

THE EFFECT OF UV RADIATION  
ON CYANOBACTERIAL MAT  
COMMUNITY STRUCTURE

by

CARRIE ELISE INMAN

A THESIS

Presented to the Department of Biology and  
Honors College of the University of Oregon  
in partial fulfillment of the requirements  
for the degree of  
Bachelor of Science

June 2000

An Abstract of the Thesis of  
Carrie Elise Inman for the degree of Bachelor of Science  
in the department of Biology to be taken June 2000  
Title: THE EFFECT OF UV RADIATION ON CYANOBACTERIAL MAT  
COMMUNITY STRUCTURE

Approved: \_\_\_\_\_

Dr. Richard Castenholz

Cyanobacteria are thought to have evolved during the early Precambrian, 2.5-3.8 billion years ago, when there were very high levels of UV radiation reaching the Earth's surface due to a lack of absorbing gases in the atmosphere. Oxygenic cyanobacteria were one of the few photosynthetic organisms that could survive under these harsh conditions. Cyanobacteria today have also been found to employ UV tolerant strategies to cope with the damaging effects of UV radiation. In this study three cyanobacterial mats were investigated to determine if UV had an effect on the mats in the field and subsequently on clonal isolates cultured from these mats in the lab. UV exclusion experiments, using UV blocking and UV transmitting filters, were carried out in the field over a two-month period. Protein and pigment analysis on the core samples collected from the field UV treatments showed no significant difference between the treatments. However, samples

collected of new settlement cell material on ceramic tiles, did show a significant difference between the two UV treatments. UV exclusion experiments run using two strains of clonal isolates of *Leptolyngbya* sp. cultured from Mushroom Spring did show a highly significant detrimental effect of UV on growth measured by dry weights. In addition, the two strains, one cultured from the UV(-) field material and one cultured from the UV(+) material, were effected differently by UV radiation in the lab UV exclusion experiment. The isolate cultured from the UV(+) mat material was less effected by UV radiation, as measured by dry weight accumulation over time. This may imply the use of a unique UV tolerant strategy employed by this strain.

## **ACKNOWLEDGMENTS**

The author would like to express her sincere appreciation to Dr. Richard Castenholz for giving her the opportunity to work in his lab and for all the guidance he has provided her. In addition, she would like to express a very special thank you to all the members of the Castenholz lab, especially Dr. Jesse Dillon without whom this project would not have been possible, and to Dr. Scott Miller for introducing her to the world of laboratory research, and to Erich Fleming, and Lauren Wingard. She would also like to thank Professors Nathan Tublitz and Eric Selker, and all the members of the Biology Honors seminar, for helping to ease this process along. And she would like to thank Professor Joseph Fracchia for awakening her to the skills of critical thinking. Finally, she would like to thank her family for their support and understanding throughout these amazing college years.

## TABLE OF CONTENTS

| Section                        | Page |
|--------------------------------|------|
| I. INTRODUCTION.....           | 1    |
| II. METHODS AND MATERIALS..... | 6    |
| III. RESULTS.....              | 12   |
| IV. DISSCUSSION.....           | 15   |
| V. BIBLIOGRAPHY.....           | 18   |

## LIST OF FIGURES

| Figures                                            | Page |
|----------------------------------------------------|------|
| 1. Field UV Exclusion Experimental set up.....     | 21   |
| 2. Clonal Isolate Experimental Design .....        | 22   |
| 3. Lab UV Exclusion Experimental set up.....       | 23   |
| 4. Protein and Pigment analyses of cores.....      | 24   |
| 5. Protein and Pigment analyses of tiles.....      | 26   |
| 6. Lab UV Exclusion, log dry weight over time..... | 28   |
| 7. Lab UV Experiment, Average Chlorophyll a.....   | 30   |

## **Introduction**

### **Cyanobacteria:**

Cyanobacteria (formerly blue-green algae) are photosynthetic prokaryotes of the domain Bacteria. They are distinguished from other photosynthetic bacteria because they perform oxygenic photosynthesis. Whereby water is split to provide electrons for photosynthesis with the release of oxygen. Cyanobacteria exhibit a diversity of morphological types including both unicellular and filamentous forms, which can be branched or unbranched. Like eucaryotic algae and plants, cyanobacteria possess two photosystems, which allow them to harvest light energy from the sun with water as the reductant (Ho and Krogmann, 1982).

Cyanobacteria are commonly found living in aggregations that make up what is called a microbial mat. Mat-forming cyanobacteria make multiple species-specific layers, which together constitute what is commonly called a “mat”. Mat-like stromatolite formations have been found in the fossil record, suggesting that mat-forming cyanobacteria were common 2.5 billion years ago, during the mid-Precambrian period (Schopf, 1996).

Cyanobacteria are widely distributed, and have representatives in almost every kind of ecosystem, from the open ocean to the desert floor (Castenholz, 1997). Cyanobacteria are also well known for their tolerance of extreme habitats such as hot springs or Antarctic pools and lakes (Castenholz, 1997). The ability to inhabit and tolerate extreme habitats may have enabled their survival through billions of years.

### **Ultraviolet Radiation on Early Earth:**

During the early Precambrian period (2.5-3.8 billion years ago) there were very high levels of ultraviolet radiation reaching the Earth's surface (Kasting et al. 1989). The high level of radiation at that time was the result of a different atmospheric composition. The early atmosphere was highly reducing and did not contain oxygen (O<sub>2</sub>) or ozone (O<sub>3</sub>) (Kasting et al. 1989). Today, oxygen and ozone are responsible for absorbing a significant proportion of the ultraviolet radiation before it reaches the Earth's surface. However, organisms, such as cyanobacteria, which lived before the rise of oxygen in the atmosphere, had to deal with very high levels of radiation.

### **Ultraviolet Radiation Today:**

Today we are faced with the effects of the depletion of the ozone layer, which is gradually increasing the levels of ultraviolet radiation reaching the surface of the earth, specifically radiation in the UVB range (280-320nm) (Kerr and McElroy, 1993). Studies on the effect of increased levels of UVB have shown that it is detrimental to many types of organisms, including humans (Baker et al. 1997, Basiouny et al. 1978, Caldwell et al. 1995, Jordan 1996, Kruschel and Castenholz 1998, Cockell and Rothschild 1999, Karentz et al. 1991, Brenowitz and Castenholz 1997). The photosynthetic processes in both plants and in bacteria are negatively effected by ultraviolet radiation (Allen et al., 1998). UVB, specifically, is damaging to both the light and dark reactions of photosynthesis (Vincent and Roy, 1993). As a result, numerous studies have found that UVB inhibits primary production (Vincent and Roy 1993, Häder and Worrest 1991, Jokiel and York 1984, Cullen and Neale 1994, Prezelin et al. 1994). As well as inhibiting primary production,



other specific negative effects of UV radiation include DNA damage (Harm 1980, Karentz et al.1991) and inhibited nitrogenase activity (Sinha et al., 1996).

