ABSTRACT

Bacterial Growth on UV-B Photolytically Procuded Dissolved Organic Matter

Advisor: Owen T. Lind, Ph.D.

The effect of ultraviolet radiation into the Lake Chapala trophic processes was investigated in this study. Responses of bacterial populations to changes in UV radiation exposed water were significantly greater (bacterial biomass increased 57% and cell concentration increased 92%) compared to those populations that were grown in water covered by glass as UV blocker.

Measurements for penetration of ultraviolet radiation in the water column of Lake Chapala were made at midday and to a depth of 0.45 m in one of the clearest parts of the lake (Station 11). Ten per cent of the UV radiation that reached the surface of the lake was still present at 0.2 m of depth. The extinction coefficient was 10.1 m^{-1}.

For a lake with low phytoplankton productivity, the supply of organic carbon via photolysis of refractory material may be an important supplement to bacteria in the water column.

Bacterial Growth on UV-B Photolytically Produced Dissolved Organic Matter

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Requirements for the Degree

of

Master of Science in Limnology

By

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Most importantly, I want to express my gratitude to Antonio and to my parents, Ramón and Conchita Velarde and my siblings Patricia, Alfonso and Melissa for their unconditional love, encouragement and faith in me.

CHAPTER 1

Introduction

What is the role of UV-B (280 to 320 nm) radiation in the trophic processes of Lake Chapala, México? Although long known is the negative impact on microbial life, is it possible that this source of electromagnetic energy may be beneficial as usable organic substrate to bacteria?

Concentrations of dissolved organic matter (DOM) in lakes are often an order of magnitude greater than concentrations of particulate organic matter (POM), and DOM plays an important role (as carbon sources for growth, as antibiotics, as growth substances and nonessential organic micronutrients, or as chelators of trace metallic ions) in the regulation of the productivity of phytoplankton, higher vascular aquatic plants and heterotrophic bacteria (Wetzel 1975, Allen 1976).

DOM, which can be of either allochthonous or autochthonous origin, is chemically diverse and ranges from simple compounds (glycol acid, glucose, glycogen) to recalcitrant and highly polymerized macromolecules formed through partial degradation of plant structural components commonly refereed to as humic substances (HS) as cellulose, hemicellulose and lignin. HS have high molecular weights (200 - 80,000 daltons) and consist of diverse benzenecarboxylic and phenolic acids with esterified n-fatty acids and adsorbed alkanes. Their ecological importance is as excellent metal complexation agents

that are resistant to further microbial degradation. They can also lower the light transmittance of the upper layer of the water (due to its concentration that commonly imparts a yellowish brown color to the water) and thus disturb primary productivity. If they are broken down, they can be used as a carbon, phosphorus, or nitrogen source for microorganisms (Sederholm et al. 1973, Ghosh and Schnitzer 1980, Steward and Wetzel 1981).

Aquatic HS can be altered by UV-B irradiation in several ways: photolysis, by means of chemical oxidation after light initiated processes affect the redox processes in aqueous systems, and by a high pulse dose of organic nutrients that enhance its degradation by heterotrophic bacteria (Geller 1986, Kieber et al. 1990, De Haan 1993). Once these substances have been broken down into low molecular weight substances, they can be used as an energy source by heterotrophic organisms to enhance their growth. Such enhancement will be modified by the exposure times of lake waters to UV radiation. Exposure time will be a function of water transparency, time of the day, incidence angle of UV radiation and water circulation rate. Transparency of seawater for UV-B radiation is greater than freshwater lakes because the low concentration of chlorophyll a and DOM (Smith and Baker 1979). Penetration in seawater extends as far as 20 meters, being reduced 14 per cent per meter depth (Jerlov 1950).

The incidence of solar UV-B radiation upon oceans and lakes has increased as a consequence of anthropogenic diminishing of the ozone layer in the stratosphere by atmospheric pollution. This may have a significant effect on primary producers (photoinhibition) and other organisms (detrimental effects on eggs and larvae of northern anchovies or amphibians among others) in natural waters (Smith and Baker 1979, Jokiel and York 1984, Sayed 1988, Blaustein 1994).

Little work has been done in the measurement of HS, their photodegradation rates or their association with the mechanisms of daily photosynthesis in turbid and humic

systems (De Haan 1993). This is an important topic in current limnology because photodegradation of humic substances can be a way for the turbid or humic system to compensate for the low primary production by making available DOM of low molecular weight for the stimulation of heterotrophic organisms.

It has been found that the rates of photodegradation of aquatic HS are of the same order of magnitude as the mean daily primary production of typical oligotrophic and humic lakes. So this photodegradation of HS, in terms of carbon in the first 6 cm of the water column, is equivalent to the C fixation by phytoplankton in the euphotic zone, usually > 1, m in humic and turbid lakes. The UV-B photodegradation of HS produces biologically usable substrates, so equally high amounts of carbon may be channeled to higher trophic levels by these two different light-driven processes. Also the photodegradation of HS can cause some changes in the turnover rate of organic C in humic and turbid lakes, and this has not been taken into account so far (Kieber et al. 1989, De Haan 1993).

The objectives of this research were to (1) demonstrate the role of UV-B on lake trophic processes through the stimulation of bacterial population growth enhanced by the breakdown of DOM of high molecular weight, and (2) determine the volume of water so affected in the water column in Lake Chapala, Mexico. If UV-B affects the breakdown of DOM, Lake Chapala would have an alternative light-driven process to channel simple DOM to higher trophic levels (bacterioplankton, zooplankton and fishes). This would be of great importance to the Chapala food chain study that has been carried out during the past 5 years, because there is a question that is not yet answered: What supports the large fishery in the lake? (Lind and Dávalos-Lind 1991). This work can also lead us to a better understanding of the role allochthonous DOM plays in aquatic ecosystems.

On the other hand, it has been established that UV-B has a bactericidal effect damaging bacterial DNA (it has been widely used as a means of sterilization) and also can

disrupt photosynthetic processes, though the algae can increase the tolerance of UV-B by synthesizing protective UV absorbing compounds and by repairing damaged DNA (Noorudeen and Kulandaivelu 1982, Bothwell et al. 1994). There is far more literature on negative effects of UV-B radiation, and only recently scientists have become interested in the possible positive effects that UV-B radiation can have in aquatic ecosystems.

CHAPTER 2

Literature Review

Increase in Ultraviolet Radiation

The increase in the solar UV radiation due to anthropogenic diminishing of the ozone layer in the stratosphere has been a general concern (Jerlov 1950, Smith and Baker 1979, Calkins and Thordardottir 1980). Spectral measurements of UV radiation made at Toronto, Canada, since 1989, indicate that the intensity of radiation at wavelengths near 300 nanometers (UV-B) has increased by 35% per year in winter and 7% per year in summer (Kerr and McElroy 1993). The increases vary according to season and zenith angle of the sun. This increase might have a significant effect upon primary producers (photoinhibition) and other organisms (bacteria, larvae and eggs of insects, amphibians) or substrates (HS and pesticides) in natural waters (Zepp and Cline 1977, Jokiel and York 1984, Scotto et al. 1988).

