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Title: Physiological Responses of the Marine Phytoplankton Dunaliella tertiolecta to UV-B Enhanced Solar Radiation

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Robert C. Worrest

Recent concern over a possible decrease in the atmospheric ozone concentration due to increased chlorofluorocarbon release has resulted in several investigations into the possible biological effects from increased levels of ultraviolet-B radiation (UV-B, 290-320 nm). Studies have shown UV-B radiation to be harmful to a variety of organisms.

Cultures of Dunaliella tertiolecta Butcher were exposed to solar visible radiation and an artificial UV-B spectrum in a greenhouse located at the Oregon State University Marine Science Center in Newport, Oregon. The UV-B spectrum was shaped to simulate a natural UV-B spectrum using a sunlamp/filter system. Cell number, average cell volumes, fluorescence measurements, and ^{14}C-uptake were used as a measure of cell growth and photosynthetic activity. Results indicated an initial depression in growth rate, fluorescence, and ^{14}C-uptake under UV-B exposure followed, after 48 hours, by a rapid return of cell numbers and fluorescence to control levels. The ^{14}C-uptake remained depressed in UV-B enhanced cultures.

Within the time limits of this study (five weeks), D. tertiolecta appeared to be relatively resistant to the levels of UV-B radiation used (129 Eff_{DNA} $J \cdot m^{-2}$). Growth rates remained at control levels. The decrease in photosynthesis (^{14}C-uptake) apparently did not affect cell growth during the five-week period.

Physiological Responses of the Marine Phytoplankton *Dunaliella tertiolecta* to UV-B Enhanced Solar Radiation

by

James Douglas Scott

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Research Associate Professor of General Science
in charge of major

Redacted for privacy

Chairman of Department of General Science

Redacted for privacy

Dean of Graduate School

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Typed by Linda Chalker for James Douglas Scott

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PHYSIOLOGICAL RESPONSES OF THE MARINE PHYTOPLANKTON DUNALIELLA TERTIOLECTA TO UV-B ENHANCED SOLAR RADIATION

James D. Scott and Robert C. Worrest

Department of General Science

Oregon State University

Corvallis, Oregon 97331, USA

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I. SUMMARY

Recent concern over a possible decrease in the atmospheric ozone concentration due to increased chlorofluorocarbon release has resulted in several investigations into the possible biological effects from increased levels of ultraviolet-B radiation (UV-B, 290-320 nm). Studies have shown UV-B radiation to be harmful to a variety of organisms.

Cultures of *Dunaliella tertiolecta* Butcher were exposed to solar visible radiation and an artificial UV-B spectrum in a greenhouse located at the Oregon State University Marine Science Center in Newport, Oregon. The UV-B spectrum was shaped to simulate a natural UV-B spectrum using a sunlamp/filter system. Cell number, average cell volumes, fluorescence measurements, and ^{14}C-uptake ($NaH^{14}CO_3$) were used as a measure of cell growth and photosynthetic activity. Results indicated an initial depression in growth, fluorescence, and ^{14}C-uptake under UV-B exposure followed, after 48 hours, by a rapid return of cell numbers and fluorescence to control levels. The ^{14}C-uptake remained depressed in UV-B enhanced cultures.

Within the time limits of this study (five weeks), *D. tertiolecta* appeared to be relatively resistant to the levels of UV-B radiation used (22.1 $kJ \cdot m^{-2}$, 129 Eff_{DNA} $J \cdot m^{-2}$). Growth rates remained at control levels. The decrease in photosynthesis (^{14}C-uptake) apparently did not affect cell growth during the five-week period.

II. INTRODUCTION

Predictions of anthropogenic depletion of ozone in the stratosphere have led to concern over the possible effects of the resultant enhanced solar ultraviolet radiation levels (NAS 1979a, b, 1982). Dotto and Schiff (1978) and Caldwell (1979) discuss the role of atmospheric ozone and the history concerning the present controversy regarding potential enhancement of solar UV-B radiation. Current studies are attempting to characterize the impact of UV radiation on organisms and ecosystems. To date, these investigations indicate that exposure to a simulated solar UV spectrum can have detrimental effects on marine algae, bacteria, and zooplankton: all essential components of the marine food web (Worrest et al. 1978, 1980, 1981; Karanas et al. 1979, 1981; Damkaer et al. 1980, 1981; Thomson et al. 1980a, b). Marine phytoplankton are the primary producers within the marine ecosystem and, as such, any effects which occur at this level of the food chain resulting from increased UV-B radiation levels could affect the higher trophic levels.

Most studies concerning possible UV-radiation effects have been conducted under artificial laboratory conditions. Therefore, results obtained in the laboratory may not be totally valid when extrapolated to natural systems. Previous laboratory studies (Wolniakowski, 1980) have shown that Dunaliella tertiolecta Butcher, a marine phytoplankton species, exhibits definite adverse physiological responses to enhanced UV-B exposure. The present study was undertaken to determine if cultures of D. tertiolecta exposed to natural levels of visible solar radiation and a simulated solar UV-B spectrum would exhibit these same responses and, if so, to what degree.

III. MATERIALS AND METHODS

Unialgal stock cultures of Dunaliella tertiolecta Butcher were grown in 20-l plastic carboys under a bank of four F40 CW fluorescent lamps (General Electric) and four 40W Vita-Lites set on a 24-hour photoperiod (L24:D0). Sterilized natural seawater enriched with f/2 nutrients (Guillard and Ryther 1962) was used as the culture medium. Silicate was not added.

The experimental containers consisted of twelve 300-l fiberglass tanks enclosed within a greenhouse located at the Oregon State University Marine Science Center in Newport, Oregon (44°37'N, 124°03'W). The greenhouse was constructed of 0.32 cm thickness Sunadex glass (ASG Industries, Kingsport, Tennessee). This is a low-iron content, high UV-transmitting glass. The tanks were filled with natural seawater rendered pathogen-free by sand filtration followed by UV (254 nm) irradiation. The flow rate through each tank was adjusted to approximately 0.25 l/min. Equal volumes of the stock algal culture were added to each tank to give an initial cell concentration of 300-500 cells/ml. One hour after addition of the stock culture, samples were withdrawn for initial determination of cell number, mean cell volume, and fluorescence. All cell counts were performed using a Coulter Counter Model YMI equipped with a Coulter Counter Model M_2 Volume Converter. Fluorometric readings were performed using a Turner Designs Model 10 Fluorometer.

