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**EFFECTS OF ULTRAVIOLET RADIATION ON BENTHIC ALGAE IN A  
MIDWESTERN LAKE AND A STREAM**

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## **ABSTRACT**

*This experiment was conducted at the University of Notre Dame Environmental Research Center and was designed to determine the effects of ultraviolet radiation on the chlorophyll a and phaeophytin levels of the benthic algae in a lake and a stream. Two treatment groups were used to determine the effects of the UV light. Through the use of plexiglas shields, one group was able to be shielded from the UV light and the other was unshielded. Chlorophyll a levels in the stream showed no significant difference between the +UV and -UV treatments. In the lake, however, the chlorophyll a of the -UV treatment was significantly higher than that of the +UV treatment during the middle of the experiment but then showed no difference at the final sample date (day 40). Phaeophytin levels were also measured but showed no distinct pattern in either the stream or lake.*

## **INTRODUCTION**

With the continued depletion of the ozone layer by chlorofluorocarbons and other atmospheric pollutants, the impact of ultraviolet radiation (UVR) on the aquatic environment has become an emerging area of concern in ecology. UVR travels from the sun unaltered until it enters the atmosphere of the earth (Madronich, 1993). Upon reaching the atmosphere, the absorption and scattering of UVR by many gases and particles modify the radiation. As this modified radiation travels toward the terrestrial and oceanic biospheres, the majority of the wavelengths most harmful to organisms are filtered out. Human activities are now changing the composition of the atmosphere, raising serious concerns about how the wavelength distribution and quantity of ground-level UVR will be affected (Madronich, 1993).

While many studies of UVR effects have been conducted with terrestrial organisms, effects on aquatic organisms are less well known (Holm-Hansen, 1993). Effects on aquatic organisms are less certain because spectral irradiance changes dramatically with depth in the water column. Because many aquatic organisms move up and down in the water column, quantification of UVR effects must consider this vertical migration. As with terrestrial plants, UVR can affect

several physiological aspects of aquatic plants such as photosynthetic rates, photosynthetic pigments, and cell growth and division.

Of the various aquatic organisms affected by UVR, algae serve as important models for study for several reasons. For example, much of what is known about photosynthesis was discovered through algal studies (Prescott 1968). Algae are of major limnological importance because they respond to water chemistry and light, and form the basis for most aquatic food webs (Prescott 1968). Finally, algal studies have become important to the medical field in areas of cancer research.

Of the many processes affected by UVR, one of the most important is algal photosynthesis. Characteristic to all ecosystems, photosynthesis is the basis for primary production that supports the food web. Examining the effects of UVR on algal photosynthesis allows the determination of either harmful or beneficial effects that can affect primary production and possibly entire food webs. Recent studies with unicellular and multicellular algae confirm that UVR with wavelengths down to 320 nm can be used in photosynthesis. However, at shorter wavelengths of UVR, known as UVB radiation, a general decline in rates of photosynthetic production has been observed with increased UVR (Holm-Hansen, 1993).

UVR also can affect other aspects of algae such as pigments and cell growth (Bothwell et al., 1994). Studies with UVR can help to locate the site of pigment degradation. This is accomplished by determining whether UVR affects chlorophyll *a* levels by inhibiting synthesis of the pigment, or destroying the pigment directly. In addition, algal studies focusing on UVR can measure excessive growth or inhibition of cells due to varied amounts of this radiation.

Knowledge concerning the effects of solar UVR on aquatic organisms has advanced considerably during the past decade, but it is obvious that there are

still enormous gaps in our understanding. With continued studies of UVR, we can continue to accumulate knowledge of UVR that will enable us to better understand its impact now and in the future.

This six-week study of Tenderfoot Creek and Crampton Lake at the University of Notre Dame Environmental Research Center had three main objectives: (1) to examine the effects of UVR on chlorophyll *a* of benthic algae; (2) to examine the effects of UVR on phaeophytin levels of benthic algae; (3) to compare the effects of UVR in the lake with those of the stream.

## **MATERIALS AND METHODS**

### **Experimental Floating Racks**

Floating racks were built to study UVR effects on benthic algae in both the lake and the stream environments. Each apparatus consisted of a square wooden frame and a cross beam for stability. Four strips of PCV piping were spaced parallel to one another and secured to the frame with U-bolts. Enough space was left between each piece of piping to accommodate a row of treatment baskets. To keep the racks afloat, styrofoam was lashed to the frames and crossbeams with pieces of twine.