Measurements of UV Radiation

Only within the last several years have instruments suitable for underwater measurement of UV radiation become available, as the LiCor spectroradiometer (LI-1800UV). But several devices had been used, even though they are not for underwater use, like the UV spectroradiometer (Smith and Baker 1979, Kerr and McElroy 1993), or the Robertson - Berger meter (Berger 1976, Smith and Calkins 1976, Scotto et al. 1988).

There is also an inexpensive and versatile chemical actinometer, suitable for field as well as laboratory studies, that was developed in the early 1960's (Pitts et al. 1968). The actinometer is a thin film of methyl methacrylate in which the actinic material *o*-nitrobenzaldehyde (ONB), is dispersed. This material undergoes a photochemical rearrangement in response to UV radiation, forming *o*-nitrobenzoic acid. The difference in the amount of ONB in the film would be the cumulative UV radiation responsible for the conversion. The film is transparent to visible radiation, is easily calibrated with an Infra Red (IR) spectrophotometer and can be sized for use in small spaces (Pitts et al. 1968, Gupta et al. 1980, Fleischmann 1989).

Penetration of UV Radiation into Natural Waters

Besides the reflection and refraction effects, UV radiation depends on solar zenith angle, dissolved salts, dissolved organic matter and particulate matter, for its penetration into natural waters (Zaneveld 1975).

Among the studies done in oceanic waters, Smith and Baker (1979) made estimations of the maximum penetration of UV-B radiation into natural waters by determining the diffuse attenuation coefficient for radiation (k) for clear ocean waters; this k value ranged from $k = 0.31\ m^{-1}$ at 280 nm to $k = 0.08\ m^{-1}$ at 340 nm.

In Discovery Bay, North Coast of Jamaica, k for integrated wavelengths of UV in the 300 - 400 nm range was between 2.6 and $0.09\ m^{-1}$ with an average of $0.33\ m^{-1}$ (Fleischmann 1989).

Measurements of the penetration of solar UV-B radiation into lakes had been done (Calkins 1975). Although he did not calculate k, he found that at between 1 and 3.5 m depth the intensity of UV-B radiation is less than 2% of the surface intensity for several northern lakes (Superior, Huron, Michigan, Erie, Herrington and Douglas).

Effects of UV Radiation on Aquatic Ecosystems

Most of the work on UV effects in aquatic ecosystems has been done in the oceans (Smith and Baker 1979, Kieber et al. 1990) and regarding the damage that UV radiation causes to living organisms such as algae (Worrest et al. 1978, Jokiel and York 1984), or the entire marine food chain (Sayed 1988). There are also some studies regarding the damage that UV radiation causes on amphibians in the Lost Lake, Oregon (Blaustein 1994), or on insect larvae in river ecosystems (Bothwell et al. 1994). Very little work has been done in the chemical changes of DOM due to the UV-B radiation (De Haan 1993) although since earliest 1950's there has been an awareness of such phenomena (Jerlov 1950). In 1990, it was found that the wavelengths in the solar spectrum responsible for photoproduction of low molecular weight DOM in the sea are in the UV-B region from 280 to 320 nm (Kieber et al. 1990). In Lawrence Lake, Michigan, (Stewart and Wetzel 1981) demonstrated sunlight or high intensity UV radiation is sufficient to cause alteration in the chemical characteristics of dissolved high molecular weight humic substances. Also in a bog lake Francko and Heath (1982), found the release of orthophosphate from UV-sensitive complex P compounds as an important photodependent process involving the photoreduction of iron.

The changes in UV-B absorbance (due to photolysis) may reflect the degradability of "aquatic humus" as a photolysis phenomenon as demonstrated in a mesoeutrophic lake (Geller 1986). De Haan (1993), showed that photodegradation of humic substances was mainly caused by UV-B light between 302 and 320 nm, he also proposed a value of 22 mg C m^{-2} d^{-1} of humic substances that were degraded by UV-B radiation. This was 8% of the annual production of the Fen Lake.

This humic photolysis could be a positive effect of UV radiation upon aquatic ecosystems, although few studies have been done about it. DOM photolytically produced has several ecological implications in the carbon cycle particularly with

respect to planktonic food web dynamics in lakes and in the global carbon budget. DOM can be used by heterotrophic bacteria as an energy source for growth, as an antibiotic, as growth regulation substances, as nonessential organic micronutrients, or as chelators of trace metallic ions (Wetzel 1975, Allen 1976, Kieber et al. 1989, Francko and Heath 1982, Salonen and Tulonen 1990).

CAPTER 3

Materials and Methods

Sutudy Area

Lake Chapala is located 42 km south of Guadalajara, Jalisco, México. It is the largest natural lake in México and belongs to one of the most important drainage systems in the country: Río Lerma - Lago de Chapala - Río Santiago. The lake is the principal water source for the Guadalajara metropolitan area, and other uses of the lake are irrigation, tourism, recreation and fisheries (Limón et al. 1989). I selected this lake for the study as an example of a tropical lake and because it is one of the few turbid lakes studied in tropical areas (Lind et at. 1994).

Preparation of the Water Samples

I collected water samples from two stations described by Limón et al. (1989) Station 26 and Station 11 (Figure 1), during the summer of 1994. I selected the stations based on their suspended clay concentrations. Stations 26 and 11 have annual average Secchi depths of 0.2 m and 0.7 m, respectively (Lind et al. 1992). I took surface samples with a 2-meter integral sampler in order to get a representative sample of the water column from the photic zone.

I filtered the samples in the Chapala Ecology Station research laboratory through several filters (GF filters) to remove clay and finally through a 0.2 μm membrane filter to remove the bacteria.

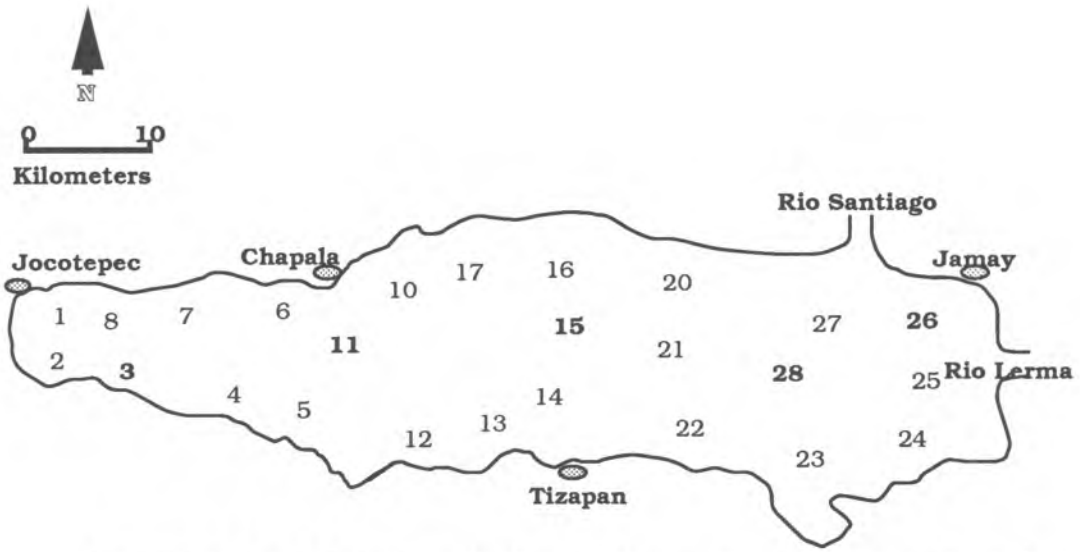

Figure 1. Map of Lake Chapala showing the smpling stations (Limón et al. 1989).

