b* is more sensitive to UV-B than chlorophyll *a*, but this effect has not been consistently observed (Hollósy 2002).

**Proteins and Enzymes**

The aromatic amino acids phenylalanine, tryptophan and tyrosine absorb strongly in the UV range, and may be damaged by this radiation (Hollósy 2002). Damage to these amino acid residues can lead to the inactivation of proteins and enzymes, including Rubisco (Jordan 1993).

**Growth and Morphology**

UV-B exposure has been shown to induce numerous changes in plant morphology, including increased leaf thickness (Sullivan *et al.* 1994); decreased foliage area (Cen and Bornman 1990; Gonzalez *et al.* 1996; Keiller and Holmes 2001); and decreased growth rates (Germ and Gaberščik 1999, Jayakumar *et al.* 2002). The decrease in growth rate is a result of DNA damage and reduced photosynthesis, but UV-B radiation has also been shown to oxidize indole-acetic acid (auxin), an important plant hormone (Tevini and Iwanzik 1986 *in* Allen *et al.* 1998), and inhibit cell expansion and division (Hopkins 1997 *in* Allen *et al.* 1998). Also, it has been suggested that UV-B exposure might influence floral induction, fertilization, and seed development (Sullivan *et al.* 1994).

**Protective mechanisms**

Higher plants have some ability to mitigate UV-B induced damage. The most common response of plants exposed to UV-B is the induction of UV-B absorbing compounds, especially flavonoids, including anthocyanins, flavones, and flavonols that absorb strongly between 230-380 nm (Cen and Bornman 1990; Strid *et al.* 1994; Day and Vogelmann 1995). UV-B irradiation induces the expression of phenylalanine ammonialyase encoding genes, the first stage in flavonoid synthesis (Allen *et al.* 1998). Leaf thickening and foliage reduction, common responses to UV-B irradiation, are adaptive responses to reduce light penetration. Other leaf properties, including trichomes and cuticular waxes may also reduce UV-B penetration into leaves (outlined in McLeod and Newsham 1997). Bornman and Vogelmann (1991) found that *Brassica campestris* showed a 45% increase in leaf thickness as a response to UV-B exposure.

***Lemna minor* and *Spirodela polyrhiza***

Not all plant species are equally sensitive to UV-B irradiation (Keiller and Holmes 2001; Searles *et al.* 2001). The impacts of UV-B on agricultural species are well documented, but relatively little is known about the effects of UV-B exposure on aquatic plants (Farooq *et al.* 2000) and non-agricultural monocots (Searles *et al.* 2001). *Lemna minor* L. and *Spirodela polyrhiza* (L.) Schleid are small, free-floating plants of the family Lemnaceae, known as lesser, and greater duckweed respectively. *Spirodela polyrhiza* occurs around the world, except for some islands (New Zealand) and areas in South America. *Lemna minor* occurs in cooler regions of North America, Europe, Africa, western Asia, southern Australia and New Zealand (Landolt 1986). The Lemnaceae are the world's smallest flowering plants, and their morphology is extremely reduced (Appendix I). The species have no distinct stem or leaf structures. The plant body is neither a leaf, since it is capable of forming flowers and daughter leaves, nor is it a shoot, since it lacks meristematic tissue at the tip (Landolt 1986). The term "frond" is the generally accepted term to describe the plant body. This term is slightly misleading, suggesting a pteridophyte connection, but it is used here for the sake of consistency with published literature.

Duckweeds are important aquatic macrophytes: as a food source for mammals, birds, fish, and gastropods (Landolt 1986); in wastewater and effluent treatment (Hasar 2002, Tripathi and Upadhyay 2003); as feed for animals and fish (Bairagi *et al.* 2002); and as a fertilizer supplement (Mbagwu and Adeniji 1988). Duckweeds form dense mats on still water and can completely dominate a water body. As a floating plant, duckweed is subject to full sun exposure, yet little is known about the sensitivity of these species to UV-B. The purpose of this experiment was to determine the effects of elevated artificial UV-B radiation on *L. minor* and *S. polyrhiza*. The parameters investigated were growth, survival, and chlorophyll

concentration. It was hypothesized that duckweed would be sensitive to UV-B exposure, and would show a decrease in the parameters investigated relative to non UV-B irradiated plants.

Duckweed are short lived plants, their fronds having an average lifespan of 12.1 (*S. polyrhiza*) to 31.3 days (*L. minor*) (Lemon *et al.* 2001). Duckweed grows continuously, with a production rate (fronds per individual per day) of 0.45 for *L. minor* and 0.08 for *S. polyrhiza* (Lemon *et al.* 2001). Although they are capable of flowering, species generally reproduce via asexual budding. Newly produced fronds may be more sensitive to UV-B radiation due to poor cuticle and epidermis development (Germ and Gaberščik 1999). However, according to Allen *et al.* (1998), exposure to UV-B during tissue development may lead to greater induction of UV-B protective mechanisms like flavonoids, reducing the deleterious effects of UV-B. It is unknown whether the Lemnaceae's short generation time confers an advantage or disadvantage with regard to UV-B damage.

Previous work on duckweed species demonstrated that *L. minor* and *S. polyrhiza* were sensitive to UV-B radiation. Farooq *et al.* (2000), found that UV-B exposure resulted in a decrease in chlorophyll, starch, protein, and free sugar content in *S. polyrhiza* (referred to in the article by a previous name, *Lemna major*). Germ and Gaberščik (1999) found that high levels of UV-B exposure resulted in decreased growth and chlorophyll content in *L. minor*. It would appear that there has been no published work comparing the sensitivities of different duckweed species following identical UV-B treatments.

*Lemna minor* is an efficient competitor (Keddy 1976), and greatly outnumbers *S. polyrhiza* in Manitoban ponds (personal observation). Fronds of *L. minor* live more than twice as long as fronds of *S. polyrhiza*, and produce about 14 times as many daughter fronds during their life span (Lemon *et al.* 2001). However, the competitive dynamics between these species

may be altered with UV-B exposure. Barnes *et al.* (1988) found that enhanced UV-B radiation resulted in a shift of competitive dynamics between wheat (*Triticum aestivum* L.) and wild oat (*Avena fatua* L.) in favour of the wheat. In this instance, UV-B induced morphological changes in wild oat that rendered the species a less efficient competitor for light.

Duckweed species are important ecological factors in their environment. *Lemna minor* and *S. polyrhiza* form dense mats on the water surface that can block over 90% of incoming light (Landolt 1986). A loss of duckweed cover as a result of UV-B sensitivity would increase light penetration into these water bodies, which could impact water chemistry and species composition. Duckweed sensitivity to UV-B would suggest that the composition of aquatic communities, in particular sub-surface photosynthetic organisms, could be altered in the presence of enhanced UV-B. Furthermore, differences in the UV-B sensitivity between sympatric species such as *L. minor* and *S. polyrhiza,* would suggest that competitive dynamics between co-occurring species could be altered in the presence of enhanced UV-B radiation.