In each floating rack, eight pairs of baskets were placed between the rows of PCV piping. Each pair of baskets consisted of one basket without a plexiglas lid (designated +UV) and one basket with a plexiglas cover secured by a rubber band (designated -UV) to shield the ultraviolet radiation. The baskets were secured to the rack with individual pieces of monofilament tied between the corners of each basket and the piping on each side of the baskets. For the baskets designated -UV, one corner was left untied to allow removal of the plexiglas lid. A sheet of styrofoam was placed at the bottom of each basket for the algae to colonize. To keep the styrofoam from floating out of the baskets, each piece was secured to the bottom of its basket with a small piece of twine.

The monofilament not only kept the baskets from floating away but also helped to keep the styrofoam in each basket submerged in the water.

### Site Selection

For the stream experiment in Tenderfoot Creek (Gogebic Co., Michigan), the floating rack was placed toward the tail of a riffle area. The site was unshaded by vegetation and provided maximum exposure to sunlight. Water depths at the site varied between 0.3 to 0.5 m.

In Crampton Lake (Vilas Co., Wisconsin), the floating rack was placed in a calm embayment approximately 6 to 9 meters from shore with a water depth of 1.8 meters. The site also was unshaded by vegetation.

The floating rack in Tenderfoot Creek was anchored to its site at all four corners of the rack. Each corner was tied to a piece of twine that had been tied to a brick. In Crampton Lake, the rack was secured to its site at one corner with a long piece of twine and brick for its anchor.

### Algal Sampling Methods

On each sampling day, a styrofoam cooler was filled with icepacks and 16 empty plastic containers per site. Each container was labeled according to a particular basket at a site such as S1+ meaning "stream site, basket 1, with ultraviolet exposure." The cooler, containers, and a coring device for sampling were taken to each site. Additional items such as monofilament, scissors, and extra rubber bands were also brought along in the event repairs were needed.

At each site, debris and other matter inhibiting water flow was cleared from the baskets every two to three days. The plexiglas was also wiped down to limit bacterial growth on the surface. Core samples were then taken from each basket using a coring device (a plastic syringe with the tip cut off). Each core was then placed in the appropriate plastic container that corresponded to the basket label, and a small amount of water was added. The core and water were then sealed

in the plastic container and placed in the cooler to prevent the degradation of the chlorophyll. When all of the baskets had been cored for the particular sampling day, the cooler with samples was then brought back to the laboratory for chlorophyll *a* and phaeophytin analysis.

#### Chlorophyll *a* and Phaeophytin Analysis

Upon returning to the laboratory, samples were removed and cleared of invertebrates. Samples were then placed core-side down in the film canisters labeled to correspond with each particular sample. To each canister, I added 10 mL of 90% buffered acetone to dissolve the styrofoam and extract the chlorophyll. The samples were placed in the freezer for 20-24 hours. Before analysis, the samples were removed from the freezer and warmed to room temperature.

Chlorophyll *a* and phaeophytin were analyzed using spectrophotometry according to the method of Steinman and Lamberti (1996). A blank was prepared by adding 3 mL of buffered acetone to a cuvette, which was then put into the spectrophotometer, and zeroed at a wavelength of 750 nm. The blank was kept in the machine throughout the analysis. For each sample, a second cuvette was prepared by pipetting 3 mL of sample from the middle of the film canister into the cuvette. The sample cuvette was then placed in the spectrophotometer and its absorbance read at 750 nm. The spectrophotometer was then changed to 664 nm and zeroed. Another absorbance reading of the sample was taken at this wavelength and recorded.

For the phaeophytin analysis, 1 drop of 0.1 N HCL was added to the sample and the cuvette was flicked 2 or 3 times to mix. However, for the first 2 sampling days, 2 drops of 3 N HCL were used. The cuvette was left to stand for 2 to 3 minutes and then placed in the spectrophotometer at 750 nm. The machine was zeroed at 665 nm and an additional absorbance reading of the

sample was also taken at this wavelength.

The chlorophyll *a* phaeophytin concentrations were calculated using the following equations (Steinman and Lamberti, 1996):

$$\text{Chlorophyll } a \ (\mu\text{g}/\text{cm}^2) = 26.7(E_{664b} - E_{665a}) \times V_{\text{ext}}/\text{area of tile}(\text{cm}^2) \times L$$

$$\text{Phaeophytin } (\mu\text{g}/\text{cm}^2) = 26.7(1.7E_{665a} - E_{664b}) \times V_{\text{ext}}/\text{area of tile}(\text{cm}^2) \times L$$

Where:

**E664b** = [(Absorbance of sample at 664 nm -- Absorbance of blank at 664 nm) -- (Abs. of sample at 750 nm -- Abs. of blank at 750 nm)] before acidification

**E665a** = [(Abs. of sample at 665 nm -- Abs. of blank at 665 nm) -- (Abs. of sample at 750 nm -- Abs. of blank at 750 nm)] after acidification