### **Cyanobacteria as Model Organisms:**

The use of cyanobacteria as model organisms for further investigation of the effect of UVB on living organisms is attractive for two major reasons. The first is due to the early exposure that ancestral forms of cyanobacteria faced during the Precambrian. These ancestral forms may have developed the ability to tolerate UV radiation to survive the high levels reaching the Earth's surface at that time. Cyanobacteria could have retained these UV tolerance strategies over evolutionary time, and employ them today. Secondly, cyanobacteria are a good study organism because they inhabit environments that are exposed to high ultraviolet radiation. Because cyanobacteria are photosynthetic organisms, and therefore rely upon solar energy for their metabolism, they cannot easily escape the damaging ultraviolet radiation of the sun.

### **Ultraviolet Tolerance Strategies:**

Cyanobacteria have been found to employ a variety of UV tolerance strategies to cope with the detrimental effects caused by ultraviolet rays. By studying UV tolerance mechanisms, not only can we learn how organisms cope with limiting factors in their environments, but we can also learn the specific ways that UV radiation negatively affects living cellular material. The strategies known today can be divided into three

categories; passive tolerance (cellular filters), avoidance (motility), and active repair of damaged cellular components (Castenholz, 1997).

Passive tolerance:

Scytonemin is found in the extracellular sheath of numerous ensheathed cyanobacteria. It was first observed by Nägeli (1849) who described it as a yellow to brown sheath pigment, but until recently its function was uncertain. Scytonemin's function as a UV screening pigment was discovered by Garcia-Pichel et al. in 1992. Scytonemin blocks UV radiation from entering the cell. Unlike mycosporine-like amino acids (MAAs), which are colorless UV absorbing compounds that typically accumulate inside the cell and absorb damaging UV radiation, scytonemin is an extracellular compound (Castenholz, 1997). However, in one species of cyanobacteria, *Nostoc commune*, MAAs were found extracellularly (Böhm et al. 1995). These extracellular MAAs are the only known example of MAAs that act as a true sunscreen (Ehling-Schulz et al. 1997).

Carotenoids are other UV protective pigments found in cyanobacteria, however, they function indirectly. As opposed to directly absorbing UV wavelengths, carotenoids quench potentially damaging cellular byproducts created by UV radiation, such as the highly reactive singlet oxygen molecules, triplet chlorophyll, and peroxy radicals (Krinsky, 1979). Carotenoids are also thought to function by inhibiting free radical reactions (Britton, 1995).

#### Avoidance:

Another UV tolerance strategy employed by motile cyanobacteria, such as gliding *Oscillatoria* spp., is to move away from areas of high UV radiation. Some mat forming cyanobacteria have been shown to respond to damaging levels of UV radiation by using the UV absorbing properties of the mat to their advantage (Castenholz, 1997). This is accomplished by motile cyanobacteria who can move deeper into the mat substrate where solar irradiance is attenuated (Kruschel and Castenholz, 1998). For example trends in downward migration were recorded over a diurnal period by Garcia-Pichel et al. in 1994.

#### Active Repair:

If UV radiation does damage the cell, cyanobacteria can actively repair or re-synthesize the damaged cellular components. DNA lesions of several types can occur during exposure to UV radiation; the most common is photodimerization of adjacent pyrimidine bases (Sage, 1993). Cyanobacteria have been shown to perform excision repair but they are thought to rely more on photoreactivation for DNA repair (Levine and Thiel 1987, Blakefield and Harris 1994). Excision repair requires a complex of enzymes that remove the portion of damaged DNA and replace the missing nucleotides (Blakefield and Harris, 1994). Photoreactivation is a process that uses light energy and the enzyme photolyase to break the bonds of the pyrimidine dimer (Blakefield and Harris, 1994). Interestingly, many organisms, including humans, can photoreactivate UV light-induced pyrimidine dimers (Levine and Thiel, 1987)

## **Yellowstone Mats:**

In this study, three cyanobacterial mats in Yellowstone National Park were investigated. The UV tolerance mechanism, if any, used by the species present within these mats is currently unknown. The purpose of this study was two-fold: first, to determine if UV radiation exposure had an effect on the cyanobacterial community structure of the mats in the field, and second, if UV radiation during laboratory experiments differentially affected cultures isolated from the mat. Answers to these questions may suggest the use of UV tolerance mechanisms by strains of cyanobacteria that were isolated from high UV conditions.

## **Materials and Methods**

### **Long Term Field Filter Experiments:**

#### **Research Sites:**

The cyanobacterial mats studied were located in the Lower Geyser Basin in Yellowstone National Park. They were chosen using four criteria. (a) Different species of cyanobacteria are present in the different layers of the mats. (b) Mat uniformity of the surface layer's color, texture and temperature. (c) The samples were collected from research areas that had to be out of sight of tourist areas. And (d), for practical reasons, it was necessary to be able to secure a filter over the mat in order to effectively cancel out UV exposure over a study area. With all of these criteria in mind we chose to study two locations: (1) in Pool B at two sight (56°C, pH = 8.90 and 36°C, pH = 9.60), and (2) one site in Mushroom Spring (46°C, pH = 9.20).

### UV Exclusion Experiment:

UV exclusion experiments were carried out over the three selected hot spring sites, for the duration of two months (July and August) in the summer of 1999. UV transmitting and UV blocking filters were placed next to each other over the mats (Figure 1). All screens transmitted equal levels of visible light (PAR 90 % ambient levels). Screens that transmitted UV radiation (UVA 80 %, UVB 75 % of ambient levels) were 3mm thick (OP-4, Multicraft Plastics, Eugene, OR). Screens that blocked UV radiation (UVA 20 %, UVB 5 % of ambient levels) were 1mm thick (OP4, Multicraft Plastics, Eugene, OR). The filters were secured approximately 4 cm above the surface of the mat to limit the extent to which solar irradiance entered from the sides during periods of low sun angle. This ensured that an area in the center of the filter remained covered at all times. Ceramic tiles were placed under both the UV(+) and the UV(-) screens on the surface of the Pool B mat to measure new settlement and growth of cells. Ceramic tiles were not placed at Mushroom Spring because there was not enough water running over the mat to submerge the tile.