# METHODS

## *Lemna minor* and *Spirodela polyrhiza* Cultures

*Lemna minor* and *Spirodela polyrhiza* were collected from a shallow pond at Fort Whyte Centre, Winnipeg, Manitoba, in September 2004 and kept in separate aquaria at the University of Winnipeg. The plants were cultured in dechlorinated water, and supplied with nutrients in the form of fish fertilizer (approximately 1 teaspoon/month of Alaska® Fish Fertilizer (Home Harvest® Garden Supply, Inc.): 5.0 % N; 2.0% $P_2O_5$; 2.0% $K_2O$; 40.0% Organic Matter). The plants were maintained at room temperature, on a 14h light (0800 – 2200 h): 10h dark cycle.

## Experimental Apparatus

The experimental apparatus consisted of a platform suspended under UV-A, UV-B, and PAR light bulbs (Figure 1). The bulbs used in these experiments were as follows: PAR – GE F48T12 CWHO (average output: 17.4 $W/m^2$) suspended 18.0 – 21.0 cm above the platform; UV-A – Phillips F40 T12/BL (average output: 14.2 $W/m^2$) suspended 7.0 – 7.5 cm above the platform; and UV-B – Phillips TL 40W/12RS (average output: 19.8 $\mu W/cm^2$) suspended 20.0 – 22.0 cm above the platform. The UV-B bulb was loosely wrapped with pre-burned cellulose acetate (previously exposed to 72 hours of UV-B radiation) to filter out the lower wavelengths. The intensity of UV-B radiation was higher than outdoor conditions to simulate a situation of ozone depletion (average solar radiation measured at Grand Beach, Manitoba on July 26, 2004: UV-A: 39.8 $W/m^2$; UV-B: 13.4 $\mu W/cm^2$ (peaked at 15.5 $\mu W/cm^2$); PAR: 403 $W/m^2$). Light intensities were determined using a model PMA2100 light meter (Solar Light Co.) with PAR (PMA2132), UV-A (PMA2111), and UV-B (PMA2104) sensors.

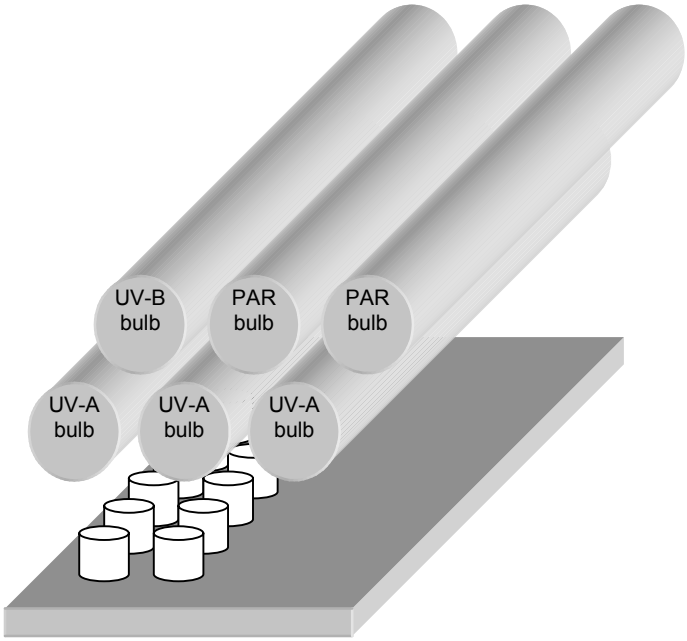


**Figure 1:** Schematic diagram of the experimental apparatus, showing the arrangement of the bulbs and the placement of the experimental dishes (not to scale). Three apparatuses, each containing 20 dishes, were used in each trial.

**Experimental Treatment**

*Lemna minor* was exposed to 5 or 11 days of artificial UV-B radiation. Due to time constraints, *Spirodela polyrhiza* received a 5-day treatment only. In total, two 5-day *L. minor* trials, two 11-day *L. minor* trails, and two 5-day *S. polyrhiza* trials were conducted. For each trial, 20 healthy (fully pigmented) fronds of *L. minor*, or 8 healthy fronds of *S. polyrhiza* were transferred from the stock culture to each of 60 dishes containing WC' media (Appendix II). The amount of WC' media per dish was not a set volume, rather, dishes were filled such that their water levels were at an equal height, which required approximately 35 ml. Twenty dishes (10 control and 10 experimental) were placed in each of three experimental apparatuses. Control dishes were covered with Mylar® (type D from Grafix® Plastics) to exclude UV-B wavelengths. Experimental dishes were covered with cellulose acetate to exclude lower UV-B wavelengths, which are not a component of the natural sunlight reaching Earth. All plants received photosynthetically active radiation (PAR) 14 hours/day (0800 – 2200h), and UV-A and UV-B light for 6 hours/day (1100 - 1700). During the experimental period, the dishes were periodically rotated between the apparatuses in order to minimize differences in UV-B exposure that might result from different bulbs. Additional WC' media was also added to the dishes at this time if evaporation had occurred.

At the end of each trial, 20 randomly sampled dishes (10 control and 10 experimental) were removed at each of the following recovery times: 0 hours (1700h on the final day of irradiation); 5 hours (2200h on the final day of irradiation); and 29 hours (2200h on the day after the last day of irradiation). During the recovery period, the duckweed was on a 14h PAR (0800 – 2200h): 10h dark cycle. Following removal from the apparatuses, the fronds were counted and allowed to dry in the dark for approximately 72 hours, in preparation for chlorophyll extraction. *Spirodela polyrhiza* have many rootlets, which were

removed prior to drying in order to prevent interference in the chlorophyll extraction. *Lemna minor* rootlets are much less prolific than those of *S. polyrhiza* and were left intact.

**Chlorophyll Determination**

The 10 dishes removed for each time period (for each treatment) were pooled into a single sample. Each trial therefore resulted in 6 samples for chlorophyll extraction: control treatments at 0, 5, and 29 hours, and experimental treatments at 0, 5, and 29 hours. Chlorophyll *a* and *b* were extracted in a solution of 1 part 0.1N $NH_4OH$ to 9 parts acetone, as outline by the United States Environmental Protection Agency Environmental Response Team (1994). Chlorophyll concentrations were determined spectrophotometrically at 645 nm and 663 nm, according to the formulas given in Hendry and Price (1993) (Appendix II), however, the final chlorophyll concentration was expressed as μg chlorophyll/g dry tissue, rather than μg chlorophyll/unit surface area.

**Data Collection and Analysis**

During the experimental period, the total number of fronds per dish and the number of unhealthy fronds per dish (defined as greater than one half white or brown) were counted daily at 1000h. The growth of the duckweed was calculated using the mean total number of fronds per dish. The daily production rate, essentially the change in population from the previous day, was calculated as the change in fronds from the previous day, divided by the number of fronds on the previous day (for each dish). Biotic potential was the highest average daily production rate for each treatment. Percent survival was calculated as the number of healthy fronds over the total number of fronds.

Two 5-day *L. minor* trials, two 11-day *L. minor* trials, and two 5-day *S. polyrhiza* trials were conducted. Pooled results were used for the mean chlorophyll concentration, the total species production, and the species biotic potential. Two-way analysis of variance (treatment

and day) was conducted on growth, survival, and daily production rate ($\alpha = 0.05$). Recovery was assessed using an analysis of variance to examine a "time" effect on duckweed survival at 0, 5, and 29 hours following UV-B irradiation ($\alpha = 0.05$). Survival and production rates were subjected to an arcsine square root transformation prior to statistical analysis. The chlorophyll concentrations were analyzed as a randomized block design, and analysis of variance was conducted on the effect of light treatment on chlorophyll concentrations ($\alpha = 0.05$). All statistical analyses were conducted using SPSS software (SPSS Inc. 2001).