Samples Exposure to Natural UV Radiation

I exposed 500 ml samples of filtered water to UV radiation in 4 cm deep glass trays immersed in a bigger tray with flowing water to maintain constant temperature (20º C). I set the trays under direct sunlight, for 2, 4 and 6 hours for Station 11 water (July 25), and for 6 hours for Station 26 water during two consecutive days because of cloudiness (I exposed water from this Station for 2 hours on August 8, stored the sample in the refrigerator and exposed again for 4 hours on August 9). I made the exposures at midday, and I also measured the Photosynthetic Active Range (PAR) at 10 minute intervals with a photometer (LI-Cor LI-1000 Data Logger). I also set control trays with filtered water. To avoid UV radiation reaching the control trays water, I covered the controls with a piece of glass 0.75 cm thick, 15 cm away of the surface of the incubation tray to avoid drops of condensation.

To measure UV-B radiation received during the exposure period of the samples, I exposed *o*-nitrobenzaldehyde (ONB) films in triplicate to sunlight at the beginning and at

the end of the water sample's exposure period. To verify the efficiency of the glass as a UV-B block, I also exposed films in triplicate under the same glass cover with the control water samples.

Bacterial Inoculation and Counts

I measured the response to UV radiation by changes in bacterial population growth rates in inoculated UV exposed water.

I placed aliquots of 20 ml of the treated water (both exposed to UV and covered with glass) in 30 ml glass assay tubes and inoculated with natural lake water (80 parts of treated water with 20 parts of fresh lake water from the same station) to allow bacterial growth. There were three incubation times: 0 h, 12 h and 24 h, and five replicates of each incubation time. I did the incubation of the tubes in an ice chest filled with lake water at a constant temperature (20º C) and in the dark.

I preserved, at the end of each incubation time, 4 ml from each glass tube in a plastic vial with formalin (final concentration of 2%).

I filtered 1 ml for Station 11 and 0.5 ml of preserved material for Station 26 through a 0.2 μm membrane filters. I did bacterial counts and cell measurements with a Nikon fluorescence microscope at 1600x using the Acridine Orange technique (Hobbie et al. 1977).

Actinometer Film Preparation

For the preparation of an actinometer film in order to measure the UV-B radiation, I prepared a 10% solution of ONB by mixing 0.5620 g of ONB (Aldrich No. N1,080-2) with 5.51 g of the polymer methyl methacrylate (Aldrich No. 18,223-0) dissolving these in dichloromethane (Aldrich No. 27,056-3) and diluting the solution to 500 ml. I pippeted 9.5 ml of this solution onto a 9 cm diameter (63.62 cm^2) glass petri dish covered with mercury and allowed the dichloromethane to evaporate for 24 h. I lifted the

film that formed from the mercury surface, cut it with scissors into 3.5 x 4.5 cm pieces and mounted them in standard cardboard 35 mm photographic slide mounts.

I cleaned the mercury before use it as a surface in the glass petri dish by sequential washes in a separatory funnel: two times in 50% HNO_3, ten times in demineralized H_2O, five times in 100% acetone, and four times in dichloromethane (Fleischmann 1989).

I did all the work with ONB under a yellow safe light KODAK Safelight Filter OC Cat-1521699. Completed films and unused chemicals were stored in the dark to avoid exposure to UV radiation.

Calibration of the ONB Film

The calibration curve of the ONB Film is shown in Figure 2 where I plotted the absorbance (at 1533 cm^{-1} wavenumber) against mg of ONB/cm^2 in the film (Gupta et al. 1980).

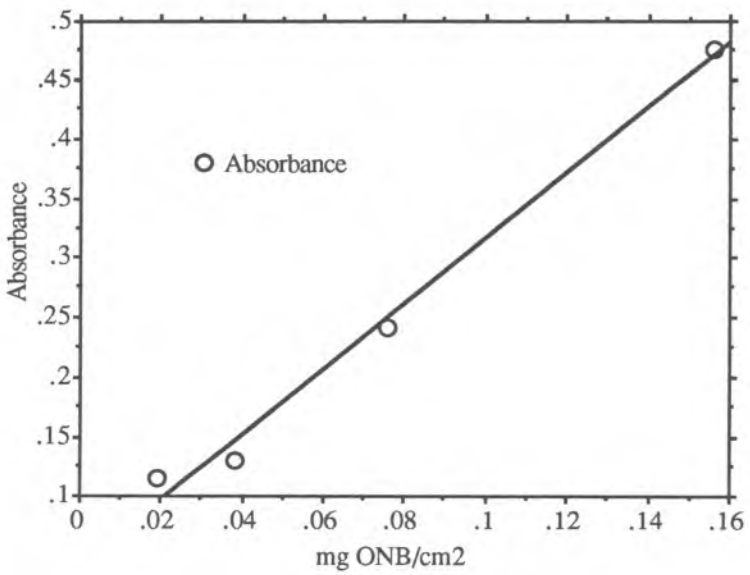

Figure 2. Calibration of the *o*-nitrobenzaldehyde (ONB) film, Absorbance at 1533 cm^{-1} wavenumber is plotted against mg of ONB cm^{2-1}. $y = 2.744x + 0.043$, $r^2 = 0.99$.

The absorbance of the film was measured in a IR Spectrophotometer FT-IR NICOLET -5PC. I calculated the photons absorbed per time of exposure as follows:

$$n = \frac{C \times 10^{-3}}{M} \times \text{minutes of exposure} \times N \times 2.38$$

Where:

n = Integrated number of photons per cm^2

C = Difference in ONB concentration in the film (mg/cm^2), before and after the exposure to UV radiation.

M = Molar weight of ONB (151)

N = Avogadro's number (6.02×10^{23})

2.38 = Correction factor for the efficiency of the actinometer.

I obtain the efficiency of the actinometer by exposing some films to a known UV source (Sun Lamp FS20T12/UVB) from Ultraviolet Resources International (Figure 3). The calculations that I made through the formula are 42% below the measured UV radiation reaching the film, so the correction factor is 2.38.

The ONB film actinometer has a reported efficiency of 50% over a range of UV radiation from 300 to 410 nm, (Pitts et al. 1968, Gupta et al. 1980, Fleischmann 1989). The 42% efficiency determined in this study may be due to the different type of lamps used for calibration, like a Mercury light source (PEK 100 watt; Pitts et al. 1968).