### Sampling:

After a two-month period, the screens were removed and samples were collected from the mat using two different techniques: the first involved coring the center of the mat, using a metal  $\frac{3}{4}$  inch corer, and the second involved collecting all the material on the tiles into sterile zip-lock bags. Thirteen core samples were taken from the center of each mat. The samples were placed upright into sterile 15 ml polypropylene centrifuge tubes (Corning). Six samples were frozen for protein assays and pigment analysis, three

samples were preserved with 4% formalin (a 37% saturated solution of formaldehyde, which kills the cells), and four samples were kept alive at room temperature for culturing. The samples that were kept alive were returned to the University of Oregon, Eugene, OR, for culturing in the lab. The new cell material, which grew covering the ceramic tiles, was analyzed with the same protein and pigment assays as the core samples.

#### Protein and Pigment analyses:

Triplicate cores from each UV treatment were used for separate protein and pigment analyses. The analyses were performed on homogenized suspensions of the top 0.2 cm of a frozen core, cut off with a razor blade, and triplicate 1 ml samples of a homogenized cell suspensions from the tile material. The protein assay was done using the bicinchoninic acid method (Smith et al., 1985) which is a colorimetric assay. The homogenized cell suspensions were placed into 15 ml disposable centrifuge tubes (Corning, Corning, NY), spun down in a centrifuge (International Clinical Centrifuge, Boston, MA) and the medium was decanted. The cells were resuspended in 1 ml distilled water and proteins were extracted with 1 ml of 1M NaOH and boiled for 20 minutes. Adding 1 ml of 1M HCL neutralized the extracted cell suspension. The colorimetric assay was performed using 100  $\mu$ l of the neutralized extract. To extract pigments, homogenized cell suspension were filtered onto a GF/F filter (Whatman, Maidstone, ENG) which were placed into a 20 ml glass scintillation vial, containing 10 ml methanol saturated with  $\text{MgCO}_3$ . Pigments were extracted over a twelve-hour period in the dark at 4°C. One ml subsamples of the extracts were placed in 1.5 ml microcentrifuge tubes and spun down in a micro 13™ microcentrifuge (Fisher, Santa Clara, CA) to prevent scatter

by filter particles. Both protein and pigment assays were measured using a spectrophotometer (Beckman Du-640). Chlorophyll *a* content was determined using the extinction coefficients from Lenz and Zeitschel 1968. Pigment values were normalized to cellular protein content.

### **Short Term Lab UV growth Experiments:**

#### **Isolations:**

Clonal lab isolates were obtained from the core samples after returning to the University of Oregon. The live cores were kept at 12°C in the dark for a three-week period before being cultured. *Synechococcus* sp. collected from Pool B and *Leptolyngbya* sp. collected from Mushroom springs were isolated on BG-11 medium agar plates (Castenholz, 1988). *Leptolyngbya* cells were isolated from mat material that was placed onto the agar plates and incubated for a 24 hour period at 45°C or until individual motile filaments separated themselves from the rest of the cell material on the plate surface. Individual filaments were excised from the plates using sterile watchmaker's forceps and placed into flasks of BG-11 liquid medium at 45°C. *Synechococcus* strains were isolated from mat material in two ways. The material, which grew on agar plates, was either streaked out multiple times until individual colonies could be picked off the agar surface using sterile forceps, or the mat material was used to make a dilution series, the cell material from which, was streaked on agar plates until individual colonies could be picked. The clonal isolates of *Synechococcus* sp. were grown at 40°, 45°, 50°, 60°C. Both

*Synechococcus* and *Leptolyngbya* isolations were grown under artificial visible light conditions provided by coolwhite florescent bulbs (Philips).

#### UV Exclusion Growth Experiment:

Two *Leptolyngbya* spp. isolates from Mushroom Spring, one from each field treatment UV(-) and UV(+) were grown in liquid BG-11 medium and tested over a ten-day period at 45°C under different UV lab conditions. Each isolate of *Leptolyngbya* sp. was re-inoculated into six 250 ml quartz flasks (Quartz Scientific, Fairport Harbor, OH). Each flask contained a 130 ml cell suspension. Three replicate samples, of each isolate, were placed under each UV treatment (Figure 2).

As in the field experiments, UV treatments were provided by the two types of filters (UV(+) and UV(-)) which were suspended in a 45°C incubator. The filters were suspended between the UVB bulbs (Philips) and the flasks, which contained cell material. Underneath the flasks were coolwhite florescent bulbs (Philips) providing the visible light. Areas along the periphery of the incubator that received equal amounts of visible and UVB radiation were marked (Figure 3). Visible and UVB levels were measured using a IL-1700 research radiometer (International Light, Newburyport, Massachusetts) and separate filter sensors calibrated to read visible light (400-700nm) and UVB radiation (280-320nm). UVB bulbs were transmitted at an average of  $0.62 \pm 0.08 \text{ W/m}^2$  by the UV(+) filter. UVB was reduced to an average of  $0.0011 \pm 0.0003 \text{ W/m}^2$  by the UV(-) filter. Visible radiation generated by coolwhite florescent bulbs (Philips) was constant under both filters at an average of  $11 \pm 2 \text{ W/m}^2$ .



### Sampling:

Triplicate 8 ml samples from each flask were removed after 0, 2, 5, and 8 days. Cell material in all flasks were homogenized, by repeated pumping using a sterile 20 cc syringe, and the flasks were randomized within the incubator daily. Samples were removed after repeated pumping of the cell suspension. Samples were filtered onto 3  $\mu$ m polycarbonate filters (Poretics) with a 25 mm glass microfiber backing filter (Whatman). Before use, filters were dried at 80°C overnight and weighed twice, using an M5 balance (Mettler, Switzerland). After filtering the cells onto the filters, they were dried overnight at 35°C and then re-weighed twice. Dry weights for the samples were calculated by subtracting the average pre-weight from the average final weight.

Pigment extracts in methanol were then performed as described above on each polycarbonate filter after it was weighed. The only difference was that 3 ml of methanol was used instead of 10 ml.

### Statistical Analysis:

Analysis of variance (ANOVA) models were built and performed using Super ANOVA computer program (Abacus Concepts, Inc., Berkeley, CA). Planned comparisons of means; chlorophyll *a* values and dry weight, for different UV treatments were performed to determine significance between means. 1-factor ANOVA's were used to test for significance in the chlorophyll *a* data from the field UV exclusion experiment.

## **Results:**

### **Long term Field Filter Experiments:**

#### **Observations:**

Following the 2 month screening there was no visible difference between the mats that were covered by the UV(-) filter compared to the UV(+) filter. Protein and pigment analyses performed on the core samples from the two treatments supported these visual observations.