# RESULTS

## Growth and Survival

Two-way ANOVA (treatment and day) showed a significant UV-B treatment effect on the total number of fronds for 5-day irradiated *L. minor* (Table 2, Figure 2), 11-day irradiated *L .minor* (Table 2, Figure 3), and 5-day irradiated *S. polyrhiza* (Table 2, Figure 4) with respect to controls. For the 5 and 11-day *L. minor* trials, there was a significant treatment effect on the number of unhealthy (white or brown) fronds, and percent survival (Table 2). For *S. polyrhiza*, there was no treatment effect on the number of unhealthy fronds, or the percent survival (Table 2).

## Production Rate and Biotic Potential

UV-B irradiated duckweed had lower production rates and biotic potentials ($r_m$) than control plants (Table 3). The difference in production rates was significant for 5 and 11-day irradiated *L. minor*, and for 5-day irradiated *S. polyrhiza* (Table 2).

## Recovery

UV-B irradiated *L. minor* had lower percent survival than controls during the 29-hour recovery period (Table 4). Percent survival of UV-B irradiated *S. polyrhiza* was greater than or equal to controls during the recovery period (Table 4). Analysis of variance revealed that there was no significant "time" effect on the percent survival of UV-B irradiated *L. minor* fronds at 0, 5, and 29h of recovery. There was a significant decline in the percent survival of control and UV-B irradiated *S. polyrhiza* fronds at 29 hours recovery in one of two trials ($p=0.047$, $p=0.259$).

**Table 2:** ANOVA results for light treatment effect on total number of fronds, number of unhealthy fronds, percent survival and production rate of *Lemna minor* and *Spirodela polyrhiza* following UV-B irradiation.[a]

| | d.f. | MS | *F*-value | Probability >*F* |
|---|---|---|---|---|
| **Total fronds** | | | | |
| 5-day *L. minor* I | 1 | 2700.0 | 118.416 | <0.001 |
| 5-day *L. minor* II | 1 | 572.920 | 115.915 | <0.001 |
| 11-day *L. minor* I | 1 | 110878.884 | 499.199 | <0.001 |
| 11-day *L. minor* II | 1 | 188419.490 | 2742.328 | <0.001 |
| 5-day *S. polyrhiza* I | 1 | 57.203 | 36.066 | <0.001 |
| 5-day *S. polyrhiza* II | 1 | 227.070 | 88.895 | <0.001 |
| **Unhealthy fronds** | | | | |
| 5-day *L. minor* I | 1 | 85.333 | 56.672 | <0.001 |
| 5-day *L. minor* II | 1 | 22.499 | 48.063 | <0.001 |
| 11-day *L. minor* I | 1 | 1556.359 | 546.835 | <0.001 |
| 11-day *L. minor* II | 1 | 1903.098 | 716.676 | <0.001 |
| 5-day *S. polyrhiza* I | 1 | 0.00333 | 0.341 | 0.560 |
| 5-day *S. polyrhiza* II | 1 | 0.030 | 0.493 | 0.483 |
| **Survival** | | | | |
| 5-day *L. minor* I | 1 | 2381.571 | 52.244 | <0.001 |
| 5-day *L. minor* II | 1 | 1009.568 | 46.896 | <0.001 |
| 11-day *L. minor* I | 1 | 18277.412 | 558.961 | <0.001 |
| 11-day *L. minor* II | 1 | 26007.067 | 635.354 | <0.001 |
| 5-day *S. polyrhiza* I | 1 | 0.917 | 0.407 | 0.524 |
| 5-day *S. polyrhiza* II | 1 | 4.877 | 0.432 | 0.511 |
| **Production rate** | | | | |
| 5-day *L. minor* I | 1 | 7075.616 | 56.010 | <0.001 |
| 5-day *L. minor* II | 1 | 2884.760 | 34.292 | <0.001 |
| 11-day *L. minor* I | 1 | 6330.633 | 68.051 | <0.001 |
| 11-day *L. minor* II | 1 | 28421.384 | 365.986 | <0.001 |
| 5-day *S. polyrhiza* I | 1 | 519.854 | 3.869 | 0.050 |
| 5-day *S. polyrhiza* II | 1 | 1488.716 | 11.616 | 0.001 |

[a] The degrees of freedom (d.f.), mean square (MS), *F*-value, and *P*-value (probability>*F*) are reported.