**V<sub>ext</sub>** = Volume of 90% acetone used in extraction (mL)

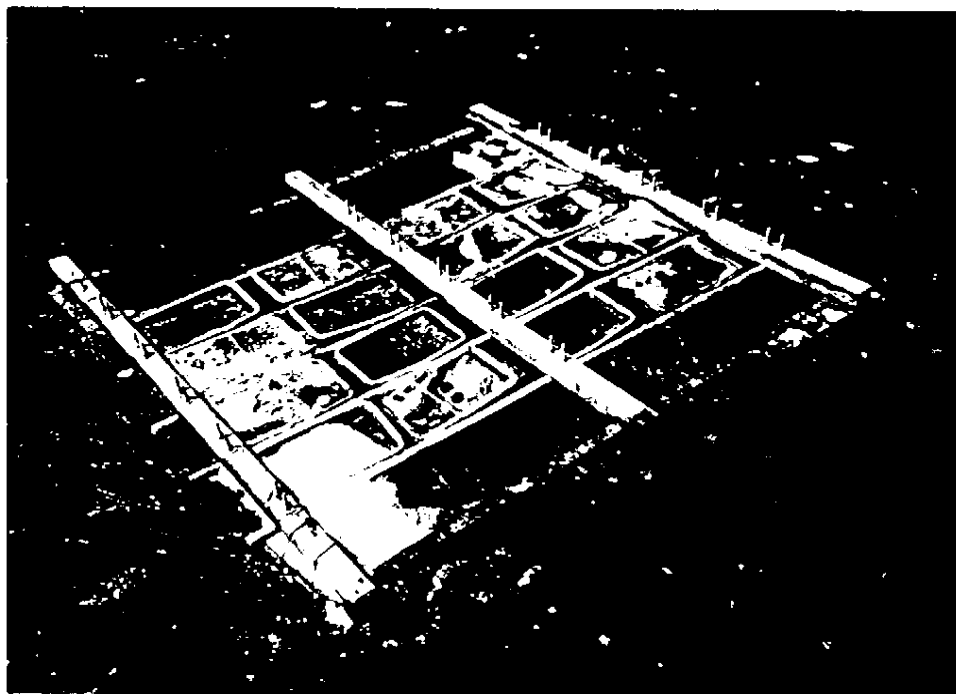
**L** = length of path light thru cuvette (cm)