#### **Pigment analysis:**

The amount of chlorophyll *a* ( $\mu\text{g}$ ) was divided by the average protein content (mg) in the cores for each treatment. There was no significant differences ( $P > 0.05$ ) between UV treatments at any of the mat locations when compared with a 1-way ANOVA. Chlorophyll *a* was not significantly different in the cores from the Pool B mat between the UV treatments, ( $P = 0.8274$ ). Chlorophyll *a* was not significantly different in the cores from the Pool B Down Stream mat between the UV treatments, ( $P = 0.0987$ ). Chlorophyll *a* was not significantly different in the cores from Mushroom Spring mat between the UV treatments, ( $P = 0.8477$ ). Figure 4 shows the average chlorophyll *a* values normalized to protein of triplicate core samples for each location; error bars are standard deviations. The locations must be analyzed separately because they are in essence different ecosystems. However, for each location the two UV treatments are comparable, but there was no difference seen at any of the locations as stated above.

Interestingly, the new settlement material that accumulated on the tiles in Pool B over the two-month period was affected by UV radiation. Figure 5 shows the average chlorophyll *a* divided by protein values of triplicate 1 ml samples from the homogenized tile cell suspension, error bars represent standard deviation. There was a nearly significant difference in chlorophyll *a* content of the tile material between the UV treatments ( $P = 0.0551$ ) using a 1 factor ANOVA. When UV was excluded there was more chlorophyll *a* present.

### **Short Term Lab UV growth Experiments:**

#### **Isolations:**

Multiple attempts at culturing the *Synechococcus* sp. collected from Pool B were not successful. Despite initial growth when streaked on agar BG-11 plates at 50°C, when they were removed from the agar and placed into liquid medium cells did not grow. *Synechococcus* isolates were placed in BG-11 medium, D-medium and ND medium, (Castenholz, 1988) at 50, 55, and 60°C, none of which were successful. As a result, the filamentous *Leptolyngbya* sp. isolates collected from Mushroom Springs were the only strains used for the UV exclusion growth experiments. Strain 1 was isolated from the UV(-) field core samples and strain 2 was isolated from the UV(+) field core samples.

#### **Dry Weights:**

The two strains of *Leptolyngbya* sp. were inoculated for an initial 48 hours before being placed under experimental conditions to ensure exponential growth rates had been

reached. The replicate samples were placed in the 45°C incubator for a period of 8 days as described in Figure 2. Figure 6 shows the change in log transformed dry weight ( $\mu\text{g}$ ) over time, for each strain under both conditions. Over time, the dry weights of each isolate under both treatments increased significantly ( $P = 0.0001$ ) as they grew. Under UV transmitting conditions UV(+) growth in both isolates was reduced. Dry weights were significantly less, as determined by a 1-factor ANOVA on the day 8 final yield values, under UV(+) conditions than dry weights under UV(-) conditions ( $P = 0.0001$ ) for both strains. This demonstrates an inhibitory effect of UVB on cell growth. Interestingly, the final yield, was significantly different between the two isolates in their response to the UV treatments ( $P = 0.0022$ ) when compared with a 1-factor ANOVA. Although they were both negatively effected by UVB, Strain 1 from UV(-) conditions, was more greatly affected by the UV(+) lab treatment than Strain 2, which came from UV(+) conditions.

### **Pigment Analyses:**

Average chlorophyll *a* content normalized to dry weight, for both strains, was highly significantly reduced by UV radiation ( $P = 0.0001$ ) when compared with a 1-way ANOVA. However, there was no significant different between the two strains chlorophyll *a* content ( $P = 0.1927$ ). In other words both strains were similarly affected by UVB radiation. Figure 7 shows average chlorophyll *a* content over the whole experiment, excluding day zero data.

## **Discussion:**

### **Long term Field Filter Experiments:**

Chlorophyll *a* values from the mats studied in Yellowstone National Park were not affected when UV radiation was excluded over a two-month period. Chlorophyll *a* is a measure of the population of photosynthetic cyanobacteria which UV radiation was expected to negatively effect. This expectation was based on previous research, which indicated that UV radiation has detrimental effects on cyanobacteria (Vincent and Roy, 1993). The fact that there was no observed effect of UV can be explained three ways; the first, is that UV does not have a negative effect on cyanobacterial mats. Second that there is a UV effect, but that it was not measured in the chlorophyll *a* analysis. And third, there is the possibility that UV tolerant strains were selected for by the UV(+) treatment, which produced equal amounts of chlorophyll *a* as the cells under the UV(-) condition. The inherent diversity in the microbial mats may have accounted for the lack of a measured difference between UV treatments. Therefore, UV may not be a limiting factor at ambient levels in these well-established mats as was previously expected, because there could be UV tolerant strains, that have moved to the surface of the UV(+) mat, thus allowing photosynthesis to occur at comparable efficiency as under UV(-) conditions. Tolerance in such strains could constitute more efficient DNA repair systems. All three of these possibilities could explain our neutral result.

The newly settled cell material, i.e. tile material, was more affected by UV radiation than the existing cell material in the cores. Though this difference was not actually significant, ( $P=0.0551$ ) it is interesting to speculate why chlorophyll *a* levels of

newly settled cell material may be more effected than well established cells in the mat. One explanation could be that the newly settled material did not have the diversity of strains to draw from to perform UV tolerant photosynthetic activities, like the already established mat may have. An additional confounding factor may have been that newly settled cells did not have the advantage of being shaded by other layers as they grew initially; therefore all cells received UV radiation as they grew.

Another reason why the chlorophyll *a* values could be different for the tiles and not the cores may be due to the fact that the cell suspensions from the cores, used in both the pigment and the protein analysis, were very difficult to homogenize. This poor cell suspension, which included more layers than just the surface layer, may account for the measured variation in these values, and therefore mask any effect of UV. Where as the tile material was much easier to homogenate. In order to avoid this source of error it might have been better to test only the top most layer of the mat, 2 mm, which would enable a more homogeneous suspension to be made.

### **Short Term Lab UV growth Experiments:**

UV radiation had a highly significant effect on cell growth, measured by dry weight, in the *Leptolyngbya* sp. isolates from Mushroom Spring ( $P=0.0001$ ). Interestingly UV radiation affected Strain 1 more than Strain 2 ( $P=0.0022$ ). Strain 1 was isolated from the field UV(-) conditions and Strain 2 was isolated from the field UV(+) conditions. This suggests that either there is simply variation among strains, or that Strain 2 possessed a UV tolerance strategy that Strain 1 lacked. The chlorophyll *a* data indicate that, in both strains, chlorophyll *a* values were significantly reduced by UV, but

that they were not affected differently ( $P = 0.1927$ ). Overall, UV radiation negatively affects cell growth. In addition, it affects strains differently, which could be due to the presence of a UV tolerance mechanism.

To further explore which mechanism, if any, is being utilized by the species more tests would have to be done. For example molecular genotyping would be essential, to determine if the strains were in fact different. But most importantly, additional *Leptolyngbya* sp. isolations from the field treatments should be tested in the lab, to investigate the constancy of this trend.