Penetration of UV Radiation

I measured the penetration of UV radiation in lake water in Station 11 (Secchi depth 0.4 m). I kept ONB films submerged in stoppered quartz tubes (2 per tube) while I exposed them to sunlight for 5 minutes at midday and at different depths. I measured UV radiation every 5 cm through the water column to a depth of 0.40 m.

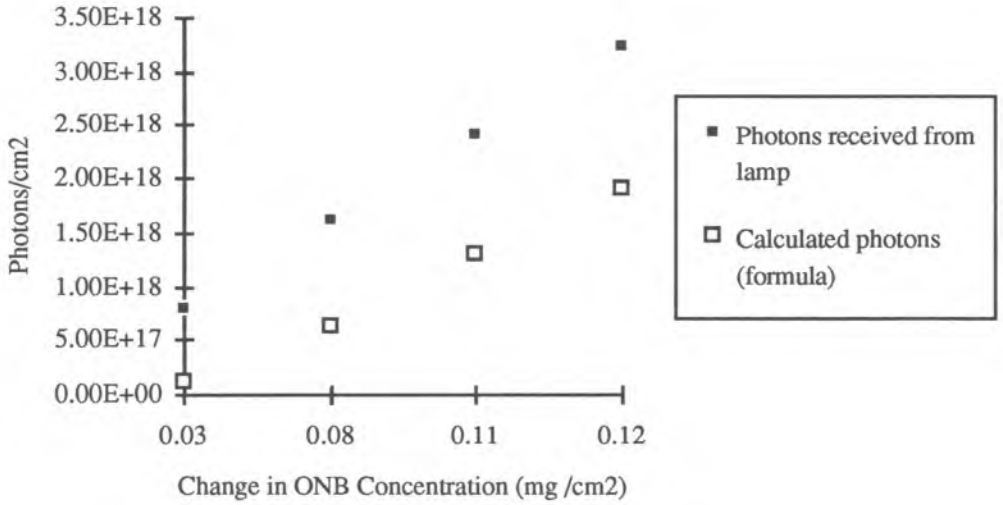

Figure 3. Difference between the photons calculated by the *o*-nitrobenzaldehyde (ONB) film exposure and the photons received from the sun lamp (FS20T12/UVB).

I calculated the extinction coefficient (n") according to the following:

$I_z = I_0 e^{-n''z}$ and, $n'' = \frac{\ln I_0 - \ln I_z}{z}$

Where:

I_z = UV radiation at depth z

I_0 = UV radiation at surface (Lind 1985).

Effectiveness of Glass

To determine the effectiveness of glass to block UV radiation, I exposed for two minutes 15 films: 5 without glass, 5 with one glass cover and 5 with 3 glass covers. I found that even though some (49%) of UV radiation can pass through the glass, there is a significant difference between the UV radiation that reached the films without glass cover

and the one that reached the films either with one or three glass covers, (ANOVA, P = 0.05; Table 1).

There was no significant difference between 1 and 3 glass covers. It has been determined that UV radiation is reduced by the glass, and the UV light that is transmitted has different percentages according to the wavelength: 73% of the 360 nm light is transmitted; 60% of the 350 nm light and 0% of the 300 nm light (Geller 1985). This may explain why there was some light passing through 1 glass that did not pass through 3 glass covers.

Table 1. Effectiveness of glass as a UV radiation block.

Treatment	Mean (Photons)	Standard deviation
3 glass	7.18E18	6.09E17
1 glass	9.11E18	3.71E18
No glass	1.65E19*	5.31E17

* Significantly different (P = 0.05)

CHAPTER 4

Results

Effects of UV Radiation on Bacterial Growth

Water that was previously exposed to UV radiation led to a more significant growth of bacteria populations than the water that was covered by glass blocking the UV radiation.

Biomass

The bacterial biomass increased at a greater rate in water exposed to UV radiation (Figures 4 5 and 6) at both Stations. After 4 h and 6 h of exposure(Station 11) and 6 h of exposure (Station 26), the difference between the biomass at 24 h of incubation was significantly different (ANOVA P = 0.05) from the control (glass covered). For the 2 h of exposure in Station 11 I did not found any significant difference in biomass between the UV exposed water and the water covered by glass, see Table 2.

Cell Concentration

I found a significant increase in the numbers of bacteria cells (ANOVA P = 0.05) between the water that had been exposed to UV radiation (4 h and 6 h in station 11 and 6 h in station 26) and the control (glass covered), but no significant difference for the

water from station 11 exposed to UV radiation for only 2 h (Figures 7, 8 and 9, and Table 3).

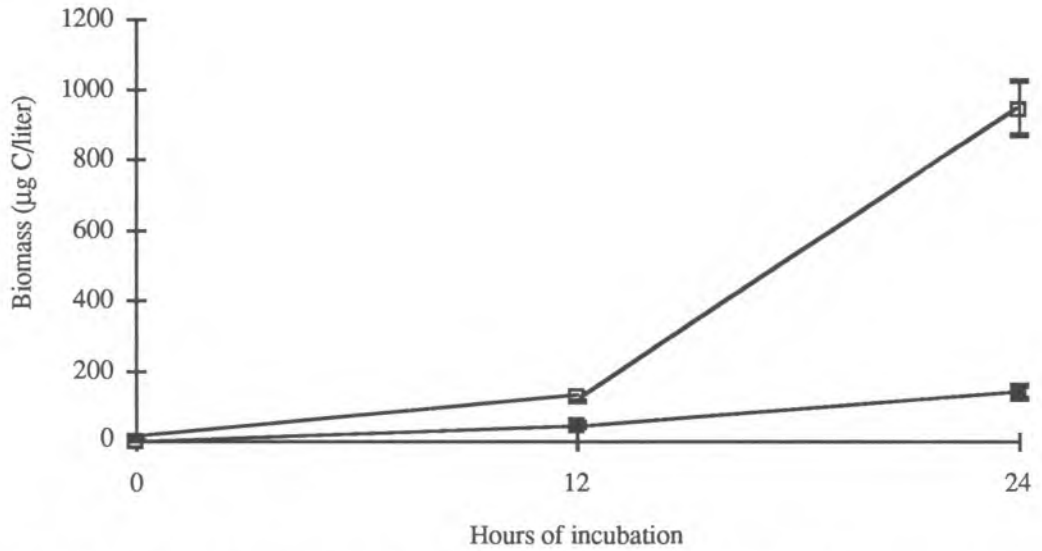

Figure 4. Bacterial Biomass Station 11. 6 h of UV exposure, UV exposed water (white squares) and Glass covered water (black squares).