**26.7** = absorbance correction

**1.7** = maximum ratio of E664b: E665a in absence of phaeopigments



1. Experimental floating rack in Crampton Lake as observed on Day 40.



2. Experimental floating rack in Tenderfoot Creek as observed on Day 41.

Chlorophyll a in Tenderfoot Creek

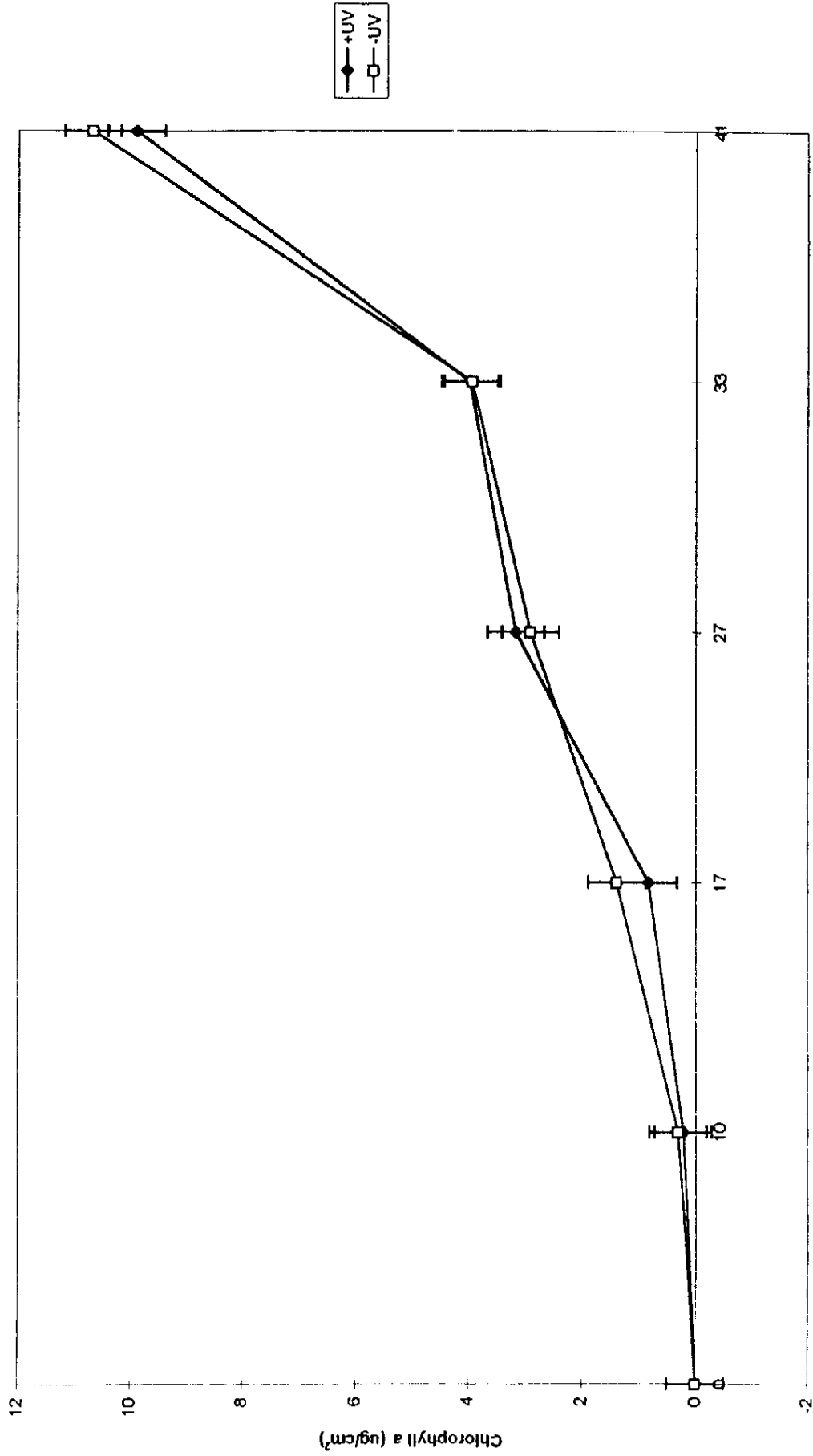


Figure 1. Figure 1 illustrates chlorophyll a for the +UV and -UV treatments in Tenderfoot Creek over a 41-day period.

\* Disregard error bars on Day 0.