## BIBLIOGRAPHY:

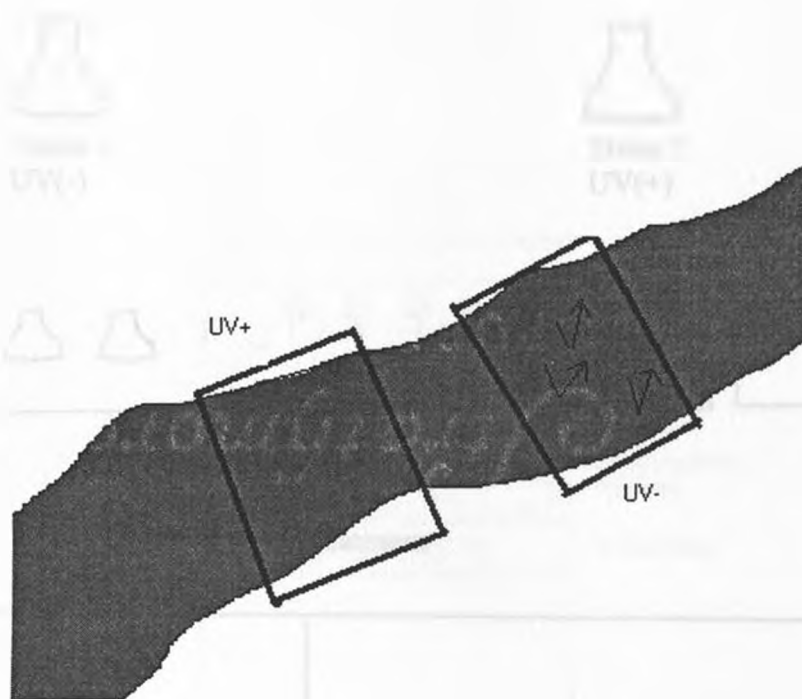
- Asato, Y. 1972. Isolation and characterization of ultraviolet light-sensitive mutants of the blue green alga *Anacystis nidulans*. *Journal of Bacteriology*. 110:1058-1064.
- Allen, D.J., Nogues, S. & Baker, N.R. 1998. Ozone depletion and increased UV-B radiation: is there a real threat to photosynthesis? *Journal of Experimental Botany*. 49:1775-1788.
- Baker, N.R., Nogues, S. and Allen, D.J. 1997. Photosynthesis and photoinhibition. In: Lumsden PJ, ed. *Plants and UV-B: responses to environmental change*. Cambridge University Press, 95-111.
- Basiouny, F.M., Van T.K. and Biggs, R.H. 1978. Some morphological and biochemical characteristics of C<sub>3</sub> and C<sub>4</sub> plants irradiated with UV-B. *Physiologia Plantarum*. 42:29-32.
- Blakefield, M., and Harris, D.O. 1994. Delay of cell differentiation in *Anabaena aequalis* caused by UV-B Radiation and the roll of photoreactivation and excision repair. *Photochemistry and Photobiology*. 59:204-208.
- Böhm, G.A., Pfeiderer, W., Boger, P., and Scherer, S. 1995. Structure of a novel oligosaccharide mycosporine-like amino acid ultraviolet A/B sunscreen pigment from the terrestrial cyanobacterium *Nostoc commune*. *Journal of Biol. Chemistry*. 270:8536-8539.
- Brenowitz, S., and Castenholz R.W. 1997. Long-term effects of UV and visible irradiance on natural populations of a scytonemin-containing cyanobacterium (*Calothrix* sp.). *Microbiology Ecology*. 24:343-352.
- Britton, G. 1995. *FASEB J*. 9:1515.
- Brock, T.D. 1978. *Thermophilic microorganisms and life at high temperatures*. Springer-Varlag New York: Library of Congress Cataloging in Publication of Data.
- Caldwell, M.M., Teramura, A.H., Tevini, M., Bornman J.F., Bjorn, L.O., and Kulandaivelu, G. 1995. Effects of increased solar ultraviolet radiation on terrestrial plants. *Ambio*. 24:166-173.
- Castenholz, R.W. 1997. Multiple strategies for UV tolerance in cyanobacteria. *Spectrum*. 10:10-16.
- Cockell, C.S. and Rothschild, L.J. 1999. The effects of UV radiation A and B on diurnal variation in photosynthesis in three taxonomically and ecologically diverse microbial mats. *Photochemistry and Photobiology*. 69(2):203-210.
- Cullen, J.C. and Neale, P.J. 1994. Ultraviolet radiation, ozone depletion, and marine photosynthesis. *Photosynthesis Research*. 39:303-320.
- Ehling-Schulz, M., Bilger, W., and Scherer, S. (1997) UV-B induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium. *Journal of Bacteriology*. 179:1940-1945