Supplemental UV-B radiation (290-320 nm) was provided for six of the tanks using a combination of fluorescent sunlamps (FS20 Westinghouse Electric) having a peak emission at 310 nm, and a filter of cellulose acetate (0.25 mm thick). The cellulose acetate was partially photo-

degraded before use and changed weekly during the study to compensate for photodegradation by the sunlamps. Enhanced UV radiation occurred during a 5.5-h exposure centered around solar noon. The daily fluence in the UV-B waveband at the surface of the UV-enhanced tanks was 22.1 $KJ \cdot m^{-2}$ (126 Eff_{DNA} $J \cdot m^{-2}$) (Table 1). Six tanks were subjected to the the same levels of photosynthetically active radiation (PAR) but received no supplemental UV-B radiation. The sunlamps over these tanks were covered with a filter of 0.18 mm Mylar "D", a UV-B opaque material. These cultures received a daily fluence of 3.1 $KJ \cdot m^{-2}$ (1.2 Eff_{DNA} $J \cdot m^{-2}$) and served as controls to assess the effects of the UV-B enhanced spectrum. Ultraviolet irradiance at the surface of the water was measured with an Optronic Laboratories Model 742 spectroradiometer whose response was characterized at the U.S. National Bureau of Standards. The study was conducted twice, once during June 1981, and again during July 1981, with each experiment lasting five weeks.

Periodically samples were taken from three depths in each tank (2.5, 38, and 74 cm below the water surface). Determination of cell counts, mean cell volume, and fluorescence were performed as described previously. Additionally, during the first two weeks of the study, radiocarbon uptake studies were performed to test for differences in the photosynthetic rates between the two treatment groups.

For determination of radiocarbon uptake, 60 ml of water were drawn from a depth of 38 cm and placed in a quartz bottle. Samples from this depth were also placed in dark bottles. Each bottle received 2.0 ml of a 1.0 μCi/ml $NaH^{14}CO_3$ solution (New England Nuclear) and was returned to its appropriate tank and suspended at depth (38 cm). They were allowed to incubate from 6 a.m. until 12 noon. At the end of this period,

aliquots were taken from each bottle and filtered onto 0.45 μm membrane filters (HAWP 02500, Millipore Corp.). They were allowed to desiccate for 12 hours over indicating silica gel, under vacuum, and were then fumed with HCl under vacuum. Scintillation fluor (Readi-Solv HP, Beckman Corp.) was added and the samples were counted using a Beckman Series 8000 Liquid Scintillation Spectrometer system. Quench correction was done by H-number determination. Due to relatively close counting efficiences for the samples, the data are reported in terms of cpm rather than converted to dpms.

IV. RESULTS AND DISCUSSION

Cultures exposed to enhanced UV-B radiation showed a significant ($p < 0.1$) decrease in cell counts when compared to cultures receiving no UV-B radiation at 2.5 and 38 cm below the water's surface through days 1 and 2 (Figure 1). There was no statistically significant decrease in cell numbers at the lower level (74 cm) in the UV enhanced tanks when compared to control tanks. This initial decrease in cell numbers was short-lived with no statistically significant difference in cell numbers between treatments past day 2. The same pattern in cell numbers was noted by Wolniakowski (1980). Vertical cell distribution in both the UV enhanced and control tanks showed similar arrangements. Before UV exposure (day 0), the cell concentration was uniform throughout the tanks. After day 1 (six hours of UV-B exposure), both treatments exhibited higher cell counts near the top of the tank. Cell counts decreased with depth. At day 2, cell counts were highest near the bottom of the tanks for both treatments with approximately equal numbers at the top and middle of the tanks.

After day 2, the cell counts were highest at the bottom, intermediate in the middle, and lowest at the top of the tanks for both treatments. This distribution remained unchanged for the remainder of the study, with differences in cell numbers among depths gradually decreasing. Since both the control and UV enhanced groups have the same type of vertical distribution in cell concentration, Dunaliella tertiolecta does not appear to exhibit any directed migration in response to exposure to UV-B radiation. Halldal (1958) found that the phototactic responses to three Dunaliella species exhibited maximum sensitivity at 493 nm (shoulder 435 nm), well out of the UV-B region.

Eppley et al. (1968) found the diurnal vertical migration of Cochanina niei to be independent of UV radiation. This species avoided the surface layer at midday when exposed to lights that produced neither excessive intensity nor UV radiation (Forward, 1976). These results suggest that initially the growth rate of Dunaliella tertiolecta is suppressed by exposure to UV-B radiation. Within approximately two days, the organism is able to compensate, either behaviorally or metabolically, to the UV induced stress and the growth rate of the population returns to control levels. Halldal (1967) and Qasim et al. (1972) proposed that the chloroplasts may orient themselves in response to different light regimes, possibly to avoid photooxidative damage to the photosynthetic enzymes. Wallen and Green (1971) proposed a physiological rather than a genotypic change in response to specific light changes.

The fluorescence/cell for Dunaliella exposed to UV radiation declined at all depths in the tank on Day 1; however, the decrease was not significant (Figure 2). Subsequently, the fluorescence/cell was greater for UV enhanced cultures at all depths than for non-exposed cultures, albeit not significantly. The difference in fluorescence/cell between the two treatments increased for approximately two weeks. The values remained relatively constant after this (Table 2). Variation within each treatment group prevented the characterization of any statistically significant changes in fluorescence/cell. The trend seen is nonetheless suggestive of a buildup of pigments (chlorophyll, carotenoids, etc.) in the cells exposed to enhanced UV-B radiation. Qasim et al. (1972) proposed that phytoplankton species have the capability to regulate pigment levels in response to changes in the

spectral qualities of incident radiation. The increase in pigment levels (fluorescence/cell) by D. tertiolecta, upon exposure to UV-B radiation, may be an adaptation resulting in the reduction of damage to the photosynthetic apparatus by synthesis of protective pigments. These pigments may absorb UV-B radiation before it interacts with the photosynthetic pathways. Alternatively, there may be an increase in production of chlorophyll and its accessory pigments to compensate for loss of any pigments from UV damage.

Cell volume showed no statistically significant changes between UV exposed and non-exposed groups (Table 3). No differences were noted between depths within the treatments. These data appear to indicate that D. tertiolecta does not respond to UV radiation by a reduction in cell volumes as postulated by Wolniakowski (1980). She reported a reduced ^{14}C-uptake in cells exposed to enhanced UV-B radiation and theorized that cell divisions were perhaps producing smaller cells which required a lower photosynthetic rate.