Phaeophytin in Tenderfoot Creek

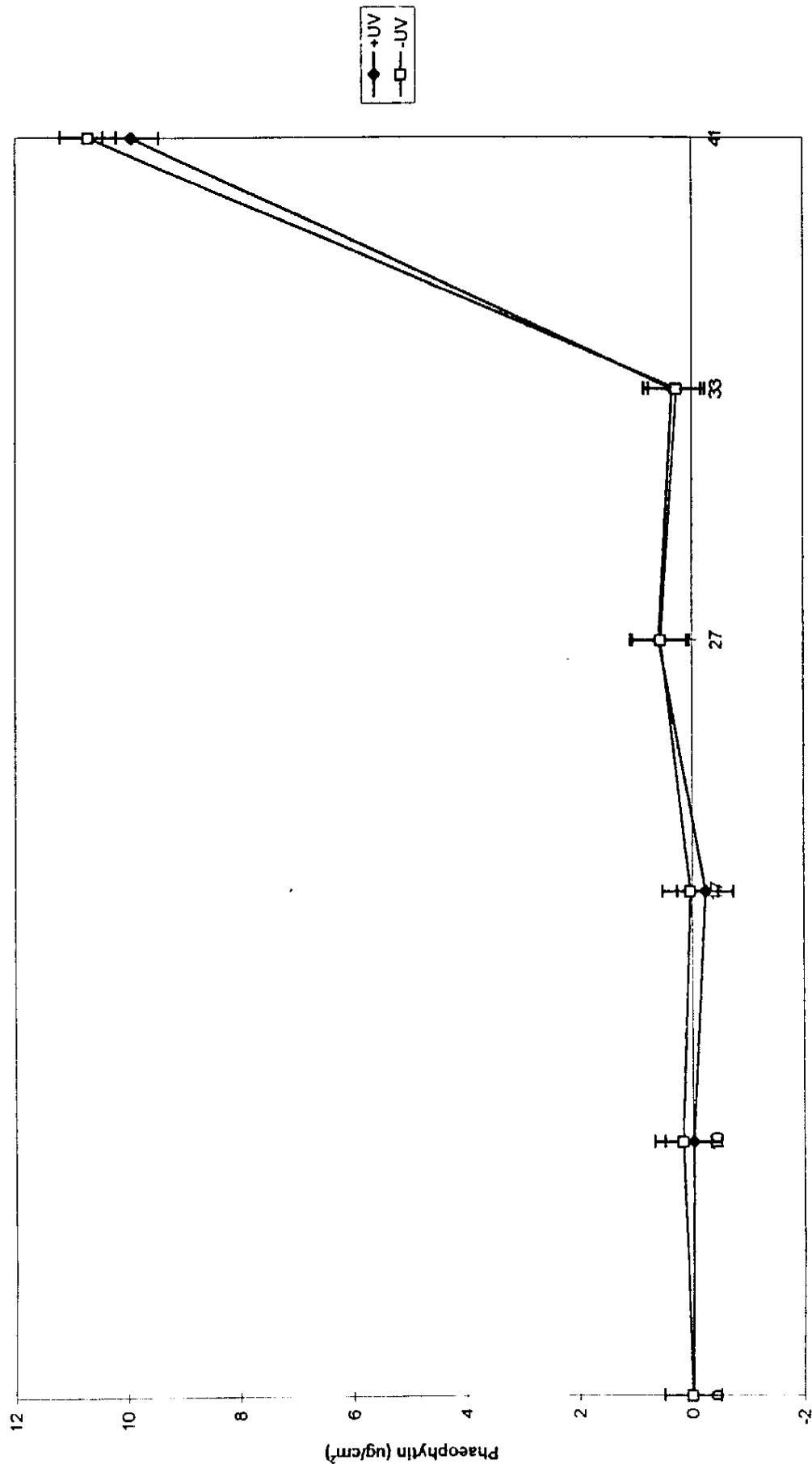


Figure 2. Figure 2 illustrates phaeophytin for the +UV and -UV treatments in Tenderfoot Creek over a 41-day period.

\*Disregard error bars on day 0.

**Table 1. Mean values (n = 7) of chlorophyll *a* and phaeophytin for algae in Tenderfoot Creek receiving UVR (+UV) at each of the 5 sampling dates.**

<b><u>Sampling Date</u></b>	<b><u>chlorophyll <i>a</i></u></b>	<b><u>Phaeophytin</u></b>
	<b><u>(mg/cm<sup>2</sup>)</u></b>	<b><u>(mg/cm<sup>2</sup>)</u></b>
<b>Day 10</b>	<b>0.2224</b>	<b>-.00101</b>
<b>Day 17</b>	<b>0.8338</b>	<b>-0.2333</b>
<b>Day 27</b>	<b>3.169</b>	<b>0.5852</b>
<b>Day 33</b>	<b>3.982</b>	<b>0.3412</b>
<b>Day 41</b>	<b>9.900</b>	<b>9.931</b>

**Table 2. Mean values (n = 7) of chlorophyll *a* and phaeophytin for algae in Tenderfoot Creek not exposed to UVR (-UV) at each of the 5 sampling dates.**

<b><u>Sampling Date</u></b>	<b><u>chlorophyll <i>a</i></u></b>	<b><u>Phaeophytin</u></b>
	<b><u>(mg/cm<sup>2</sup>)</u></b>	<b><u>(mg/cm<sup>2</sup>)</u></b>
<b>Day 10</b>	<b>0.3133</b>	<b>0.1748</b>
<b>Day 17</b>	<b>1.391</b>	<b>0.0318</b>
<b>Day 27</b>	<b>2.906</b>	<b>0.5483</b>
<b>Day 33</b>	<b>3.937</b>	<b>0.2658</b>
<b>Day 41</b>	<b>10.486</b>	<b>10.670</b>

## RESULTS

Tenderfoot Creek showed mean chlorophyll *a* ranging from 0.2224 mg/cm<sup>2</sup> to 9.900 mg/cm<sup>2</sup> in the +UV treatment (Table 1). In the -UV treatment, chlorophyll *a* ranged from 0.3133 mg/cm<sup>2</sup> on day 10 to 10.486 mg/cm<sup>2</sup> on day 41 (Table 2). Chlorophyll *a* showed similar accrual patterns in both treatments (Figure 1). Both treatments (-UV, +UV) demonstrated a gradual increase in chlorophyll *a* from day 10 to day 33 (Figure 1). Between days 33 and 41, both treatments showed a sharp increase in chlorophyll *a* (Figure 1). Over the course of the entire experiment, the -UV treatment showed a slightly higher mean chlorophyll *a* than the +UV treatment, although this difference was not statistically significant (Figure 1).

In Crampton Lake, chlorophyll *a* for the +UV treatment ranged from 0.0051 mg/cm<sup>2</sup> on day 10 to .05521 mg/cm<sup>2</sup> on day 40 (Table 3). Chlorophyll *a* for the -UV treatment ranged from 0.1011 mg/cm<sup>2</sup> on day 10 to 0.5711 mg/cm<sup>2</sup> on day 40 (Table 4). Both treatments showed similar algal growth patterns between days 10 and 26 with the -UV treatment having chlorophyll *a* about 0.1 mg/cm<sup>2</sup> higher than the +UV treatment. On sample day 32, the -UV treatment appeared to taper off while the +UV continued to increase (Figure 2). On sample day 40, both +UV and -UV treatments showed a substantial increase in chlorophyll *a* but little difference between the two treatments.