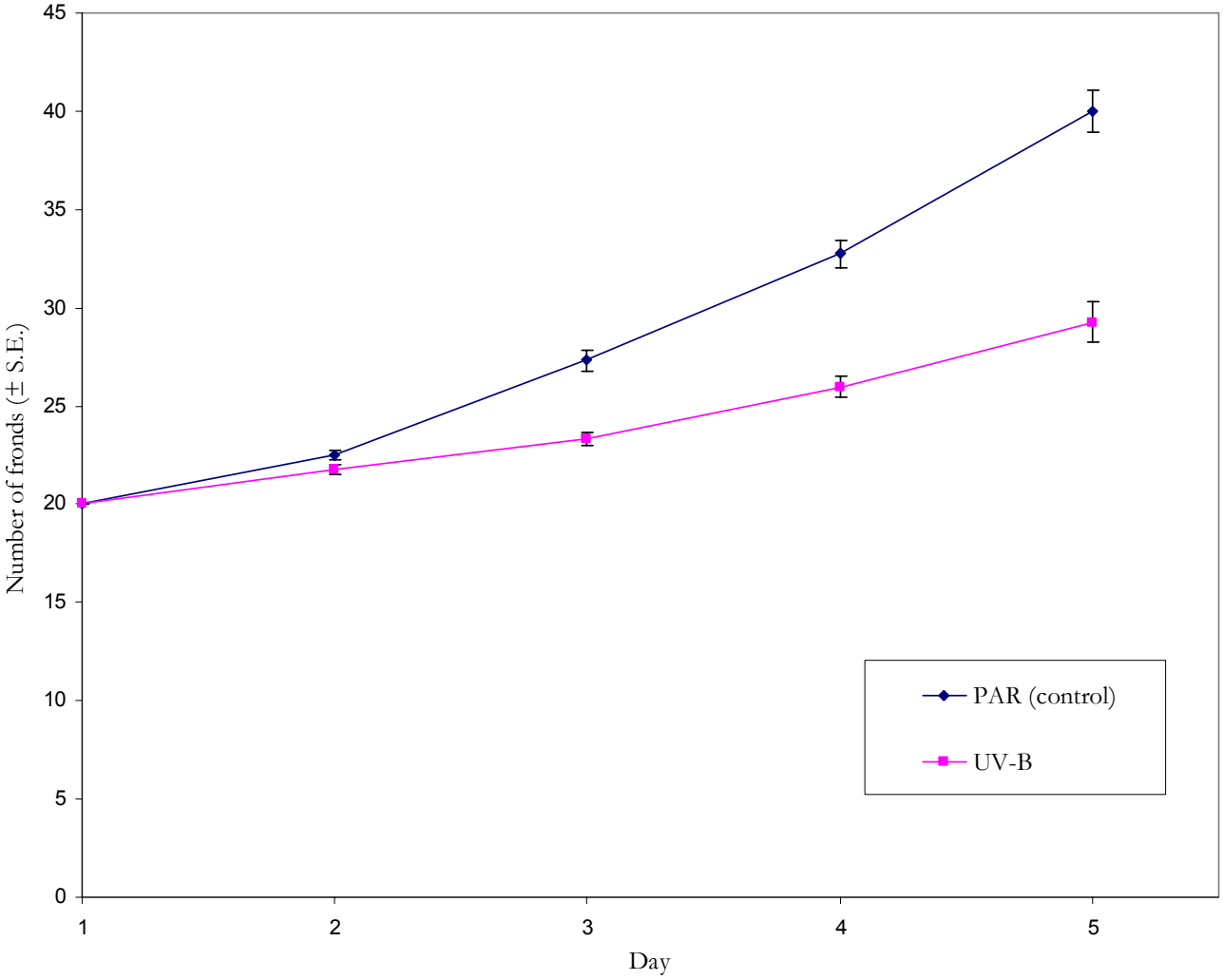


**Figure 2:** Average growth of *Lemna minor* over 5 days of UV-B irradiation.

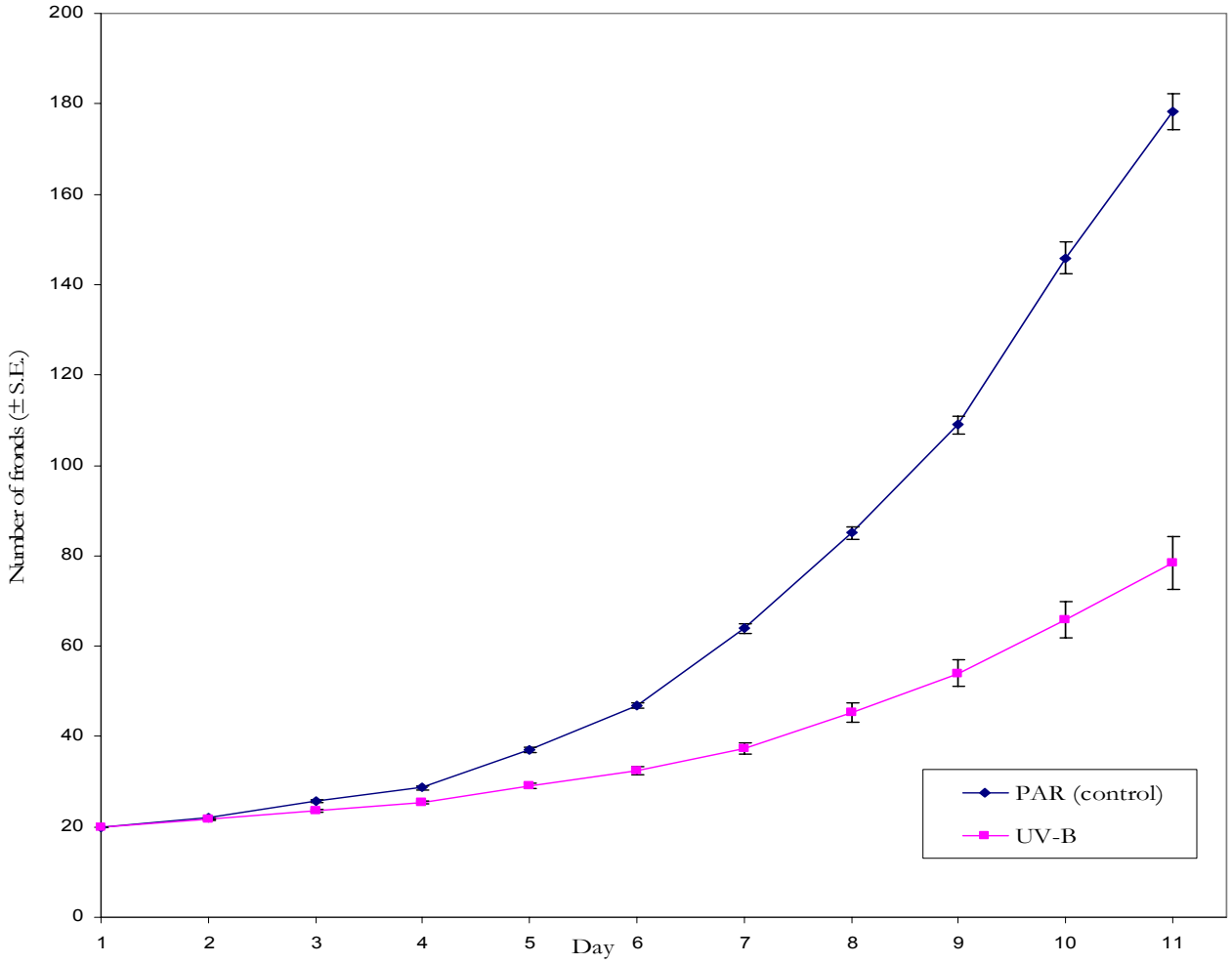


**Figure 3:** Average growth of *Lemna minor* over 11 days of UV-B irradiation.

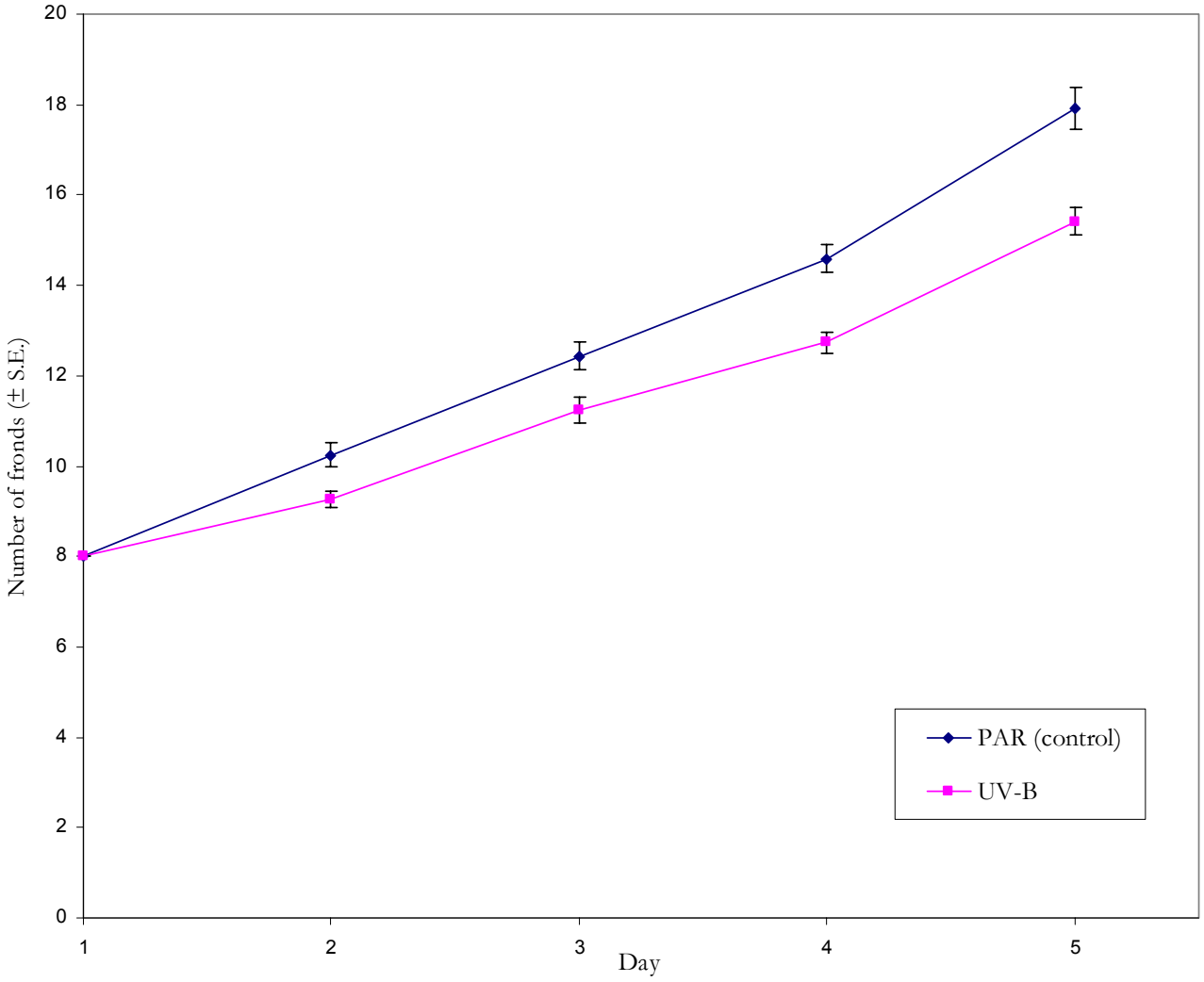


**Figure 4:** Average growth of *Spirodela polyrhiza* over 5 days of UV-B irradiation.

**Table 3:** Mean (±S.E.) production rate and biotic potential ($r_m$) of *Lemna minor* during 5 or 11-days of UV-B irradiation, and *Spirodela polyrhiza* during 5-days of UV-B irradiation.

| Species | Production rate (fronds per individual per day) | | Biotic potential ($r_m$) | |
|---|---|---|---|---|
| | PAR | UV-B | PAR | UV-B |
| *L. minor* (5 days) | 0.190 (0.0081) | 0.097 (0.0070) | 0.218 (day 4-5) | 0.116 (day 4-5) |
| *L. minor* (11 days) | 0.252 (0.0067) | 0.136 (0.0055) | 0.368 (day 6-7) | 0.194 (day 9-10) |
| *S. polyrhiza* (5 days) | 0.228 (0.011) | 0.183 (0.0093) | 0.281 (day 1-2) | 0.216 (day 4-5) |

**Table 4:** Average percent survival (± S.E.) of *Lemna minor* and *Spirodela polyrhiza* at 0, 5, and 29 hours of recovery following 5 or 11-days of UV-B irradiation.

| | 0 hours recovery | | 5 hours recovery | | 29 hours recovery | |
|---|---|---|---|---|---|---|
| | PAR | UV-B | PAR | UV-B | PAR | UV-B |
| 5-day *L. minor* | 97.7 (0.5) | 81.9 (2.3) | 97.7 (0.5) | 83.7 (2.2) | 97.0 (0.6) | 82.4 (3.3) |
| 11-day *L. minor* | 96.5 (0.2) | 80.5 (4.1) | 97.4 (0.2) | 79.8 (3.9) | 97.6 (0.1) | 83.6 (2.6) |
| 5-day *S. polyrhiza* | 99.8 (0.2) | 100.0 (0) | 99.8 (0.2) | 99.8 (0.3) | 99.6 (0.3) | 98.8 (0.6) |

**Chlorophyll Concentrations**

Following 5-days of UV-B irradiation, chlorophyll *a* and *b* concentrations were lower in UV-B irradiated *L. minor* than in control plants at 0, 5, and 29 hours of recovery (Table 5, Figure 5), but this difference was not statistically significant. The lack of statistical significance may be a product of low statistical power; but this difference could still be biologically important. Following 11-days of UV-B irradiation, there was no difference in the chlorophyll concentrations between UV-B irradiated *L. minor* and control plants (Table 5, Figure 6). Following 5-days of UV-B irradiation, there was no difference in the chlorophyll concentrations of UV-B irradiated *S. polyrhiza* and control plants (Table 5, Figure 7). Chlorophyll *b* was no more sensitive to UV-B than chlorophyll *a* (Table 5).