Radiocarbon uptake (cpm/cell) in this study was significantly depressed ($p < 0.001$) upon exposure to UV-B radiation (Figure 3). This depression in photosynthetic activity persisted through day 8, after which absorption of UV-B radiation by the upper layer of the tank probably prevented significant penetration of UV-B to the depth where ^{14}C-uptake studies were conducted. These results suggest that UV-B may act as an inhibitor of productivity. Lorenzen (1979) and Smith et al. (1980) found current natural levels of UV-B radiation reduce productivity in phytoplankton populations. There are several possible explanations for the observed decrease in ^{14}C-uptake. Chloroplasts may have repositioned themselves in arrangements which receive minimum

exposure to UV-B radiation, but resulting in a consequent decrease in photosynthesis due to a less than optimal exposure to photosynthetically active radiation (PAR). Alternatively, chlorophyll production may have decreased in response to increased production in protective pigments, or finally the reactive centers of Photosystem II may have been inactivated. These results, obtained under conditions approximating those likely to be found in the environment, indicate that Dunaliella tertiolecta experiences no drastic detrimental effects following exposure to UV-B radiation.

Species distribution within the water column probably influences the magnitude of any UV-induced effects. Most species do not occur solely at the water's surface, nor are they distributed evenly throughout the water column. Rather, they exist in some defined but changeable pattern within the water column with a maximum abundance at some depth below the water's surface. The vertical distribution of a species may change through vertical mixing of the water column, or through migratory changes in depth by the organisms. These migratory changes are usually in response to some environmental factor such as temperature, salinity, or light intensity. This last factor is usually associated with changes in the intensity of long-wave radiation (greater than 320 nm), but some species may respond to changes in the level of UV-B radiation. Therefore, any changes seen in an organism's responses or physiological processes may be due to direct interaction with UV-B radiation in the form of damage to the organism, or it may be indirect, such as the result of a migration to a less desirable location (perhaps in terms of lower levels of PAR) to avoid increased levels of UV-B radiation.

Most of the studies done to date have dealt with the effects of ultraviolet radiation upon organisms or assemblages under artificial laboratory conditions. Studies done in the laboratory usually restrict themselves to the study of one or a few variables at a time, keeping other factors constant. This is unlike the natural system where almost all of the various components are in a state of constant change. Therefore, it is difficult to extrapolate the results of studies conducted under laboratory conditions to conditions as they exist in the environment. Too many factors differ between the laboratory and "nature" to allow this comparison with any high degree of accuracy. For example, in the laboratory generally small volumes of water are subjected to irradiation. Therefore, there is little attenuation of UV-B by the water. This condition does not allow the organism under study to possibly "choose" among any spectrally different regions of the water and thus perhaps actively avoid the UV-B radiation. Also, while the natural UV-B spectrum can be reproduced in the laboratory with some degree of accuracy, there tends to be a higher proportion of radiation in the shorter wavelengths of the UV-B spectrum present than is found under natural conditions. These shorter wavelengths tend to be the more biologically active wavelengths. Additionally, it is generally not possible to achieve the levels of PAR in the laboratory which are found under natural conditions. Therefore, under laboratory conditions, photoreactivation, which requires long-wave ultraviolet or visible radiation, may not play as important a role in repairing UV-induced DNA damage as it might in a natural environment. For these and other reasons, it is desirable to conduct the experiments under as natural conditions as are possible.

While the results of this study and those of Wolniakowski (1980) cannot be directly compared due to differences in experimental conditions mentioned above, the similarity in results appears to indicate that, in this case, laboratory studies may provide an accurate indication of possible UV effects in the environment.

The decrease in photosynthetic rate noticed during this study does not appear to affect the growth rate of Dunaliella tertiolecta after the first two days. This may indicate that prior to UV exposure the photosynthetic rate of D. tertiolecta was higher than was necessary for optimal growth, with the excess carbohydrates being stored or perhaps released into the surrounding water. It is known that many types of phytoplankton produce carbohydrates in excess of their needs with part of the excess being released into the water. These released carbohydrates are a possible food source for other organisms such as bacteria. The UV-B induced decrease in photosynthesis noted in Dunaliella tertiolecta is apparently not severe enough to prevent the organism from meeting its growth and reproductive energy requirements. There may, however, be a reduction in the amount of carbohydrates released by Dunaliella. Thus, organisms which use these carbohydrates as a nutrient may be indirectly affected by enhanced UV exposure.

Models predicting the growth rate of phytoplankton exposed to UV-B radiation based upon ^{14}C-uptake rates (Worrest et al. 1981) may not give an accurate indication of expected changes in the abundance of species exposed to UV-B radiation. Long-term growth rates need to be incorporated into these models.

In summary, short-term exposure of D. tertiolecta to UV-B radiation did not appear to affect its ability to survive and reproduce. Some

type of physiological or morphological change may have occurred within the first 48 hours of UV exposure to allow Dunaliella to cope with the stress of UV-B radiation. These adjustments may include increased production of protective pigments or orientation of chloroplasts to minimize UV damage. No vertical migration to avoid high UV-B levels was noticed. The apparent UV resistance of Dunaliella teriolecta to levels used in this experiment may indicate that if environmental levels of UV-B radiation increase as predicted, Dunaliella may increase in abundance as more sensitive species are excluded. The implications of possible shifts in community structure are at present unknown and require further investigation.