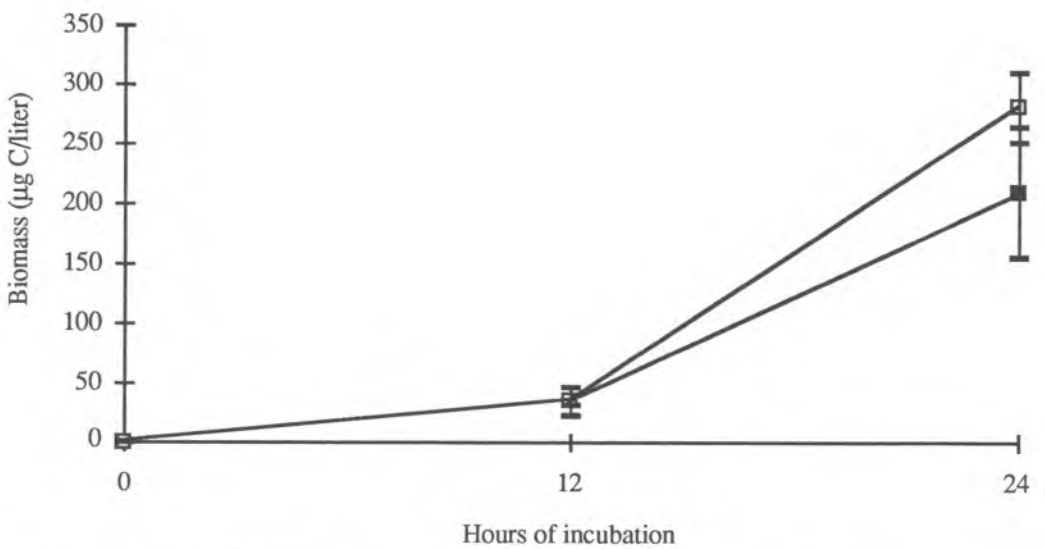

Figure 5. Bacterial Biomass Station 11. 4 h of UV exposure, UV exposed water (white squares) and Glass covered water (black squares).

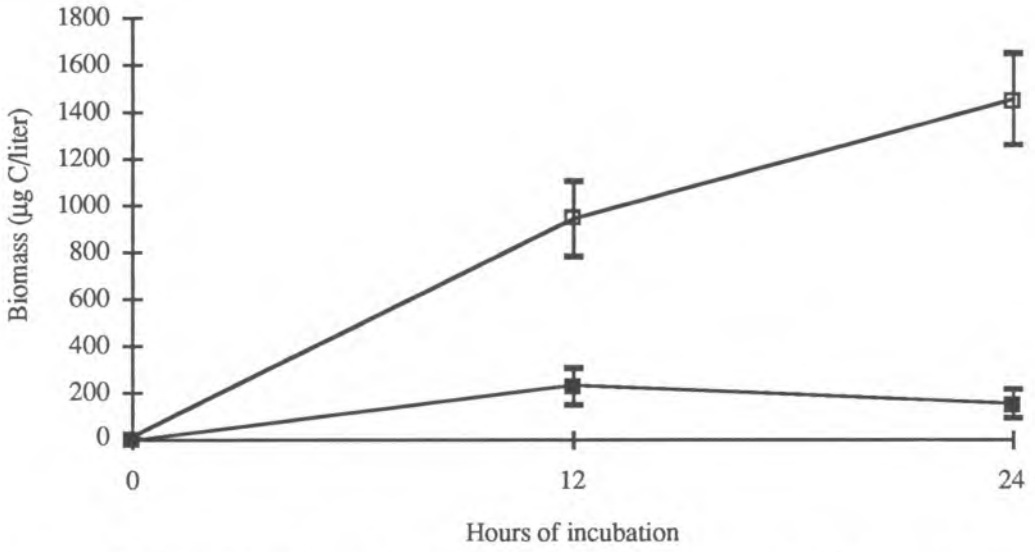

Figure 6. Bacterial Biomass Station 26. 6 h of UV exposure, UV exposed water (white squares) and Glass covered water (black squares).

Table 2. Bacterial biomass (ug C/liter) mean (standard deviation).

	UV Exposed		Glass Covered	
Incubation Time	12 h	24 h	12 h	24 h
Station 26(6 h)	937.7 (164.9)	1445.7 (193.1)	217.3 (73.5)	143.9 (59.3)
Station 11 (2 h)	35.8 (3.4)	87.2 (7.0)	34.1 (4.7)	83.9 (83.9)
Station 11 (4 h)	34.1 (3.8)	279.3 (29.2)	33.0 (11.1)	208.5 (53.8)
Station 11 (6 h)	122.1 (10.1)	941.3 (79.5)	42.6 (9.2)	134.3 (20.2)

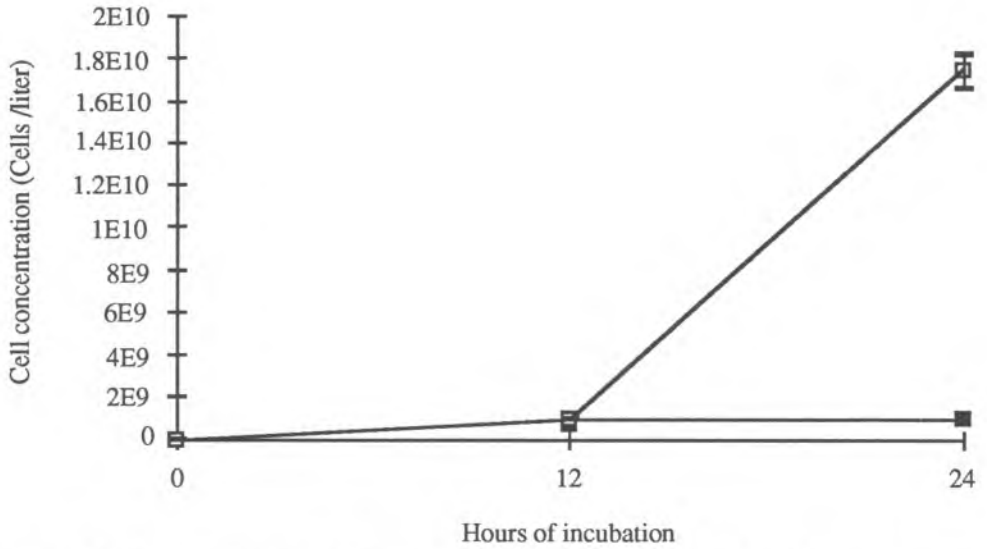

Figure 7. Cell concentration Station 11. 6 h of UV exposure, UV exposed water (white squares) and Glass covered water (black squares).

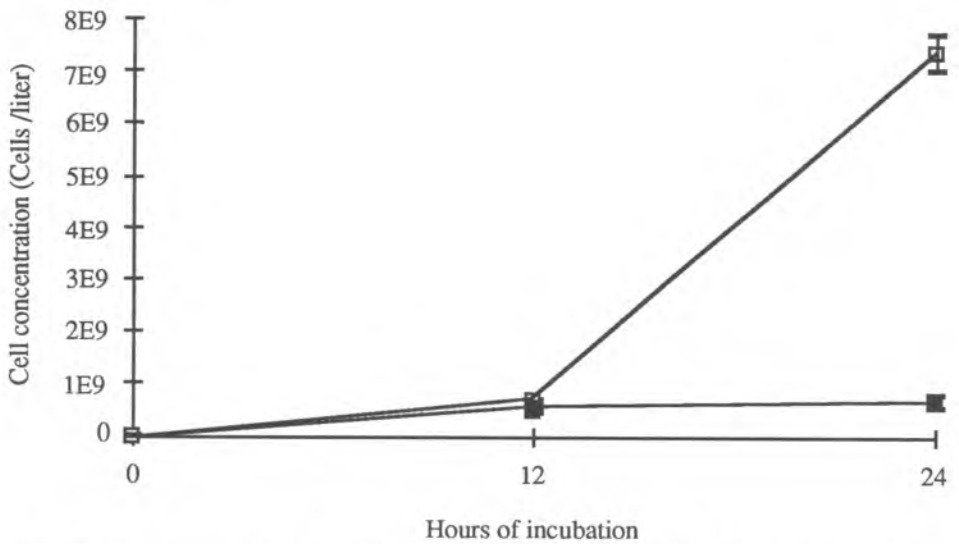

Figure 8. Cell concentration Station 11. 4 h of UV exposure, UV exposed water (white squares) and Glass covered water (black squares).