**Table 5:** Average chlorophyll concentrations (± S.E.) of *Lemna minor* and *Spirodela polyrhiza* from stock tank cultures, and at 0, 5, and 29 hours of recovery following UV-B irradiation.

| | Chlorophyll *a* (μg/g dry tissue) | Chlorophyll *b* (μg/g dry tissue) | Total Chlorophyll (μg/g dry tissue) |
|---|---|---|---|
| **Duckweed from stock tank** | | | |
| *L. minor* | 6826.0 (206.3) | 2734.3 (128.0) | 9560.6 (324.6) |
| *S. polyrhiza* | 4510.6 (125.1) | 2381.3 (27.0) | 6891.9 (151.6) |
| ***L. minor* 5-day trial** | | | |
| PAR (0h recovery) | 6366.4 (3459.0) | 12171.2 (3585.7) | 18537.6 (126.6) |
| UV-B (0h recovery) | 4142.2 (1276.6) | 4167.4 (419.2) | 8309.5 (857.4) |
| PAR (5h recovery) | 6912.2 (587.3) | 4088.4 (1111.9) | 11000.6 (524.7) |
| UV-B (5h recovery) | 4095.5 (891.1) | 3175.4 (1025.7) | 7271.0 (134.5) |
| PAR (29h recovery) | 8140.6 (733.2) | 8731.9 (6006.3) | 16872.5 (6739.5) |
| UV-B (29h recovery) | 7428.9 (1349.6) | 4247.2 (175.8) | 11676.1 (1173.8) |
| ***L. minor* 11-day trial** | | | |
| PAR (0h recovery) | 12699.8 (1586.8) | 4485.6 (207.9) | 17185.4 (1794.7) |
| UV-B (0h recovery) | 12593.1 (1811.7) | 4162.9 (35.5) | 16756.0 (1776.2) |
| PAR (5h recovery) | 12115.8 (1313.2) | 4315.0 (343.4) | 16430.8 (1656.7) |
| UV-B (5h recovery) | 12576.5 (3136.3) | 4229.8 (577.9) | 16806.4 (3714.2) |
| PAR (29h recovery) | 10895.5 (364.0) | 3612.8 (78.9) | 14508.3 (285.1) |
| UV-B (29h recovery) | 11531.3 (192.1) | 3456.0 (177.7) | 14987.3 (369.8) |
| ***S. polyrhiza* 5-day trial** | | | |
| PAR (0h recovery) | 9731.3 (566.6) | 3712.2 (296.0) | 13443.5 (862.7) |
| UV-B (0h recovery) | 9094.5 (164.7) | 3252.8 (53.8) | 12347.3 (110.9) |
| PAR (5h recovery) | 10024.8 (604.3) | 3706.3 (62.9) | 13731.1 (667.1) |
| UV-B (5h recovery) | 9329.6 (43.0) | 3273.5 (123.0) | 12603.1 (166.0) |
| PAR (29h recovery) | 10090.9 (19.8) | 3364.0 (58.9) | 13455.0 (39.1) |
| UV-B (29h recovery) | 9530.4 (11.8) | 4044.8 (609.3) | 13575.2 (621.1) |