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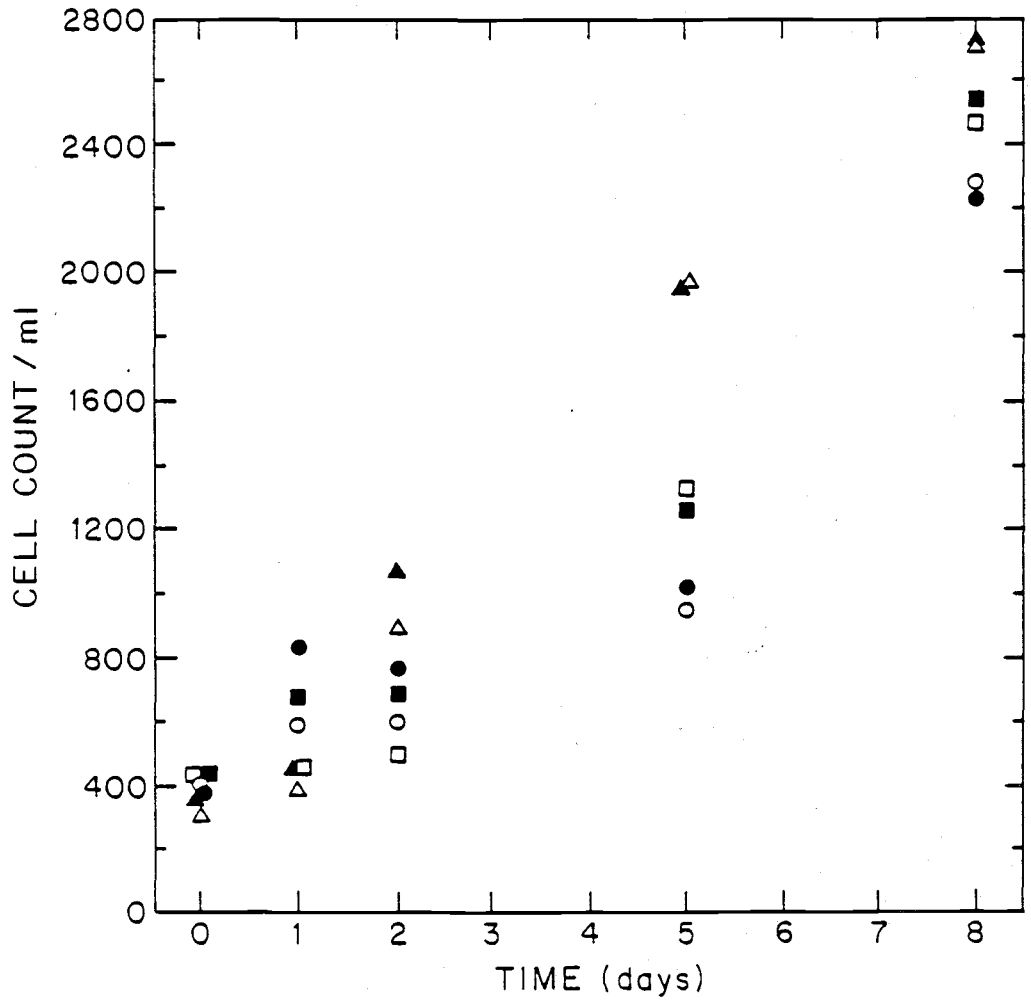

Figure 1. Density of cells within the UV-B enhanced and control microcosms at three depths for days 0 to 8. Filter and depths are as follows: Δ = cellulose acetate (CA), 74 cm; ▲ = Mylar, 74 cm; □ = CA, 38 cm; ■ = Mylar, 38 cm; ○ = CA, 2.5 cm; ● = Mylar, 2.5 cm.

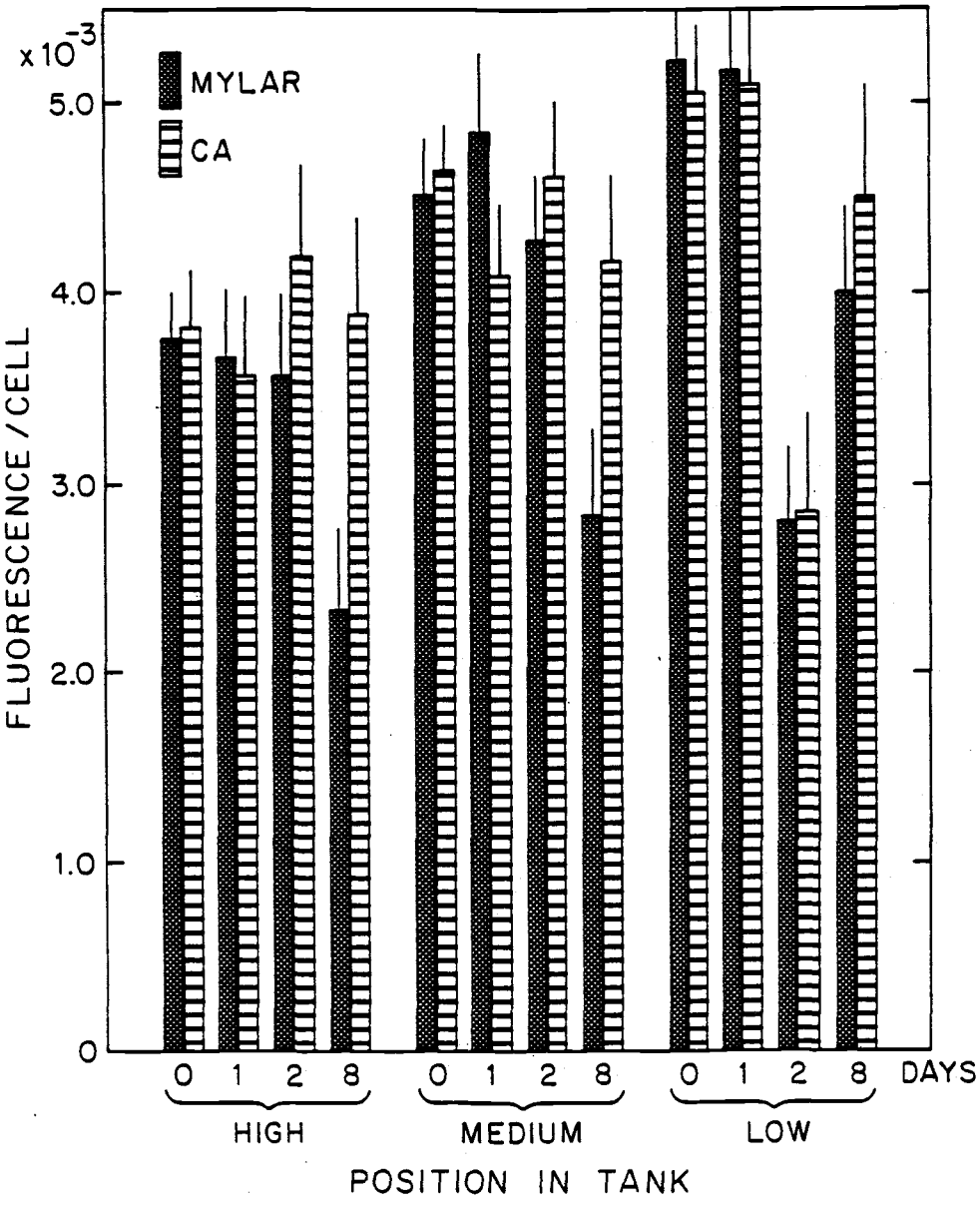

Figure 2. Fluorescence per cell in both UV-B enhanced and control microcosms at three depths for days 0 to 8. Positions in tank are High (2.5 cm), Medium (38 cm), and Low (74 cm).