Phaeophytin +UV treatments in Tenderfoot Creek ranged from -0.0101 mg/cm<sup>2</sup> on day 10 to 9.931 on day 41 (Table 1). The -UV treatment showed a phaeophytin range of 0.1748 mg/cm<sup>2</sup> at day 10 to 10.670 mg/cm<sup>2</sup> at day 41. Phaeophytin for both treatments demonstrated a growth pattern similar to chlorophyll *a* (Figure 3). Phaeophytin showed little difference between treatments (Figure 3). Similar to chlorophyll *a*, phaeophytin

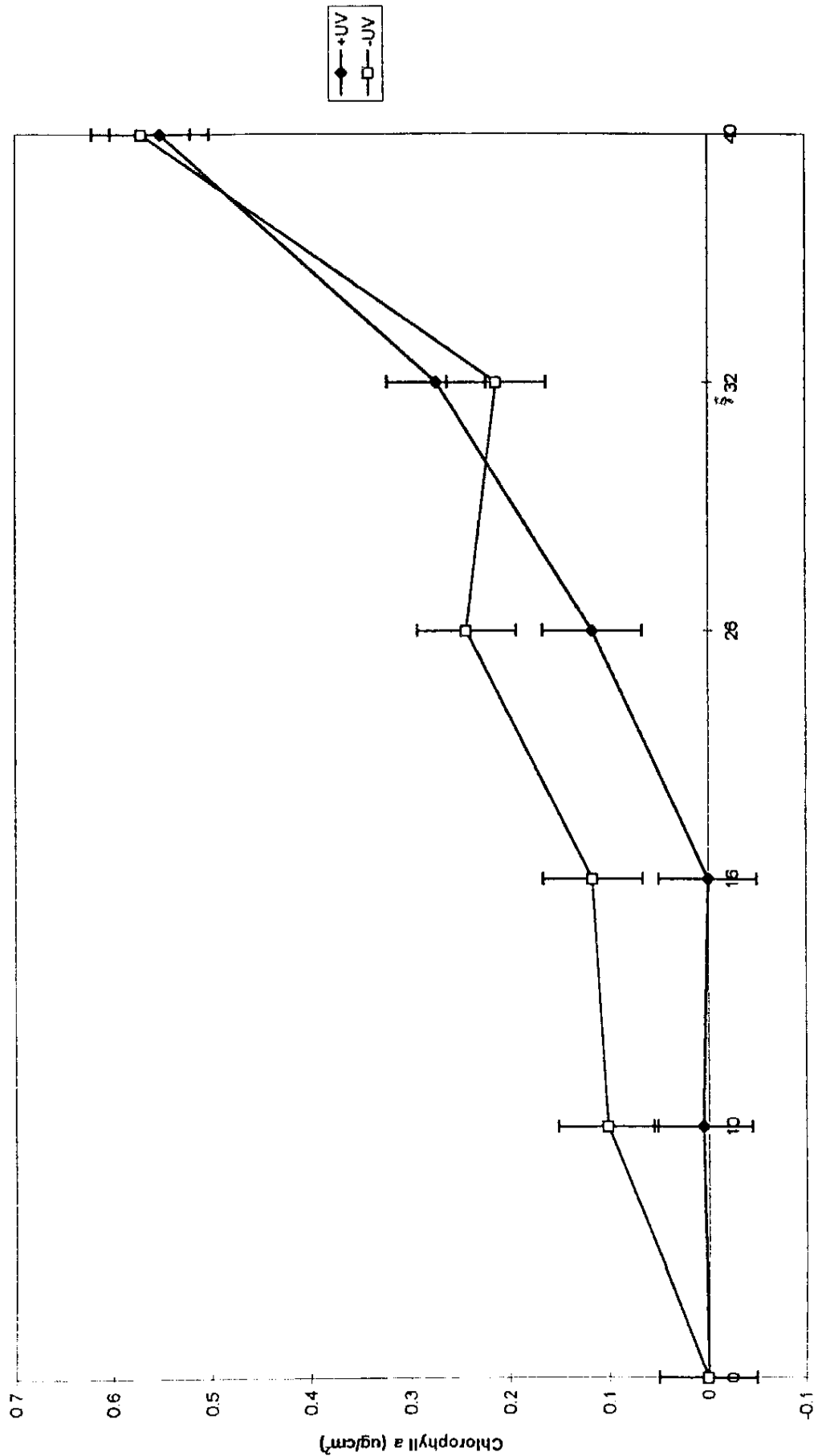
**Table 3. Mean values (n = 7) of chlorophyll *a* and phaeophytin for Crampton Lake algae receiving UVR (+UV) at each of the 5 sampling dates.**