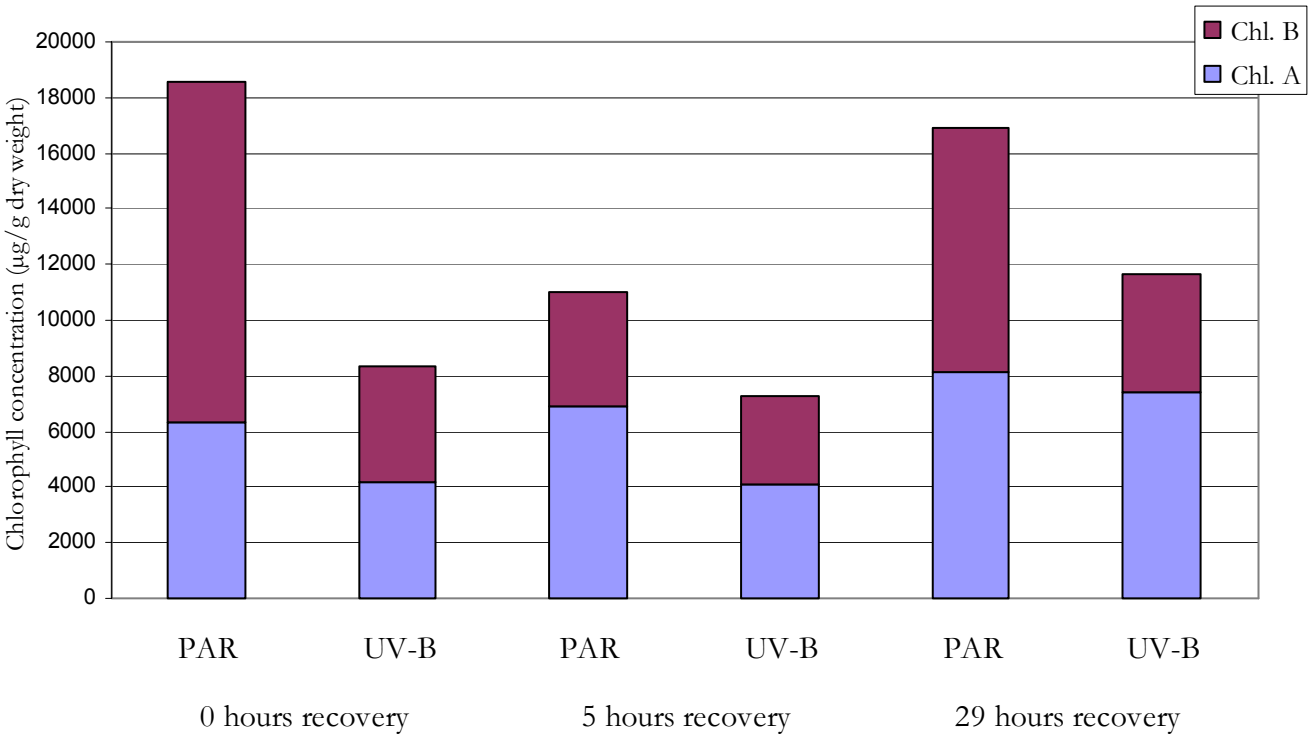


**Figure 5:** Average chlorophyll concentrations of *Lemna minor* at 0, 5 and 29 hours of recovery following 5 days of UV-B irradiation (n=2).

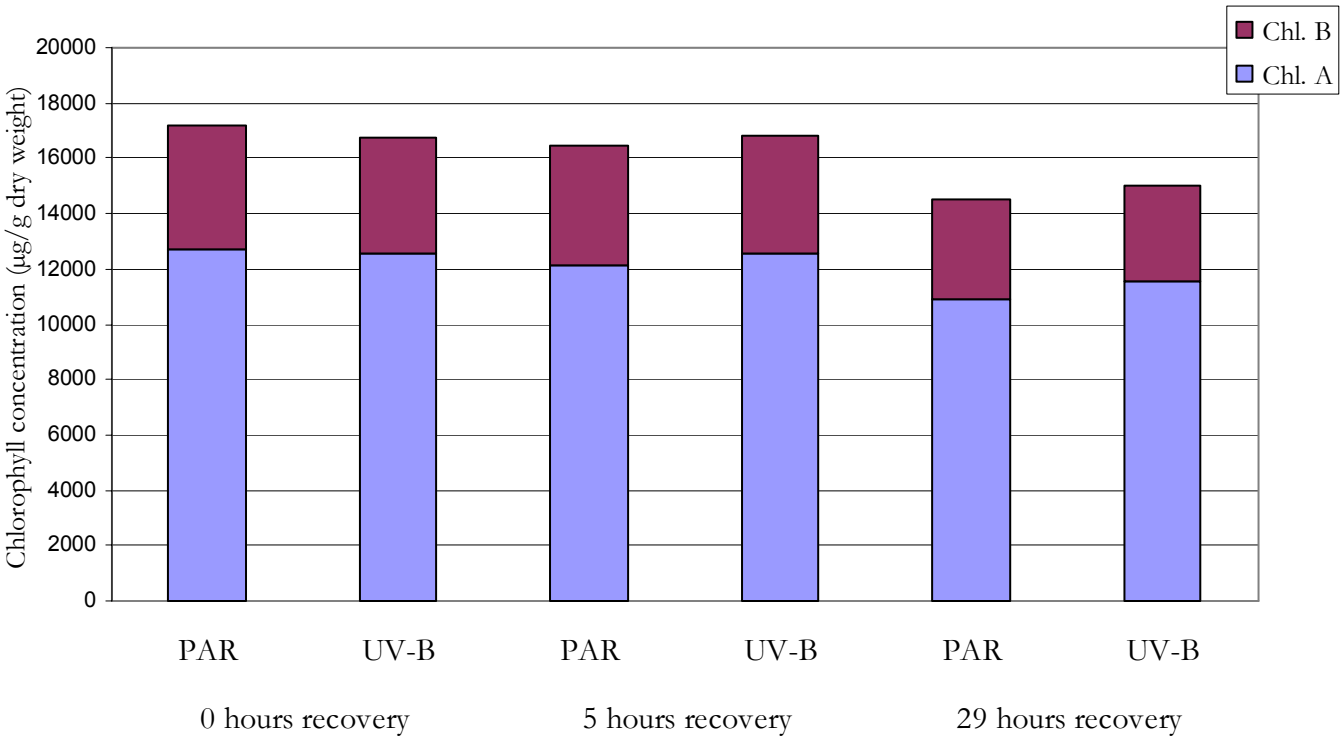


**Figure 6:** Average chlorophyll concentrations of *Lemna minor* at 0, 5 and 29 hours of recovery following 11 days of UV-B irradiation (n=2).