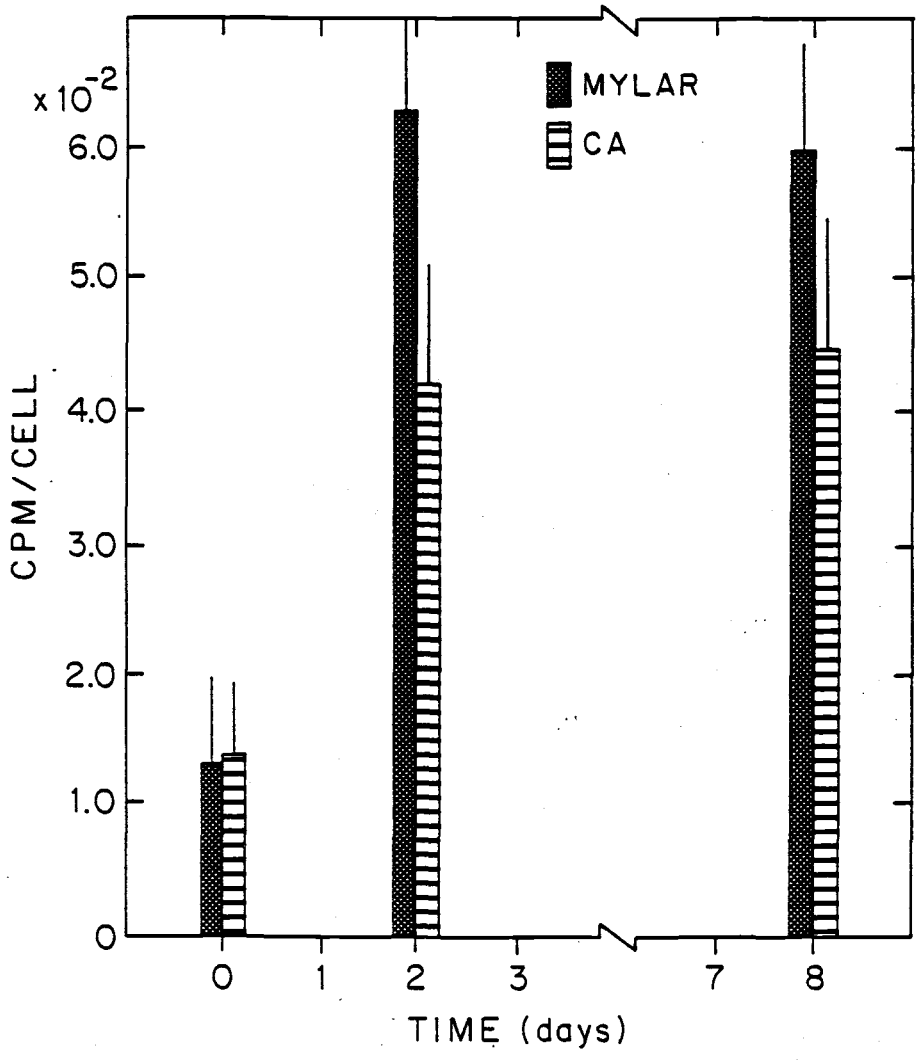

Figure 3. Radiocarbon uptake in cpm/cell for both UV-B enhanced and control cultures for days 0, 2, and 8. Uptake results were determined at mid-depth (15 cm) in each tank.

Table 1. Spectroradiometer readings for experimental area (290-320 nm).

Wavelength (nm)	Natural Light Through Greenhouse		0.18 mm Mylar "D"		0.25 mm Cellulose Acetate	
	Absolute Fluence ($W \cdot m^{-2}$)	Eff_{DNA} Fluence ($W \cdot m^{-2}$)	Absolute Fluence ($W \cdot m^{-2}$)	Eff_{DNA} Fluence ($W \cdot m^{-2}$)	Absolute Fluence ($W \cdot m^{-2}$)	Eff_{DNA} Fluence ($W \cdot m^{-2}$)
290	---	---	6.976×10^{-8}	1.806×10^{-8}	1.117×10^{-5}	2.891×10^{-6}
291	---	---	8.433×10^{-6}	1.895×10^{-8}	2.882×10^{-5}	6.477×10^{-6}
292	---	---	1.104×10^{-5}	1.952×10^{-6}	1.149×10^{-4}	2.212×10^{-5}
293	---	---	1.139×10^{-5}	1.858×10^{-6}	3.773×10^{-4}	6.141×10^{-5}
294	---	---	1.464×10^{-5}	1.985×10^{-6}	1.150×10^{-3}	1.560×10^{-5}
295	---	---	1.838×10^{-5}	2.045×10^{-6}	2.578×10^{-3}	2.869×10^{-4}
296	---	---	2.193×10^{-5}	1.973×10^{-6}	5.311×10^{-3}	4.777×10^{-4}
297	---	---	2.610×10^{-5}	1.867×10^{-6}	8.635×10^{-3}	6.175×10^{-4}
298	---	---	2.716×10^{-5}	1.520×10^{-6}	1.231×10^{-2}	6.892×10^{-4}
299	---	---	3.578×10^{-5}	1.542×10^{-6}	1.628×10^{-2}	7.011×10^{-4}
300	---	---	4.085×10^{-5}	1.333×10^{-6}	2.059×10^{-2}	6.717×10^{-4}
301	2.980×10^{-6}	7.244×10^{-8}	5.157×10^{-5}	1.254×10^{-6}	2.538×10^{-2}	6.171×10^{-4}
302	1.968×10^{-5}	3.512×10^{-7}	7.312×10^{-5}	1.304×10^{-6}	2.982×10^{-2}	5.320×10^{-4}
303	6.208×10^{-5}	8.908×10^{-7}	1.192×10^{-4}	1.538×10^{-6}	3.323×10^{-2}	4.287×10^{-4}
304	1.361×10^{-4}	1.253×10^{-6}	1.938×10^{-4}	1.784×10^{-6}	3.574×10^{-2}	3.289×10^{-4}
305	2.678×10^{-4}	1.737×10^{-6}	3.192×10^{-4}	2.070×10^{-6}	3.878×10^{-2}	2.495×10^{-4}
306	4.581×10^{-4}	2.071×10^{-6}	4.767×10^{-4}	2.155×10^{-6}	4.094×10^{-2}	1.851×10^{-4}
307	7.764×10^{-4}	2.425×10^{-6}	7.851×10^{-4}	2.452×10^{-6}	4.313×10^{-2}	1.347×10^{-4}
308	1.202×10^{-3}	2.577×10^{-6}	1.136×10^{-3}	2.436×10^{-6}	4.520×10^{-2}	9.687×10^{-5}
309	1.614×10^{-3}	2.365×10^{-6}	1.461×10^{-3}	2.139×10^{-6}	4.707×10^{-2}	6.892×10^{-5}
310	2.411×10^{-3}	2.406×10^{-6}	2.201×10^{-3}	2.196×10^{-6}	4.922×10^{-2}	4.911×10^{-5}
311	3.751×10^{-3}	2.550×10^{-6}	3.385×10^{-3}	2.302×10^{-6}	5.271×10^{-2}	3.854×10^{-5}
312	5.059×10^{-3}	2.350×10^{-6}	4.449×10^{-3}	2.067×10^{-6}	6.282×10^{-2}	2.918×10^{-5}
313	6.372×10^{-3}	2.031×10^{-6}	5.870×10^{-3}	1.871×10^{-6}	7.080×10^{-2}	2.257×10^{-5}
314	7.881×10^{-3}	1.736×10^{-6}	7.287×10^{-3}	1.605×10^{-6}	6.593×10^{-2}	1.452×10^{-5}
315	9.230×10^{-3}	1.417×10^{-6}	8.908×10^{-3}	1.368×10^{-6}	6.104×10^{-2}	9.371×10^{-6}
316	1.092×10^{-2}	1.182×10^{-6}	1.167×10^{-2}	1.263×10^{-6}	6.212×10^{-2}	6.721×10^{-6}
317	1.448×10^{-2}	1.118×10^{-6}	1.691×10^{-2}	1.305×10^{-6}	6.641×10^{-2}	5.125×10^{-6}
318	1.674×10^{-2}	9.337×10^{-7}	2.220×10^{-2}	1.239×10^{-6}	6.904×10^{-2}	3.852×10^{-6}
319	1.976×10^{-2}	8.902×10^{-7}	2.890×10^{-2}	1.833×10^{-6}	7.272×10^{-2}	2.977×10^{-6}
320	2.359×10^{-2}	7.197×10^{-7}	3.779×10^{-2}	1.533×10^{-6}	7.725×10^{-2}	2.358×10^{-6}
290-320 nm	1.242×10^{-1}	3.090×10^{-5}	1.544×10^{-1}	5.445×10^{-5}	1.116×10^{0}	6.376×10^{-3}