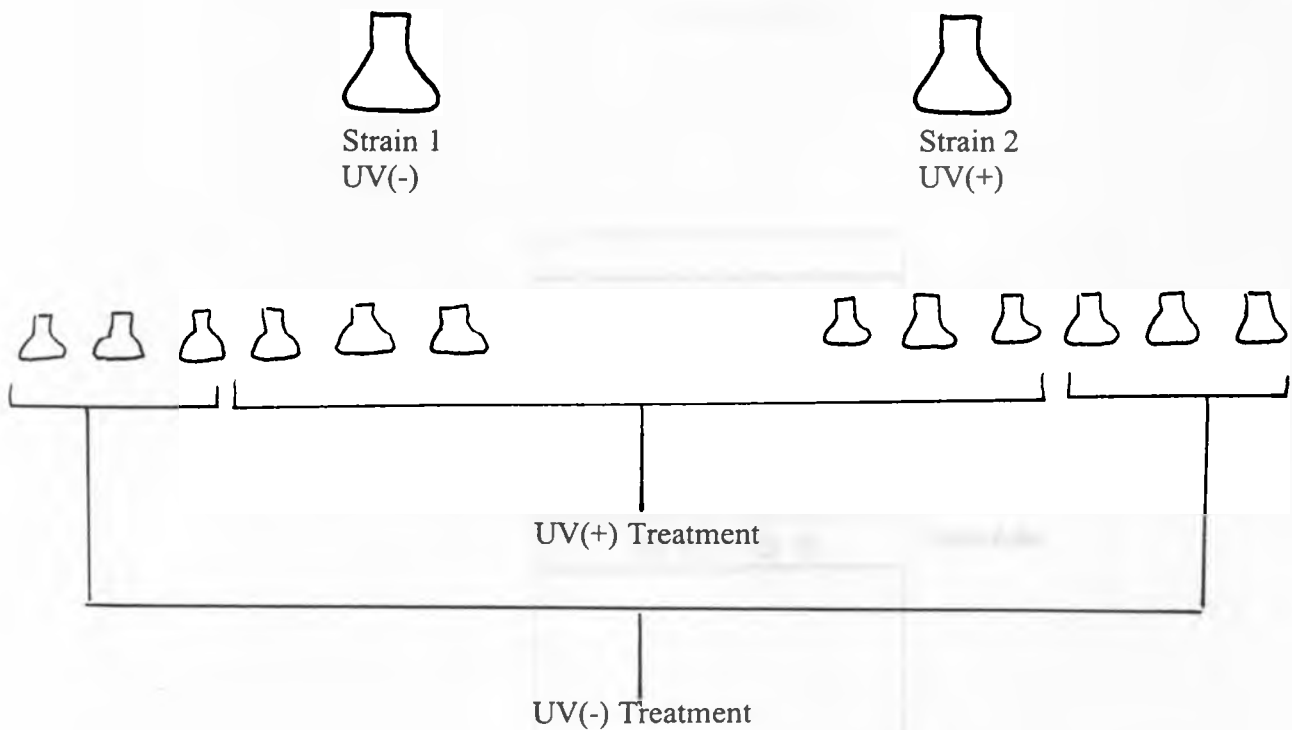
- Fenchel, T. 1998a. Artificial cyanobacterial mats: structure and composition of the biota. *Aquatic Microbial Ecology*. 14:241-251.
- Fenchel, T. 1998b. Formation of laminated cyanobacterial mats in the absence of benthic fauna. *Aquatic Microbial Ecology*. 14:235-240.
- Garcia-Pichel, F. & Castenholz, R.W. 1994. On the significance of solar radiation for the ecology of microbial mats. In Stahl, L.J. & Caumette, P. [Eds.]. *Microbial Mats, Structure, Development and Environmental Significance. NATO ASI Series, Vol. G35*. Heidelberg, Springer Verlag, pp. 149-166.
- Garcia-Pichel, F., Mechling, M. & Castenholz, R.W. 1994. Diel migrations of microorganisms within a benthic, hypersaline mat community. *Applied. Environmental Microbiology* 60:1500-1511.
- Garcia-Pichel, F., and Sherry, N.D. and Castenholz R.W. 1992. Evidence for an ultraviolet sunscreen role of the extracellular pigment scytonemin in the terrestrial cyanobacterium *Gloeocapsa* sp.. *Applied Environmental Microbiology*. 59:170-176.
- Häder, D.P., and Worrest, R.C. 1991. Effects of enhanced solar ultraviolet radiation on aquatic ecosystems. *Photochemistry Photobiology*. 53:717-725.
- Harm, W. 1980. *Biological Effects of Ultraviolet Radiation*. IUPAB Biophysics Series I. Cambridge, Cambridge University Press.
- Ho, K.K., and Krogmann, D.W. 1982. *Photosynthesis*. in *The Biology of Cyanobacteria*. Blackwell Scientific Publications. Oxford, London.
- Jokiel, P.L. and York, Jr. R.D. 1984. Importance of ultraviolet radiation in photoinhibition of microalgal growth. *Limnology Oceanography*. 29:192-199.
- Jordan, B.R. 1996. The effects of ultraviolet-B radiation on plants: a molecular perspective. *Advances in Botanical Research*. 22:97-162.
- Karentz, D., Cleaver, J.E. & Mitchell, D.L. 1991. Cell survival characteristics and molecular responses of Antarctic phytoplankton to ultraviolet-B radiation. *Journal of Phycology*. 27:326-341.
- Kasting, J.F., Zahnle, K.J., Pinto, J.P. and Young, A.T. 1989. Sulfur, ultraviolet radiation, and the early evolution of life. *Origins Life Evolution Biosphere* 19:95-108.
- Kerr, J.B. and McElroy C.T. 1993. Evidence for large upward trends of ultraviolet-B radiation linked to ozone depletion. *Science*. 262:1032-1034.
- Krinsky, N.I. 1979. *Pure Appl. Chem*. 51:649.
- Kruschel, C., and Castenholz R.W. 1998. The effect of solar UV and visible irradiance on the vertical movements of cyanobacteria in microbial mats of hypersaline waters. *FEMS Microbiology Ecology*. 27:53-72.

- Lenz, J., and Zeitschel B. 1968. Zur bestimmung des extinktionkoeffizienten für chlorophyll a in methanol. *Kiel. Meeresforsch.* 24:41-50.
- Levine, E., and Thiel, T. 1987. UV-Inducible DNA repair in the Cyanobacteria *Anabaena spp.*. *Journal of Bacteriology.* 169(9): 3988-3993.
- Miller, S., Wingard, C. & Castenholtz, R.W. 1998. Effects of visible light and UV radiation on photosynthesis in a population of a hot springs cyanobacterium, a *Synechococcus* sp., subjected to high-temperature stress. *Applied and Environmental Microbiology.* 64:3893-3899.
- Nägeli, C., and S. Schwenderer. 1877. Das Mikroskop, 2<sup>nd</sup> ed. Willhelm Engelmann Verlag, Leipzig.
- Prezelin, B.B., Boucer, N.P. and Schofield, O. 1994. Evaluation of field studies of UV-B radiation effects on Antarctic marine primary productivity. In *Stratosphere Ozone Depletion/UV-B Radiation in the Biosphere*. Springer-Verlag, Berlin.
- Sage, E. 1993. Distribution and repair of photolesions in DNA: genetic consequences and the role of sequence context. *Photochem. Photobiology.* 57:163-174.
- Schopf, J. W. 1996. Cyanobacteria: Pioneers of the early Earth. *Nova Hedwigia Beih.* 112:13-32.
- Sheridan, R.P. 1999. The role of ultraviolet radiation in maintaining the three dimensional structure of a cyanobacterial mat community and facilitating nitrogen fixation. *Division of Biological Sciences*. University of Montana, Missoula.
- Sinha, R.P., Kumar, H.D., Kumar, A., and Häder, D.P. 1995. Effects of UVB irradiation on growth, survival, pigmentation and nitrogen metabolism enzymes in cyanobacteria. *Acta Protozool.* 34:187-192.
- Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartmer, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J. and Klenk, D.C. 1985. Measurement of protein using Bicinchoninic Acid. *Analytical Biochem.* 150:76-85.
- Vincent, W.F., and Roy, S. 1993. Solar ultraviolet-B radiation and aquatic primary production: damage, protection and recovery. *Environ. Rev.* 1:1-12