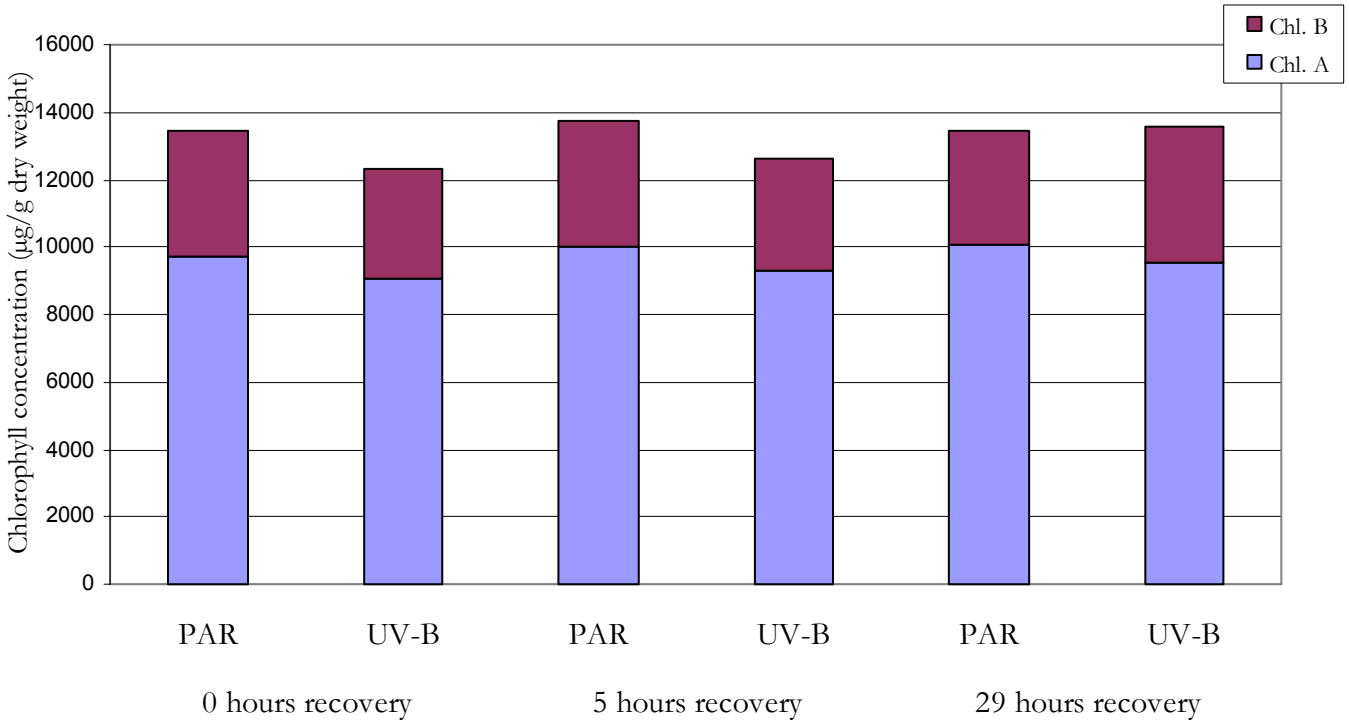


**Figure 7:** Average chlorophyll concentrations of *Spirodela polyrhiza* at 0, 5 and 29 hours of recovery following 5 days of UV-B irradiation (n=2).

## DISCUSSION

*Lemna minor* and *Spirodela polyrhiza* were both sensitive to UV-B radiation, showing a significant decrease in growth relative to control treatments. If levels of UV-B at the Earth's surface increase, there may be a reduction in available food for aquatic herbivores, and the ability of duckweed to function as an animal feed and fertilizer may be impaired.

Floating duckweed species such as *L. minor* and *S. polyrhiza* can form dense mats and coat the water surface. *Lemna minor* and *S. polyrhiza* mats block up to 93% and 99% of incoming light respectively (Landolt 1986). Lack of light penetration in duckweed-covered water bodies reduces oxygen production by phytoplankton, and leads to a decline in aerobic decomposition. Organic matter therefore accumulates in these water bodies, as do the products of anaerobic decomposition, especially hydrogen sulfide ($H_2S$). Additionally, duckweed cover reduces daily temperature fluctuations in water bodies, and the water temperature is approximately 2 – 4°C cooler than open water (outlined in Landolt 1986). The shading effects of *L. minor* mats are known to suppress populations of *Lemna trisulca* L., a sub-surface species (McIlraith *et al.* 1989). In a situation of enhanced UV-B, the populations of *L. minor* and *S. polyrhiza* may decline due to UV-B sensitivity. The resulting increase in light penetration in formerly duckweed covered water bodies could alter water chemistry and community composition. Populations of sub-surface photosynthetic organisms, like algae, could undergo rapid expansion and there could be a change in the dominant species in the water body.

*Spirodela polyrhiza* was less sensitive to UV-B radiation than was *L. minor*. Although the total number of fronds was significantly affected by UV-B radiation in both species, exposure had no effect on the survival, number of unhealthy fronds, or chlorophyll concentrations in *S. polyrhiza*. There was, however, a significant treatment effect on the

production rate in one of two *S. polyrhiza* trials. This difference in sensitivities at the species level could have important ramifications in aquatic communities. *Lemna minor* and *S. polyrhiza* commonly co-occur, but *L. minor* greatly outnumbers *S. polyrhiza*, and appears to be the better competitor (personal observation). Barnes *et al* (1988) showed experimentally that UV-B exposure could shift the interspecific competitive balance of two species. In a situation of elevated UV-B, *S. polyrhiza* may be able to out-compete *L. minor*.

The induction of flavonoid synthesis is an important defense mechanism for plants exposed to UV-B. Although *L. minor* and *S. polyrhiza* are taxonomically very close, they do produce different chemical compounds. In a chemotaxonomic study of duckweed species, McClure and Alston (1966) found that *L. minor* contained only glycoflavones, while *S. polyrhiza* contained glycoflavones, anthocyanins, and flavones. The diversity of flavonoid compounds in *S. polyrhiza* may confer a greater protective benefit on the species with regard to UV-B radiation. Another explanation for the differing UV-B sensitivities of *L. minor* and *S. polyrhiza* may be differences in light absorption at the frond surface. Both species are reported to have a similar cuticle and epidermis (Landolt 1986), but information is lacking on the reflective capacities of these cuticles.

*Lemna minor* did not show recovery during the 29 hours following UV-B irradiation. Recovery was analyzed by comparing the percent survival of the species at 0, 5, and 29 hours. Recovery would be interpreted as a significant increase in percent survival from one time period to the next. In one of two trials, *S. polyrhiza* survival at 29-hours was significantly lower than at 0, and 5-hours, but this is not interpreted as biologically significant because survival at 0 and 5-hours of recovery was 100% (therefore 0 variance) for control and UV-B treatments. At 29-hours, survival declined to 98.8% and 99.5% for UV-B and control treatments respectively. This only represents one unhealthy frond in the control treatment,

and two unhealthy fronds in the UV-B treatment, and is therefore not interpreted as biologically significant.