Table 2. Values (x 10^3) of fluorescence per cell for days 14, 21, 28, and 35 for UV-B enhanced and control microcosms.

Day	Position in Tank			Filter
	High	Middle	Low	
14	3.79 ± 0.46	3.99 ± 0.38	5.66 ± 0.51	Mylar
	3.96 ± 0.52	4.15 ± 0.41	6.77 ± 0.57	CA
21	3.81 ± 0.39	4.03 ± 0.44	6.11 ± 0.56	Mylar
	3.86 ± 0.45	4.19 ± 0.48	6.21 ± 0.61	CA
28	3.77 ± 0.55	4.16 ± 0.64	6.11 ± 0.72	Mylar
	3.88 ± 0.63	4.07 ± 0.58	6.28 ± 0.68	CA
35	3.81 ± 0.56	4.09 ± 0.69	6.07 ± 0.70	Mylar
	3.89 ± 0.58	4.02 ± 0.61	6.33 ± 0.75	CA

Mylar = Mylar "D"
CA = Cellulose acetate
High = 2.5 cm
Middle = 38 cm
Low = 74 cm

Table 3. Cell volume at three depths for cellulose acetate (CA) and Mylar "D" (Mylar) covered microcosms.

	Cell Volume (μm^3)			
	Position in Tank			
Day	High	Middle	Low	Filter
0	227 ± 35	236 ± 41	229 ± 46	Mylar
	224 ± 31	240 ± 39	231 ± 40	CA
1	230 ± 19	235 ± 32	228 ± 18	Mylar
	229 ± 23	240 ± 13	238 ± 14	CA
2	246 ± 10	257 ± 15	263 ± 15	Mylar
	246 ± 16	261 ± 9	259 ± 12	CA
3	259 ± 12	271 ± 22	259 ± 15	Mylar
	251 ± 16	279 ± 18	268 ± 21	CA
8	262 ± 14	265 ± 28	253 ± 23	Mylar
	255 ± 21	279 ± 25	271 ± 19	CA
14	288 ± 20	274 ± 32	266 ± 26	Mylar
	273 ± 24	281 ± 23	288 ± 28	CA
21	291 ± 31	274 ± 34	275 ± 35	Mylar
	269 ± 37	283 ± 29	288 ± 33	CA
28	284 ± 32	286 ± 38	291 ± 41	Mylar
	273 ± 34	292 ± 30	277 ± 37	CA
35	293 ± 38	287 ± 42	299 ± 36	Mylar
	290 ± 29	301 ± 44	274 ± 39	CA

High = 2.5 cm
Middle = 38 cm
Low = 74 cm

APPENDIX. LITERATURE REVIEW

Ozone (O^3) is the major mid-ultraviolet (UV-B, 290-320 nm) absorbing component in the earth's atmosphere. It is formed in the atmosphere by the photolysis of oxygen (O_2):

$$O_2 + h\nu_1 \longrightarrow 2\ O^*$$

$$O^* + O_2 + M \longrightarrow O_3 + M$$

and may itself be dissociated by UV absorption:

$$O_3 + h\nu_2 \longrightarrow O_2 + O^*$$

$$O^* + O_3 \longrightarrow 2\ O_2$$

where M is any background molecule and $h\nu_1$ is a photon of energy with wavelength between 40 and 280 nm (150 nm maximum) and $h\nu_2$ is a photon with wavelength less than 320 nm (260 nm maximum) (Chapman 1930; Smith 1977).

Diatomic oxygen is necessary for the production of ozone. Prior to the evolution of photosynthesis with its release of oxygen, shortwave UV radiation (UV-C, 40-290 nm) and midwave UV radiation (UV-B) would have penetrated to the earth's surface. This radiation may have supplied some of the energy necessary for the formation of the first complex organic molecules (Urey 1960). These levels of ultraviolet radiation would, however, degrade the complex molecules and the plants and animals which contain them (Giese 1968a; Caldwell 1972, 1979 Murphy, 1975; Nachtwey 1975).