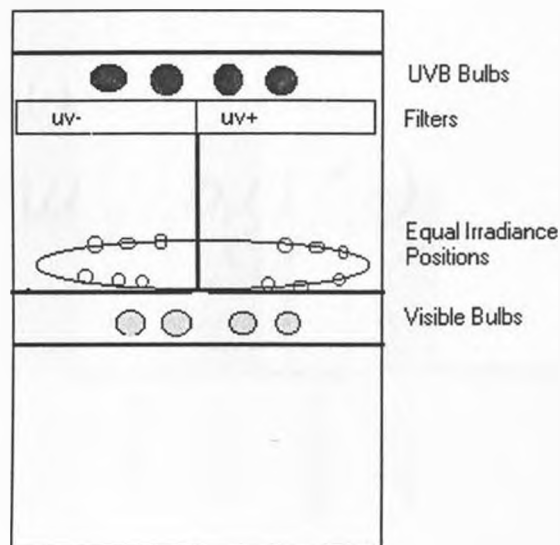
**Figure 1.** Field UV exclusion experimental set up. Where filters were placed over the cyanobacterial mat to either block UV radiation or transmit UV radiation. Both screen types transmit equal levels of visible light. Arrows indicate UV radiation being reflected by the UV(-) filter.



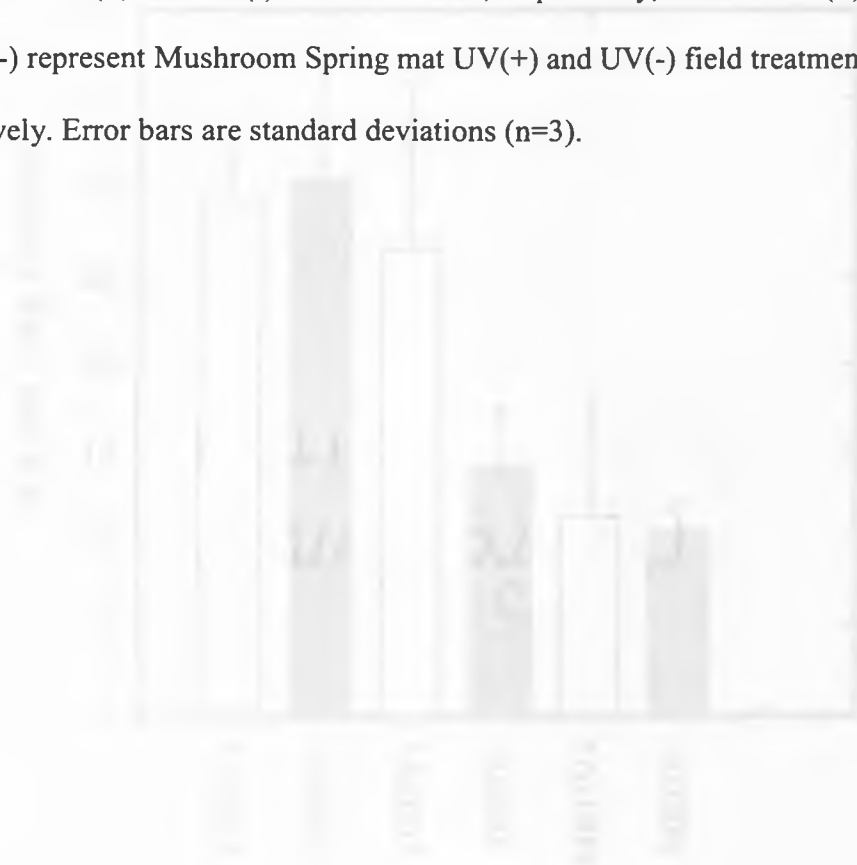
**Figure 2.** Experimental set up of lab UV exclusion experiment. Each strain was inoculated six times into replicate flasks. Three flasks of each strain were placed under both UV conditions.

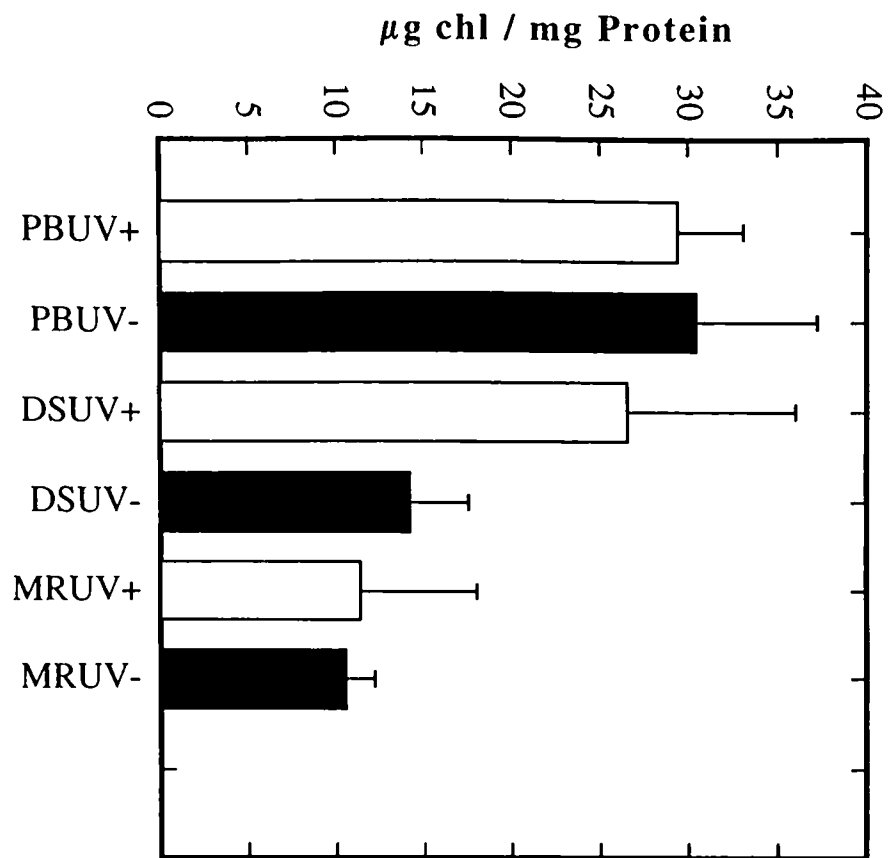


**Figure 3.** UV Exclusion experimental laboratory set up. The loop of circles indicate the equal irradiance positions where the flasks were placed, under the filters that either blocked or transmitted UVB radiation, emitted from the bulbs above. Fluorescent bulbs under the flasks provided equal amounts of visible light for both UV treatments.