<u>Sampling Date</u>	<u>Chlorophyll <i>a</i></u> <u>(mg/cm<sup>2</sup>)</u>	<u>Phaeophytin</u> <u>(mg/cm<sup>2</sup>)</u>
Day 10	0.0051	-0.0293
Day 16	0.00	0.1981
Day 26	0.1162	0.0556
Day 32	0.2729	-0.0936
Day 40	0.5521	0.6453

**Table 4. Mean values (n = 7) of chlorophyll *a* and phaeophytin for Crampton Lake algae not exposed to UVR (-UV) during each of the 5 sampling dates.**

<u>Sampling Date</u>	<u>chlorophyll <i>a</i></u> <u>(mg/cm<sup>2</sup>)</u>	<u>phaeophytin</u> <u>(mg/cm<sup>2</sup>)</u>
Day 10	0.1011	-0.01092
Day 16	0.1162	0.6894
Day 26	0.2426	-0.0702
Day 32	0.2123	0.0126
Day 40	0.5711	0.2663

Chlorophyll a in Crampton Lake



Time (days)

Figure 3. Figure 3 illustrates chlorophyll a for the +UV and -UV treatments in Crampton Lake over a 40-day period.

\*Disregard error bars on day 0.

Phaeophytin in Crampton Lake

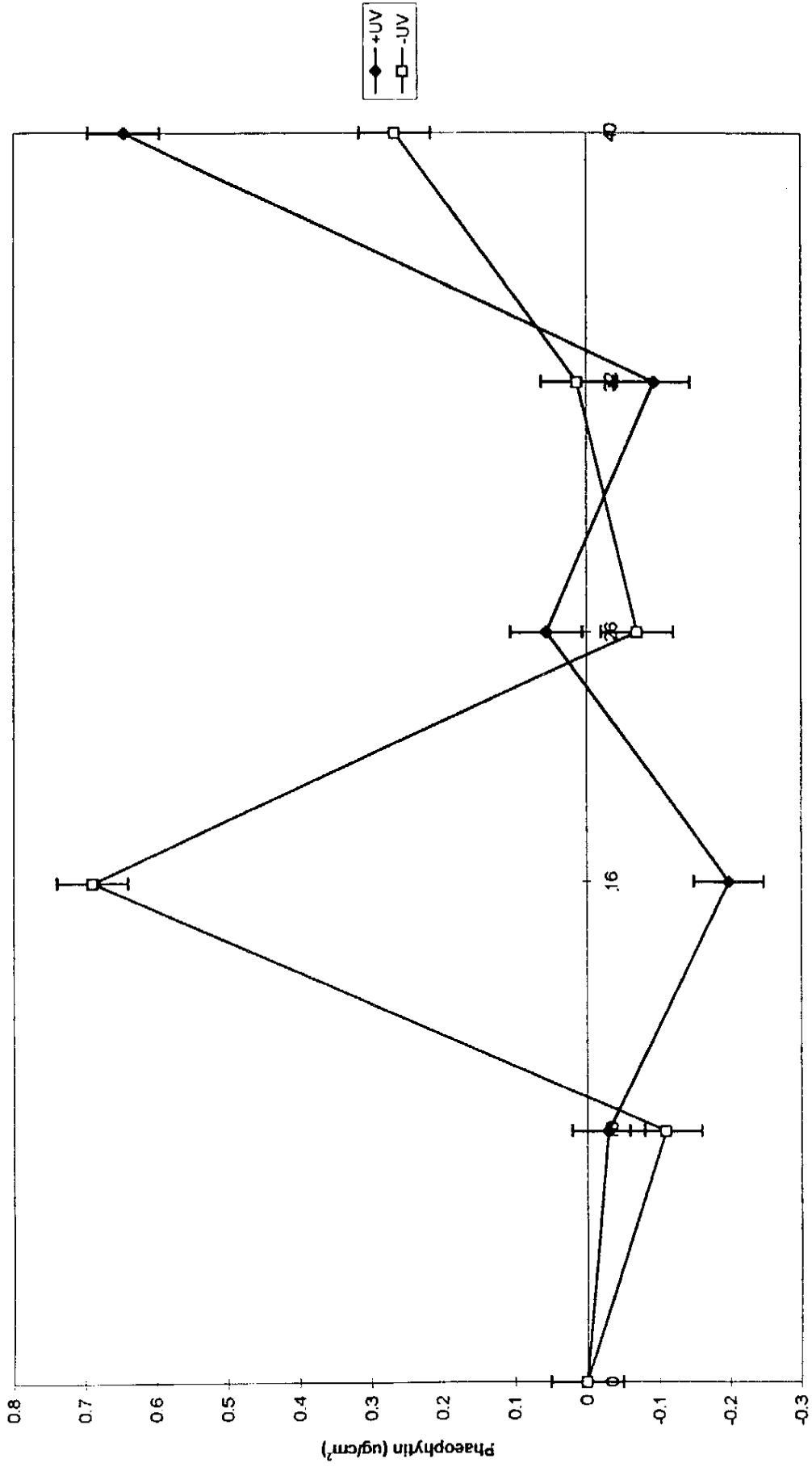


Figure 4. Figure 4 illustrates phaeophytin for the +UV and -UV treatments in Crampton Lake over a 40-day period.

\*Disregard error bars on Day 0.

showed slight increases until the last 8 days of the experiment, when phaeophytin of both treatments rose sharply (Figure 3).

Unlike Tenderfoot Creek phaeophytin, Crampton Lake phaeophytin for -UV treatments ranged from -0.1092 mg/cm<sup>2</sup> on day 10 to a high of 0.6894 on day 16 rather than on day 40 (Table 4). Phaeophytin for the +UV treatments ranged from -0.0293 mg/cm<sup>2</sup> on day 10 to 0.6453 mg/cm<sup>2</sup> on day 40 (Table 3). Phaeophytin of both treatments showed no particular growth pattern and no relationship to the chlorophyll *a* (Figures 3, 4).

## DISCUSSION

In the 6-week experiment conducted in Tenderfoot Creek, exposure of algae to UVR had no significant effect on chlorophyll *a* or phaeophytin ( $F_{1, 11} = 0.123$ ,  $p = 0.732$ ). A number of factors may have contributed to the experimental findings regarding chlorophyll *a*, the primary photosynthetic pigment of all oxygen-evolving photosynthetic organisms (Wetzel, 1983).

One factor that may have influenced the results of the experiment was the experimental design. Differences between treatment groups in addition to the dependent variable under study may have affected the chlorophyll *a* and phaeophytin level potential. Thus, the presence of plexiglas on only one of the treatment groups may have produced confounding effects of the treatment. Plexiglas may have changed the water flow over the periphyton, altered the quantity and quality of light other than UV, and protected algae from disturbance. In addition, the absence of UV under the plexiglas may have also changed the community structure of the algae within that specific treatment.