In the controls of Station 26, the cell concentration increased after 24 h of incubation (Figure 9) while the biomass decreased after the same period of 24 h (Figure 6). This reduction in mean cell size may indicate substrate exhaustion.

When the experiment for Station 26 was carried out, I conducted another control having a tray of water inside the lab (without solar radiation) and I found no significant difference in cell concentration numbers or biomass between the "glass" control and the "inside the lab" control (ANOVA P= 0.05) (Table 4).

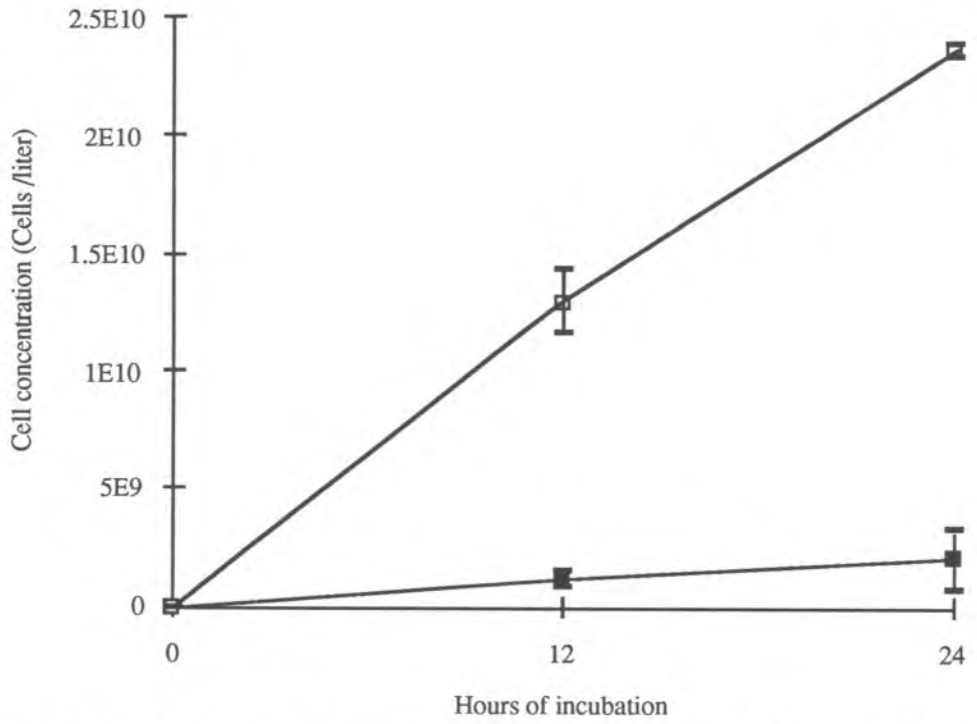

Figure 9. Cell Concentration Station 26. 6 h of UV exposure, UV exposed water (white squares) and Glass covered water (black squares).

Table 3. Cell concentration data (numbers of bacteria) mean (standard deviation)

	UV Exposed		Glass Covered	
Incubation Time	12 h	24 h	12 h	24 h
Station 26(6 h)	1.2E10 (1.4E9)	2.3E10 (3.0E8)	1.2E9 (3.1E8)	2.0E9 (1.3E9)
Station 11 (2 h)	3.4E8 (4.0E7)	5.5E8 (2.8E7)	3.4E8 (4.1E7)	5.1E8 (4.0E7)
Station 11 (4 h)	6.6E8 (2.1E7)	7.3E9 (3.3E8)	4.7E8 (5.0E7)	6.3E8 (1.2E8)
Station 11 (6 h)	9.8E8 (1.8E7)	1.7E10 (1.2E9)	6.7E8 (2.4E7)	9.5E8 (4.0E7)

Visible Light Measurements

Solar radiation during the exposure was generally continuous with little cloud cover. The exposure date for the samples at Station 11 was July 25, 1994 (Figure 10), and the dates for the ones at Station 26 were August 8 and 9, 1994 (Figures 11 and 12).

Quantity of UV and its Penetration

I measured the amount of UV radiation from 300 to 410 nm that reached the surface of the water, during the exposure of the water to natural UV radiation and was 8.60E18 photons per minute on July 25, 1.60E19 photons per square cm per minute on August 8 and 9.80E18 photons per minute on August 9.

Table 4. Bacterial biomass (ug C/liter) and Cell concentration (cell numbers) of the two controls "glass" and "inside the lab", Station 26 mean (standard deviation)

	Inside lab		Glass Covered	
	12 h	24 h	12 h	24 h
Biomass	165.7	204.3	217.3	143.9
	(19.9)	(19.5)	(73.5)	(59.3)
Cell Conc	1.3E9	1.1E9	1.2E9	2.0E9
	(3.2E8)	(1.7E8)	(3.1E8)	(1.3E9)

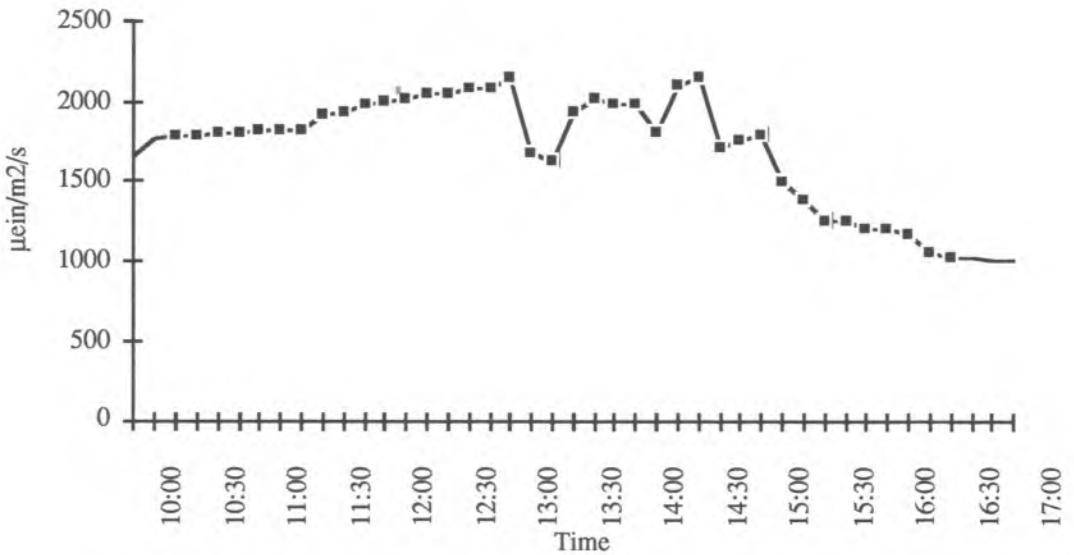

Figure 10. Solar irradiation on July 25, 1994. Black squares show the exposure period for samples at Station 11.