**Figure 4.** Chlorophyll a values ( $\mu\text{g}$ ) per (mg) protein of three core samples for each location. Where PBUV(+) and PBUV(-) represents Pool B mat UV(+) and UV(-) field treatments, respectively; and DSUV(+) and DSUV(-) represent Pool B Down Stream mat UV(+) and UV(-) field treatments, respectively; and MRUV(+) and MRUV(-) represent Mushroom Spring mat UV(+) and UV(-) field treatments, respectively. Error bars are standard deviations (n=3).

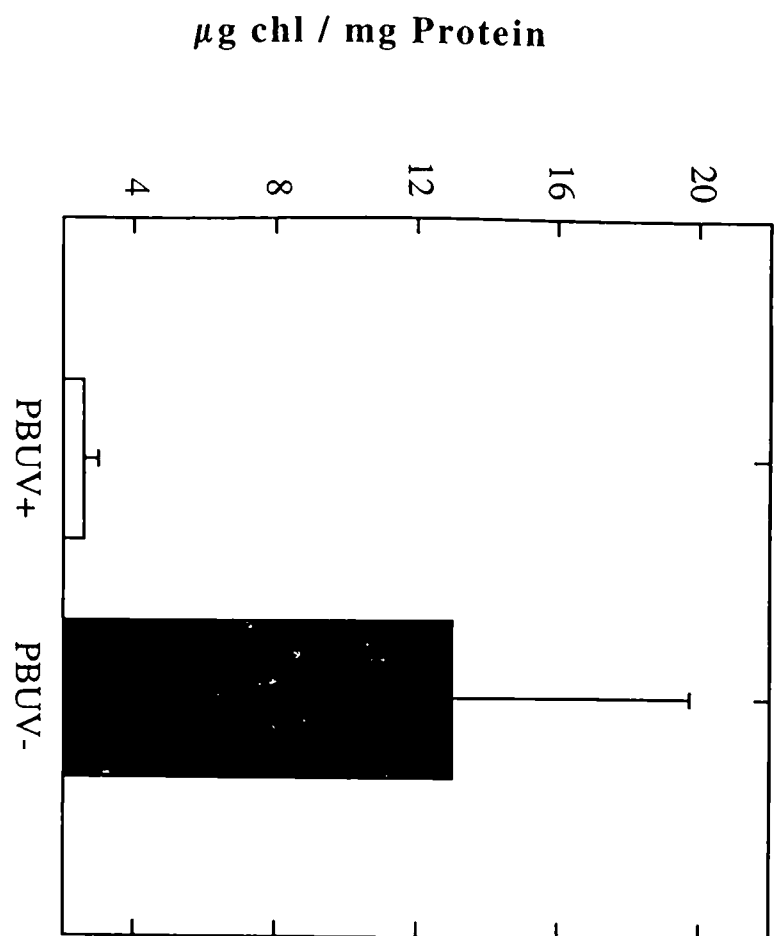




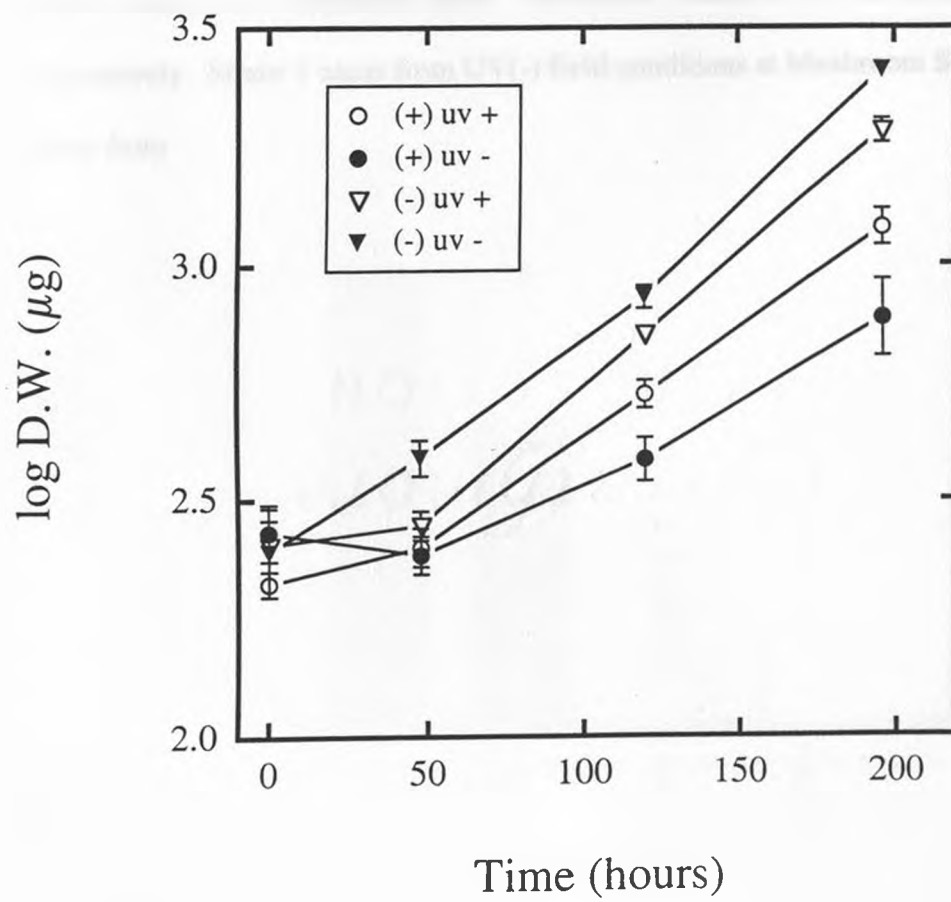
**Figure 5.** Average chlorophyll a ( $\mu\text{g}$ ) divided by protein (mg), values of triplicate 1 ml samples from the homogenized tile material cell suspension, error bars represent standard deviations. Where PBUV(+) and PBUV(-) represents Pool B mat UV(+) and UV(-) field treatments, respectively.







**Figure 6** Change in log transformed dry weight ( $\mu\text{g}$ ) over time. The triangles represent the UV(-) lab treatment and the circled represent the UV(+) lab treatment. Closed symbols, either a triangle or a circle, represents strain 1, isolated from the UV-field treatment at Mushroom Spring. And the open symbols, either triangles or a circles represent strain 2, isolated from the UV(+) field treatment at Mushroom Spring.



**Figure 7.** Average chlorophyll a content over the whole experiment, excluding day zero data. The chlorophyll a values were normalized to protein content. The values for each strain are the average of triplicate sampling over days 2, 5, and 8 (n=9). (-)UV- and (-)UV+ represent strain 1 and strain 2 under UV- lab conditions, respectively. (+)UV- and (+)UV+ represent strain 1 and strain 2 under UV(+) lab conditions, respectively. Strain 1 came from UV(-) field conditions at Mushroom Spring and strain 2 came from