In its formation and degradation, reactions involving ozone formation and degradation effectively shield the earth from radiation with wavelengths between 40 and 320 nm. Both nucleic acids and proteins

absorb in this region, with absorption maxima at 260 and 278 nm respectively (Giese 1968b). Because UV-B and UV-C irradiation was relatively intense, photosynthetic processes were probably restricted to aquatic environments where the ultraviolet radiation was attenuated. Successful organisms during this period would be expected to possess UV shielding, avoidance, and/or repair mechanisms to cope with any UV-induced damage (Caldwell 1972).

With the advent of photosynthesis, the oxygen released into the atmosphere could absorb radiation and produce ozone, which could then absorb radiation and produce oxygen again as described in the Chapman reactions previously given. This cycle, when balanced against naturally occurring ozone breakdown processes involving HO_x and NO_x compounds, gradually resulted in the production of a diffuse layer of stratospheric ozone with an average thickness of approximately 0.32 cm at standard temperature and pressure (Environmental Studies Board 1973). With the buildup of ozone and the subsequent decrease in the levels of UV-C and UV-B radiation, it is thought that life forms evolved to occupy regions which were previously uninhabitable.

Levels of ozone in the atmosphere would be expected to change if either the rate of production or destruction were altered. It is presently feared that anthropogenic interference with this equilibrium may be occurring and could eventually lead to a significant decrease in the stratospheric ozone concentration due to increased rates in ozone decomposition with no increase in production rates to maintain the equilibrium.

Attention was first drawn to this situation in the early 1970's when development of the supersonic transport (SST) gave rise to fears

that release of nitrogen oxides from the exhaust of the SST could reduce the ozone concentration (Johnston 1971; Crutzen 1972; McElroy et al. 1974). Later refinements in the SST and models for predicting their effects on the ozone layer showed that the impact would be minimal (NAS 1979a) and, in fact, its use could result in a slight increase in atmospheric ozone levels (Turco et al. 1978).

Other compounds which could possibly reduce stratospheric ozone levels include chlorofluorocarbons (Lovelock 1971; Molina and Rowland 1974; Stolarski and Cicerone 1974a), products resulting from the detonation of nuclear weapons (Koslow 1977) and industrially produced nitrogen fertilizers (Liu et al. 1977). Of these, the chlorofluorocarbons are felt to pose the greatest hazard for ozone destruction (NAS 1979a, b, 1982).

Large quantities of chlorofluorocarbons, especially CCl_2F_2 and CCl_3F (commonly called Freons) have been used as solvents, refrigerants, and propellents in aerosol cans (Howard and Hanchett 1975). They are chemically inert in the lower levels of the atmosphere and therefore were thought to pose no hazard to human health. Until recently, they were also thought to be chemically inert in the upper regions of the atmosphere, specifially the stratosphere.

In 1971, Lovelock first reported the presence of chlorofluorocarbons in the atmosphere. It was not until the work of Molina and Rowland (1974), however, that the hazards posed by the presence of these chlorofluorocarbons became apparent. They proposed that the photolysis of chlorofluorocarbons in the stratosphere provided a source of free chlorine atoms. The free chlorine atoms could then enter into a

catalytic ozone-destruction cycle first suggested by Stolarski and Cicerone (1974b):

$$Cl + O_3 \longrightarrow ClO + O_2$$

$$O_3 + h\nu \longrightarrow O_2 + O^*$$

$$ClO + O^* \longrightarrow Cl + O_2$$

$$2\ O_3 + h\nu \longrightarrow 3\ O_2 \text{ (net)}$$

Based on recent studies, the National Academy of Sciences (NAS 1982) has predicted that the release of chlorofluorocarbons into the atmosphere at 1977 levels will result in a gradual decrease of atmospheric ozone concentrations of 5% to 9%. Atmospheric models predict that continued releases of chlorofluorocarbons at 1981 levels will result in a 30-50% decrease in ozone at an altitude of 60 km (Rowland 1981). Rowland used a value of 10% for the predicted total ozone depletion.

Although the U.S. has banned the use of chlorofluorocarbons in many items and the world's total use of certain chlorofluorocarbons has therefore dropped to pre-1973 amounts, production of chlorofluorocarbons outside the U.S. has increased (Maugh 1979). In the absence of any further regulations regarding their uses, world levels of chlorofluorocarbons are expected to increase by as much as 57% in the next 20 years (Maugh 1979), possibly leading to a larger depletion in ozone concentration than the 5-9% already predicted.

If there is a gradual decrease in atmospheric ozone concentrations due to chlorofluorocarbons, it may be several years before a noticeable decrease is detected due to natural variations in the thickness of the ozone layer. Ozone concentration varies markedly with latitude, longi-

tude and season (Cutchis 1974; Johnson et al. 1976). Total ozone concentration for the Northern Hemisphere is at a maximum in the spring and a minimum in the fall. Ozone concentrations are lowest in the equatorial regions where the production of ozone is the greatest. Atmospheric currents tend to transport the ozone produced in the tropics toward the North and South Poles. There are smaller seasonal changes near the equator when compared to the polar regions. There are sizeable natural variations in the thickness of the ozone layer over both short-term periods (daily) and long-term periods (seasonal and yearly) for any given region. Therefore, it is difficult to predict cyclical patterns in ozone concentrations with any degree of accuracy at the present time (Cutchis 1974).

Currently it is thought that an annual decrease in the ozone concentration of 0.15% can be expected if chlorofluorocarbons are released at 1975 levels (Tiede et al. 1979), with larger rates if chlorofluorocarbon releases continue to rise. Tiede et al. (1979) predict that if all uses of chlorofluorocarbons were banned now, we would experience an eventual 2.7% decrease in ozone concentration, with a gradual reversal back to present levels.

The calibrated instruments needed for ozone measurement must be maintained for several years to detect decreases of 2-3% over the natural variation (Ramanathan et al. 1976). Accurate continuous measurements of ozone concentrations have not been conducted for a sufficient time to obtain reliable baseline values for any given locality at various times of the year.

When the possibility of a decrease in the ozone shield was first realized, many investigations were undertaken to determine the possible

biological effects of an increase in the level of UV-B radiation reaching the earth's surface. When compared to longer wavelength radiation, only a relatively small amount of radiation below 320 nm reaches the earth's surface. Due to the high absorption of radiation in these wavelengths by nucleic acids and proteins as previously mentioned, however, any increase in UV-B levels could be expected to have significant biological effects.