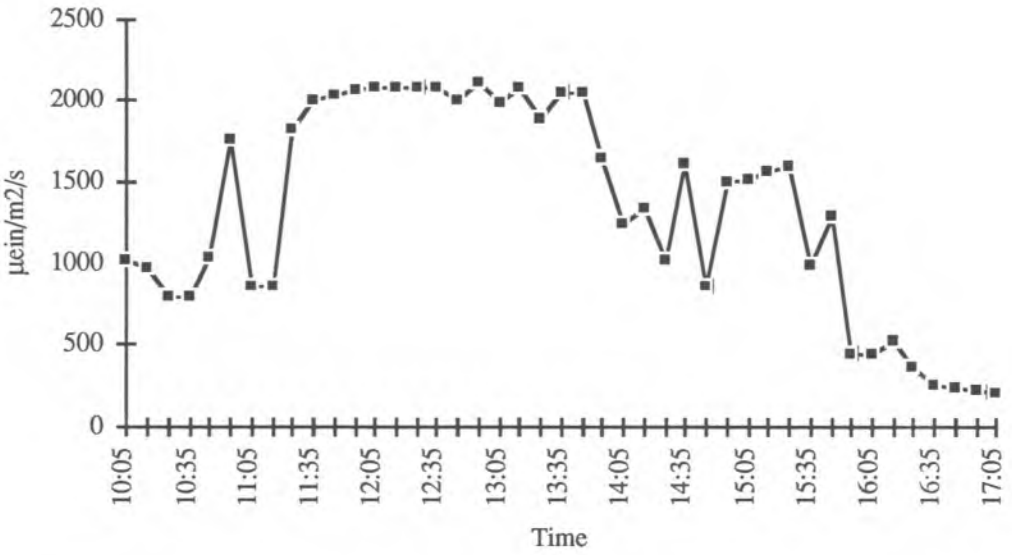

Figure 11. Solar irradiation on August 8, 1994. Black squares show the exposure period for samples at Station 26.

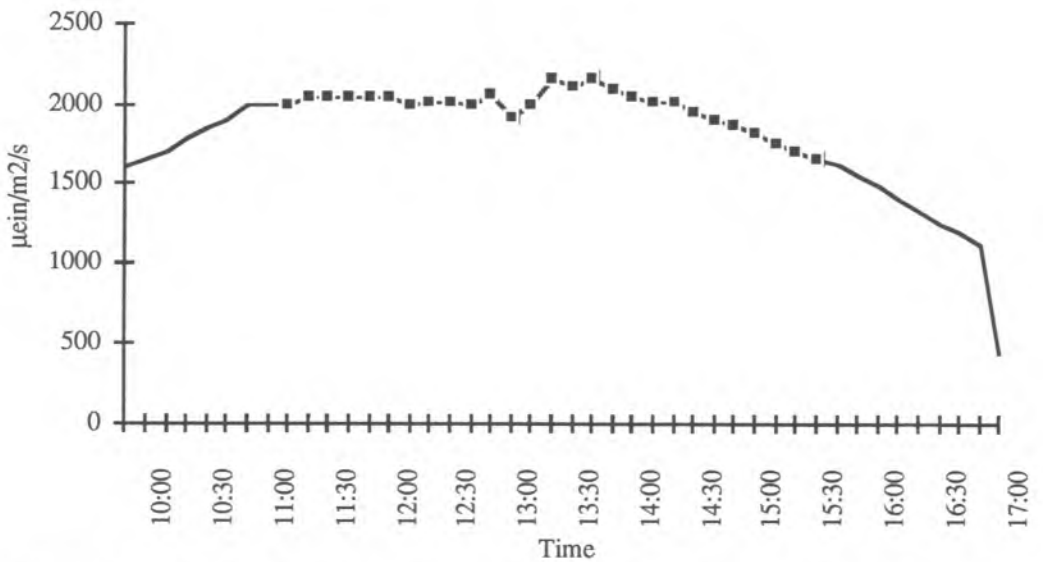

Figure 12. Solar irradiation on August 9, 1994. Black squares show the exposure period for samples at Station 26.

Extinction coefficient (n") for UV light in Station 11 ranged from 7.75 m^{-1} to 13.72 m^{-1} with an average of 10.1m^{-1}. Figure 13 shows the penetration of UV radiation into the Lake, as measured with the ONB films in a sunny day.

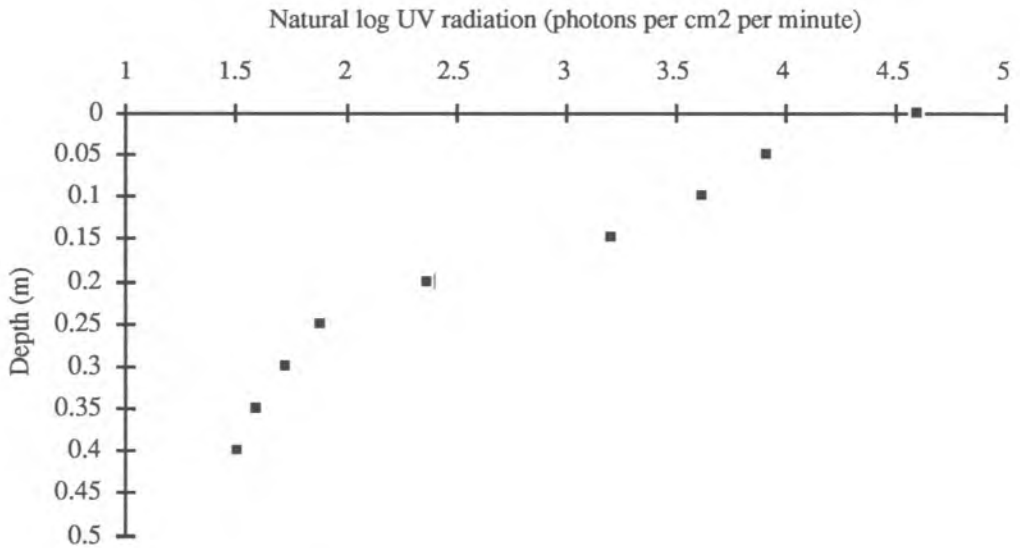

Figure 13. Penetration of UV radiation into the column of the Lake at Station 11.

CHAPTER 5

Discussion

In this study I found that bacterial growth (measured as biomass and cell concentration) was significantly stimulated in water previously exposed to natural UV radiation.