Particularly in the stream, current velocities caused foam and debris buildup on the baskets covered with the plexiglas. The debris coverage of the

plexiglas may have propagated bacterial growth on the glass itself as well as affecting the algae colonization in the baskets. Bacterial growth on the glass could have altered the amounts and intensities of the light rays entering the baskets, allowing for the colonization of a variety of algal types adapted to surviving in low light environments.

Although there was no significant effect of UVR on algae chlorophyll *a* levels, chlorophyll *a* for both the +UV and -UV treatments showed a significant increase between days 33 and 41. Weather patterns may have contributed to this increase since conditions showed extended periods of sunlight during the first two sampling periods. Over the next 17 to 20 days, the majority of the days were heavily clouded and rainy. Weather conditions improved between the final two sampling periods, and chlorophyll *a* levels rose dramatically, indicating a possible relationship between chlorophyll *a* and amounts of sunlight.

In the experiment in Crampton Lake, UVR proved to have a significant effect on chlorophyll *a* after sampling on day 26 ( $F_{1, 11} = 5.986, p = 0.033$ ). As duration of the +UV exposure time increased, the effects diminished to the point that no significant difference was observed between the chlorophyll *a* levels of each treatment after the final sample on day 40 ( $F_{1, 11} = 0.088, p = 0.773$ ). As with the stream, the plexiglas may have become a confounding variable and contributed to an alteration in the quality and quantity of light reaching the -UV treatments as well as protecting algae from disturbance.

Although the plexiglas shields were wiped periodically, green and brown films were frequently observed, indicating possible bacterial growth on the plexiglas. Similar to the stream experiment, this added film may have reduced light levels that would have been utilized to increase the chlorophyll *a*. Adding the plexiglas to both treatments might have eliminated some of the sources of experimental error.

damaging effects on algae but it apparently does not alter chlorophyll *a*. Ultraviolet light has been shown to decrease photosynthetic rates, but the decrease in photosynthetic rates is associated with photo-oxidative destruction of enzymes, and not chlorophyll (Steemann-Nielsen, 1962; Steemann-Nielsen and Jorgensen, 1962). Interactions of light with other factors such as temperature, water chemistry, and other environmental factors influence the algal communities.

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