Absorption of UV-B radiation by deoxyribonucleic acid (DNA) may lead to the formation of pyrimidine dimers, local denaturation of the double-stranded helix, cross-linkage to proteins, or chain breakage (Smith 1977). The damage to the DNA, if great enough and not repaired, may result in a significant impairment of cellular functions or even cell death. There exist in most cells enzymatic processes to repair damage to DNA. The three repair processes currently known to exist are: (1) photoreactivation; (2) excision repair; and (3) post-replication repair (Smith 1977). The current concern about possible increases in UV-B levels is that these repair mechanisms may become "overloaded"; that is, there may be more damage than can be repaired, resulting in an accumulation of mutations and eventual cell death. As an example, the NAS (1979a) predicts an increase in both melanoma and non-melanoma type skin cancers in humans.

An increase in UV-B radiation reaching the earth could therefore be expected not only to have deleterious effects on individual organisms, but also upon entire ecosystems. Within ecosystems, the most important effects may occur among the primary producers and other members of the lower trophic levels. The primary producers, through photosynthesis, ultimately provide all of the energy needed by the other trophic levels.

A decrease in primary productivity would be expected to result in less energy (food) being available to the members of the succeeding trophic levels. The NAS (1979a) has predicted a decrease in the productivity of both agricultural and non-agricultural plants as a direct consequence of ozone depletion. Marine as well as terrestrial ecosystems are expected to be affected by the predicted increase in UV-B levels.

These predictions have resulted in a great deal of research into the impact of enhanced UV-B radiation on various organisms and assemblages, including marine and estuarine ecosystems. Worrest et al. (1980) reported that previous primary productivity studies in aquatic systems probably overestimated the levels of production because they failed to include the biologically active UV-B radiation due to its absorption by the glass of the BOD bottle. This problem had been previously recognized by Steemann Nielsen (1964) and Jitts et al. (1976).

Worrest et al. (1978) also found a significant decrease in biomass, chlorophyll _a_ concentration, and community diversity of attached algae following UV-B irradiation of estuarine microcosms. It was pointed out that the shift in community structure could be one important consequence of exposure to enhanced UV-B radiation. There could be a shift towards more UV-resistant organisms and/or increased biomass of "less desirable" organisms. This could also result in a decrease in the availability of food molecules to the succeeding trophic levels.

Moehring (1980) found a decrease in the heterotrophic activity of bacterioplankton assemblages upon exposure to enhanced UV-B radiation. Thomson et al. (1980a) reported that exposure of estuarine bacterial microcosms to UV-B radiation resulted in an overall decrease in

bacterial numbers, an increase in the proportion of pigmented cells, a decrease in the number of cellulolytic organisms and an increase in the heterotrophic respiration.

Karanas et al. (1979) found that exposure to enhanced levels of UV-B radiation resulted in decreased survival and reproductive capabilities in the marine planktonic copepod Acartia clausii. Damkaer et al. (1980) found that below a threshold of UV-B irradiation, there was no significant reduction in survival or developmental rates for shrimp larvae, crab larvae, and euphausids. Above the threshold level activity, development, and survival rapidly declined. The threshold level was near present incident levels of UV-B radiation. In contrast, Chalker (1981) found no decrease in survival or reproductive capacity in the marine harpacticoid copepod Tigriopus californicus, upon exposure to UV-B levels at 300% greater than natural solar UV-B levels.

Thomson et al. (1980b) found a significantly reduced growth rate in the estuarine diatom Melosira nummuloides upon exposure to enhanced UV-B radiation. Wolniakowski (1980) found that exposure of the marine phytoplankton species Dunaliella tertiolecta to enhanced UV-B levels resulted in an initial but short-lived depression of growth rate and a persistent decrease in radiocarbon uptake in groups exposed chronically. Worrest et al. (1981) found that in terms of photosynthetic ^{14}C-uptake rates there was a significantly different sensitivity to UV-B radiation among seven species of marine phytoplankton.

The above investigations demonstrate that there is a valid reason for concern that ozone depletion and the subsequent increase in the levels of UV-B-radiation penetrance will place a harmful stress upon organisms in the lower trophic levels of the marine food web. In

assessing the impact of elevated UV-B levels on marine systems, it is important to determine the depth to which the radiation will penetrate into the euphotic zone. The euphotic zone may be considered to be the area or depth in the water column where there is still a net positive photosynthetic rate. The degree of penetrance is dependent upon many factors; the amount of suspended sediments and the quantity of dissolved or suspended organic material are two examples. Different wavelengths in the visible and UV regions have different attenuation coefficients in seawater, and the attenuation coefficient is dependent upon the water type. Therefore the spectral characteristics of ultraviolet and visible radiation in seawater will change with varying depths and water types. Jerlov (1968) has constructed a classification for oceanic and coastal waters based upon physical characteristics. The transmission of specific wavelengths as a function of depth has been characterized for each water type. UV-B radiation of 310 nm has been found to penetrate approximately the upper 10% of the euphotic zone for coastal waters before it is reduced to 1% of its surface level (Jerlov 1976). This may be approximately 15 meters in waters with a low organic content or only approximately two meters for productive coastal or estuarine waters. Smith and Baker (1979) have shown that an increase in UV-B radiation at the water's surface gives rise to a corresponding proportional increase in UV-B throughout the water column.

Because the spectral characteristics of both visible and UV radiation change with depth, a knowledge of the biological effects being investigated and the distribution of the species within the water column are necessary in order to determine the magnitude of any effect caused by increased UV-B irradiation.

To determine the penetration of any biologically effective radiation requires that a weighted spectrum be used for the biological effect to be studied. Several of these have been published, e.g., (1) the Setlow (1974) DNA action spectrum for UV-B effects upon DNA; (2) the Jones and Kok (1966) action spectrum for chloroplast inhibition; and (3) the Caldwell (1971) generalized action spectrum for plants. These action spectra provide a method for weighting each wavelength of radiation according to its effectiveness in producing a specific response. The greater the efficiency of a wavelength in producing the desired response, the higher its weighted value. Therefore, less photons of a wavelength with a high weighted value are needed to produce a given level of the designated response than are photons of a wavelength with a lower weighted value.

There is little literature available concerning the effects of UV-B radiation under natural conditions. Most studies have been conducted in the laboratory. Previous laboratory studies have indicated that Dunaliella tertiolecta Butcher, a marine phytoplankton species, exhibits definite adverse physiological responses to enhanced UV-B exposure. The present study was undertaken to determine if D. tertiolecta exposed to natural levels of visible solar radiation and a simulated UV-B spectrum would exhibit these responses and, if so, to what degree.

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