The same results were reported in Arctic coastal ponds at Barrow, Alaska (Strome and Miller 1978). They extracted humic substances from soil and decaying leaf litter, mixed it with the seawater and exposed it to sunlight or high intensity UV radiation (Hanova 450 watt). They inoculated bacteria from seawater and found that bacteria were capable of utilizing humic photolytic products for growth. In a study on coastal oligotrophic waters (Gulf of Maine and Sargasso Sea), seawater samples were incubated under sunlight and the findings were that photooxidation of biologically refractory DOM (specifically pyruvate precursors) is a significant source of DOM to bacteria, especially in oligotrophic environments that are carbon-limited in some instances (Kieber et al. 1989). The same results were found in Sjattesjon Lake in Sweden (Lindell et al. 1995). They exposed filtered and autoclaved humic lake water to simulated sunlight (UV-B, UV-A and PAR) for various periods or time. Then they inoculated the water with natural bacteria assemblages. They found that both bacterial numbers and cell volumes increased (numbers by 65% and volumes 360%) with increasing UV radiation. They did their study in the laboratory and with artificial

sunlight and found also that the absorbance (at 250 - 365 nm) of filtered lake water decreases as a function of exposure to UV radiation. This indicates the increase in availability of DOM of low molecular weight to bacteria on lake water (Lindell et al. 1995).

The increase in the UV radiation (35% per year in winter and 7% per year in summer) reaching the surface of the earth in recent years (Kerr and McElroy 1993) may have a significant effect in aquatic ecosystems, and on their food web pathways and functionality. Such an increase could affect the whole trophic structure in the system, even though the contribution of UV reaching the earth is just 5% of the total radiation of the sun (Fleischmann 1989).

The turbidity factor was excluded in this experiment because of the filtration of the water before exposure to UV radiation. To understand the role of water turbidity in this interesting pathway, I carried out a study of penetration of UV radiation into the lake.

The extinction coefficient for integrated wavelengths of UV light in the 300 to 410 nm range in Lake Chapala ranged between 7.75 m^{-1} and 13.72 m^{-1} with an average of 10.10 m^{-1}. This value is high compared to the extinction coefficients for different lakes. In a broad study of the penetration of UV radiation into various natural waters, (Zaneveld 1975) found an extinction coefficient of 1.39 m^{-1} for Lake Superior, 1.52 m^{-1} for Lake Michigan, 1.24 m^{-1} for Lake Huron, 2.98 m^{-1} for Lake Erie, 7.63 m^{-1} for Lake Herrington, Kentucky and 11.83 m^{-1} for Lake Douglas, Michigan. In a study to calculate the extinction coefficients for 10 river samples collected in southeastern U.S., values in the range of 4 to 12 m^{-1} were found (Zepp and Cline 1977).

I found that 50% of the UV radiation that reaches the surface of Lake Chapala is attenuated in the first 5 cm of the water column, at 40 cm 95.5% is attenuated. In the first 2.5 cm of Fen Lake 33% of the UV radiation was attenuated while 85% was

attenuated at 13 m, and at 25 m 10% of the surface UV radiation was still present (Fleischmann 1989).

The extinction coefficient for UV radiation has been measured more often in marine environments (Jerlov 1950, Smith and Calkins 1976, Hojerslev 1978, Worrest et al. 1978), and the values of this coefficient are much smaller for the seawater than the ones that had been reported for inland waters as lakes and rivers.

Generally extinction coefficients are higher in highly productive waters due to the particulate matter that is suspended in the water (Smith and Baker 1979). Particulate matter (soil and clay particles and decaying organic material) and dissolved "yellow substances" (humic substances) of undeterminated origin are responsible for the absorbance of UV-B radiation in waters (Calkins 1975, Lorenzen 1975). This explains the high extinction coefficient values for Lake Chapala, which is a very turbid lake (Limón et al. 1989).

The penetration of the UV radiation into the water determines the extent of the active region in which photochemical processes can be carried out in the water column with significance for the ecosystem (Jerlov 1950). In Lake Chapala, that active region would depend on the mixing rate and circulation patterns, due to the wind, because only the first 20 cm of the water column receive a significant amount of UV radiation.

As Dávalos-Lind and Lind (1993) suggest, there is an alternative (bacterial) carbon flow pathway in the trophic system of Lake Chapala. The increased availability of HS by UV-B may contribute to this unusual pathway in this turbid ecosystem. Because the percentage of HS photodegradation depends upon the turbidity of waters and the mixing patterns of the water body (Strome and Miller 1978, Geller 1986), the process becomes complex due to the great turbidity and mixing patterns of Chapala lake. This photodegradation pathway of refractory DOM may represent an important non-phytoplankton source of DOM available to bacteria in Lake Chapala.

Dissolved organic matter (DOM) in natural waters represents a large reservoir of carbon (Kieber et al. 1989). The main fraction of this DOM is generally believed to be composed of old, biologically refractory material such as HS for which the removal mechanisms remain largely unknown (Kieber et al. 1989, De Haan 1993). One potentially important removal process in oceans and lakes that has not been investigated extensively is the photodegradation of this HS in the photic zone to form biologically labile organic products (Kieber et al. 1989).

Presumably UV-B radiation altered or contributed to the break-down of large molecular weight organic substances and made available small molecular weight substances for bacterial growth. It has been shown that photodegradation of aquatic HS is mainly caused by UV-B radiation because it has energy which is sufficient to cleave C-H and C-C bonds (Allen 1976, Strome and Miller 1978, Stewart and Wetzel 1981, Geller 1985 and 1986, Kieber et al. 1990, De Haan 1993). Francko and Heath (1982) reported release of orthophosphate from UV-B sensitive complex organic phosphorus compounds in lake water, involving the photoreduction of iron, thus leading to an ecologically important photodependent process.

UV-B radiation is better known for the negative effects it induces water environments. These effects include destroying entire food webs by damaging the producers and damaging several genetic mechanisms in organisms (Smith and Baker 1979, Jokiel and York 1984, and Sayed 1988). There has been also reported the differential sensitivity to UV-B radiation among (amphibian) species and some mechanisms that they may have to protect themselves from the damages caused by the UV radiation such as producing photolyase enzyme (Blaustein et al. 1994). The positive effect of UV-B in creating available organic matter for the bacteria to consume and contributing to the food web is a phenomenon that has been under investigation recently.

It is relevant in this study especially because it has been predicted that increases in UV light would have a greater effect in temperate ecosystems (Smith and Baker 1979).

Conclusions

1. Exposure of water to UV radiation caused changes in the organic matter of the water that led to a significant growth of bacteria populations (measured as biomass and as cell numbers).
2. The minimum exposure time for Lake Chapala water to UV radiation to cause those changes that are reflected in bacterial growth is 4 hours.
3. Significant amounts of UV radiation penetrates into the water column of Lake Chapala up to a distance of 20 cm where 10% of the UV radiation that reaches the surface is still present.
4. The extinction coefficient for integrated wavelengths of UV radiation in the 300 to 410 nm range in Lake Chapala is high at 10.1 m^{-1}.

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