Plants require UV-A radiation for photorepair (Allen *et al.* 1998). However, it is nearly impossible to obtain natural UV-A levels in a laboratory setting. Therefore, the deleterious effects of UV-B on duckweed and their lack of recovery may be slightly exaggerated due to the low levels of UV-A under the present laboratory conditions.

UV-B exposure had no statistically significant effect on chlorophyll content in any trial. However, the chlorophyll concentration of UV-B irradiated *L. minor* was lower, relative to controls, after 5 days of irradiation. Although this effect is not statistically significant, it could still be biologically important, and with a larger sample size, and less variance, the difference could be statistically significant. After 11 days of UV-B irradiation, there was no difference between control and UV-B treatments. There are two possible explanations for the apparent differences between the 5 and 11-day trials. *Lemna minor* may be capable of inducing protective mechanisms during the 11-day trial. Alternatively, *L. minor*'s prolific production rate means that many of the individuals present at the end of the experiment developed in an environment of UV-B, therefore these individuals may be better adapted for an environment of high UV-B radiation.

*Lemna minor* may be capable of mitigating some of the negative effects of UV-B irradiation over longer time periods. During the 11-day irradiation period, *L. minor* might be able to respond to the UV-B, for example, by the induction of flavonoids or by changes in cuticle or leaf thickness, in ways that it could not over the shorter (5-day) time period. Bassman *et al.* (2002) found that Douglas-fir trees exposed to UV-B radiation showed a decline in growth during the first year, but they recovered in subsequent years. Although the *L. minor* irradiation periods were much shorter than those of Bassman *et al.*, *L. minor* is much

shorter lived than a fir-tree, therefore, adaptive responses may take less time to manifest themselves.

*Lemna minor* has a rapid frond production rate. Many of the fronds present on the 11$^{th}$ day of irradiation were produced during the experimental period, and therefore developed in an environment of high UV-B. There is some debate as to whether exposure to UV-B at the developmental stage is detrimental or advantageous to tissues. Germ and Gaberščik (1999) hypothesized that newly produced fronds of *L. minor* would be more sensitive to UV-B irradiation than mature fronds due poor development of the cuticle and epidermis. Also, tissues exposed to UV-B radiation at a younger stage would accumulate more radiation throughout their life span, and therefore might be expected to show proportionally greater effects. However, Allen *et al.* (1998) showed that *Brassica napus* (canola) leaves exposed to UV-B during development were less sensitive to UV-B than mature leaves. This suggests that plants that are exposed to UV-B during tissue development may be better able to mitigate the deleterious impacts of UV-B.

Duckweed is an important aquatic plant, and there is much more to learn about its sensitivity to UV-B. Future studies could explore the competitive consequences of differences in UV-B sensitivity at the species level by placing more than one species in each experimental dish and observing how competitive interactions change with UV-B exposure. Alternatively, a study could compare the effects of UV-B on mother fronds (those that developed without UV-B exposure) versus daughter fronds (those fronds which developed in an environment with UV-B radiation). Lastly, different exposure and recovery periods could be examined.

As the ozone layer declines it is essential that we learn more about the effects of this radiation. As Williamson (1995) stated, with regard to the potential impacts of UV-B on

freshwater ecosystems, "complex rather than simple responses are likely to be the rule" (p. 389). It is therefore important to understand more about the impacts of UV-B on all organisms…even the very tiniest flowering plants.

## CONCLUSIONS

1. UV-B exposure had a significant impact on the total number of fronds of *Lemna minor* and *Spirodela polyrhiza*.
- This effect was more pronounced in *L. minor*
- Neither species showed recovery during the 29 hours following irradiation

2. *Spirodela polyrhiza* is less sensitive to UV-B radiation than *L. minor*

3. In *L. minor*, chlorophyll concentrations were lower in UV-B exposed plants than in control plants following 5-days of irradiation, but the difference was not statistically significant. However, there was no difference in the chlorophyll concentrations of UV-B exposed plants and control plants following 11-days of UV-B irradiation.
- Over longer time periods, *L. minor* may be capable of mitigating some of the deleterious effects of UV-B, or
- Younger fronds may be less sensitive to UV-B

4. If the levels of UV-B reaching the Earth's surface increase and duckweed populations decline, aquatic ecosystems may be altered
- Also, *S. polyrhiza* may be able to out-compete *L. minor*, shifting the competitive balance between these species

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**Appendix I:** Experimental organisms

**A**

**B**


**A.** *Lemna minor* (~5mm)
<http://naturalaquariums.com/discus/messages/282/804.html?1108483055>
**B.** *Spirodela polyrhiza* (~12mm)
<http://www.eeb.uconn.edu/Courses/eeb204/eeb204f00/index/PICFILES/SPIROPOL.JPG>

## **Appendix II:** WC' medium

The following compounds should be added to distilled water in the order given, and the final volume adjusted with distilled water to 1L. The final pH should be adjusted with HCL or NaOH such that it is between 7.48 and 7.52. The medium should be autoclaved for 45 minutes on a "liquid" cycle to kill any bacteria.

| | **Compound** | **Concentration of compound in stock solution** | | **Volume of stock solution in 1L of WC' media** | |
|---|---|---|---|---|---|
| **Major Elements** | $NaNO_3$ | 8.50 | (g/L) | 10.0 | (ml) |
| | $KH_2PO_4$ | 13.61 | | 0.1 | |
| | KCL | 7.46 | | 0.4 | |
| | $MgSO_4 \cdot 7H_2O$ | 24.65 | | 1.5 | |
| | $CaCl_2 \cdot 2\ H_2O$ | 14.71 | | 2.5 | |
| | $NaHCO_3$ | 8.40 | | 1.5 | |
| | $Na_2SiO_3 \cdot 9\ H_2O$ | 28.42 | | 2.0 | |
| **Buffer** | Tricine | 17.92 | (g/L) | 10.0 | (ml) |

For trace elements and vitamins, stock solutions are pre-mixed in the following concentrations. For 1L of WC' media, 2.5 ml of trace element stock solution and 1.0 ml of vitamin stock solution are used.

| | | | |
|---|---|---|---|
| **Trace Elements** | $Na_2EDTA$ | 874.0 | (mg/L stock solution) |
| | $FeCl_3 \cdot 6\ H_2O$ | 630.0 | |
| | $H_3BO_3$ | 200.0 | |
| | $MnCl_2 \cdot 4\ H_2O$ | 36.0 | |
| | $NaMoO_4 \cdot 2\ H_2O$ | 1.2 | |
| | $ZnSO_4 \cdot 7\ H_2O$ | 4.4 | |
| | $CoCl_2 \cdot 6\ H_2O$ | 2.0 | |
| | $CuSO_4 \cdot 5\ H_2O$ | 2.0 | |
| **Vitamins** | Thiamine | 10.0 | (mg/100ml stock solution) |
| | Cyanocobolamine | 0.05 | |
| | Biotin | 0.05 | |

**Appendix III:** Formulas for calculating chlorophyll concentrations

From Hendry and Price (1993):

Chlorophyll *a* (mg/L) = 12.7 x A663 – 2.69 x A645

Chlorophyll *b* (mg/L) = 22.9 x A645 – 4.68 x A663