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Reduction of stratospheric ozone caused by human perturbation with halocarbon compounds and the consequent increase in mid-wavelength ultraviolet radiation (UV-B radiation, 290-320 nm) reaching the earth's surface could have deleterious ecological effects on marine primary producers. Unialgal and mixed cultures of Thalassiosira pseudonana and Thalassiosira weisflogii were exposed to three levels of UV-B radiation (1.26 Wm^{-2}, 1.58 Wm^{-2}, and 0.02 Wm^{-2}) in three experiments, each of four day duration. The effects of these three levels of UV-B irradiance on cell numbers, doubling times, and relative biomass were analyzed. Two analyses were done: a two-treatment (UV-B/no UV-B) and a three-treatment (UV-B intermediate/UV-B enhanced/no UV-B) analysis. Results of the two-treatment study show that UV-B radiation depresses population growth of the two diatoms. An apparent competitive shift in favor of the larger, less UV-sensitive diatom (Thalassiosira weisflogii) was also

noted, although it was not statistically significant ($p>0.05$). Similar results were obtained with the three-treatment analysis, with the UV-B enhanced cultures generally showing the greatest depression of cell numbers, the UV-B intermediate cultures intermediate, and the UV-B deficient cultures the least growth depression. No significant community shift toward the larger species Thalassiosira weisflogii was noted for the three-treatment study ($p>0.050$).

Some Effects of Ultraviolet-B Radiation (290-320 nm) on Unialgal and Mixed Cultures of Two Centric Diatoms

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Some Effects of Ultraviolet-B Radiation (290-320 nm) on Unialgal and Mixed Cultures of Two Centric Diatoms

INTRODUCTION

Our planet is a megacosm of biological, physical and chemical systems that are open, interacting, and in many respects interdependent. Changes in the physical and chemical environment can have profound effects on biological systems, particularly when change occurs over time frames much shorter than evolutionary or geologic time. The accumulation of the so-called "greenhouse gases", primarily carbon dioxide, methane, oxides of nitrogen, and halocarbons from human industrial activity, is implicated in the relatively rapid and accelerating changes that have been detected in the earth's climate over the past several years. One focus of concern is the apparent deterioration of the stratospheric ozone layer as a result of the reactions of ozone with chloroflourocarbon gases (CFC's), enormous quantities of which are discharged into the atmosphere from global production and use of spray can propellants, foam insulation, and refrigerant compounds.

Atmospheric ozone protects the biosphere by absorbing the harmful shorter wavelengths of solar ultraviolet (UV) radiation, most notably UV-B radiation (290-320 nm), or mid-wavelength UV. The known effects of

UV-B radiation in humans include sunburn, skin cancer and eye damage such as cataracts. The effects of enhanced (above ambient) levels of UV-B exposure on biological communities and ecosystems, and on the food web are less clear. Nonetheless, accumulating evidence suggests that increased UV-B radiation can result in profound changes in the grazing efficiency of the primary consumers, hence on the quality of nutrients passed on from the primary producers to the higher trophic levels. Implications of these findings to the world's supply and quality of marine, fresh water and terrestrial food sources are cause for concern.

The objective of this study is to attempt to answer two fundamental questions: (1) Does UV-B radiation have a measurable effect on the population growth rates and/or community composition of certain marine producers, and if so, (2) are there discernible effects between ambient and experimentally-enhanced exposure? Two species of centric diatoms were used in this study, Thalassiosira pseudonana and Thalassiosira weisflogii (T. fluviatilis), both common inhabitants of northern temperate marine environments.

MATERIALS and METHODS

The experimental exposure apparatus consisted of a rectanguloid frame enclosed by white reflective styrofoam at the ends, front and bottom, and the room wall with its attendant electrical outlets bounding the structure in the rear. Flourescent sunlamps (Q-Panel UVB-313) and flourescent white lamps (General Electric F40SW) were suspended from the top of the structure, serving as sources of mid-wavelength ultraviolet (UV-B) and photosynthetically active radiation (PAR). Along the length of the floor centerline, there were six irradiance regions, or two three-region blocks symmetrical about an imaginary line running from front to back in the center of the chamber. The six regions were designated regions A through F (Appendix Figure A.1). Three experimental conditions were represented by the six regions: (1) regions A and F were UV-B enhanced, (2) regions C and D were UV-B intermediate (ambient), and (3) regions B and E were UV-B deficient.

Schott glass filters, 1 mm thick WG320 for regions A, C, D, and F, and 3 mm thick WG335 for regions B and E, were placed over beakers containing unialgal and mixed cultures of Thalassiosira pseudonana and Thalassiosira weisflogii. "Intermediate" UV-B irradiance for this study was defined as DNA effective irradiance at solar noon on June 15th., latitude 44.6^{o}N (Green et al., 1980: 0.342 cm

ozone, 2.0 aerosol scaling coefficient). Absolute filtered UV-B irradiances were 0.016 +/- 0.002 Wm^{-2} for the low or "UV-B deficient" exposure, 1.26 +/- 0.13 Wm^{-2} for the intermediate or "UV-B ambient" exposure, and 1.58 +/- 0.20 Wm^{-2} for the high or "UV-B enhanced" exposure. Table 1 shows the spectral distribution of these irradiances. The PAR component of visible radiation was 12 +/- 1 Wm^{-2}.

The filtered visible and UV irradiance in each region was quantified before and after each experiment using a spectroradiometer (Optronics model 742) interfaced to a microcomputer (Hewlett-Packard 9815A). Standard lamps traceable to the U.S. National Bureau of Standards (NBS) were used in the calibration of the spectroradiometer. Biologically weighted UV summations were based on action spectra for DNA effective (Setlow, 1974) and plant effective (Caldwell, 1971) irradiance, normalized to 300 nm.

Four 250-ml beakers were placed in each region, one containing a culture of Thalassiosira pseudonana, another containing Thalassiosira weisflogii, and the remaining two beakers containing a mixture of the two diatoms. Autoclaved and 0.45 um Millipore-filtered seawater (31-35 ppt salinity) for the cultures was obtained from Yaquina Bay, Oregon ($44^{o}37'N/124^{o}02'W$). The seawater was enriched with nutrients (Matthiessen and Toner, 1966) and used for

Table 1. Spectral distribution of filtered UV-B radiation for three exposure levels. '-' = low UV-B exposure, 'N' = intermediate UV-B exposure, and '+' = high UV-B exposure. Units are in Wm^{-2}.

λ	-	N	+
290	0.00	0.00	0.00
291	0.00	0.00	3.18×10^{-6}
292	0.00	1.59×10^{-5}	2.64×10^{-5}
293	0.00	6.56×10^{-5}	8.31×10^{-5}
294	0.00	1.78×10^{-4}	2.36×10^{-4}
295	0.00	4.58×10^{-4}	5.69×10^{-4}
296	0.00	1.09×10^{-3}	1.35×10^{-3}
297	0.00	2.04×10^{-3}	2.53×10^{-3}
298	0.00	3.44×10^{-3}	4.56×10^{-3}
299	0.00	5.89×10^{-3}	7.49×10^{-3}
300	0.00	9.05×10^{-3}	1.19×10^{-2}
301	0.00	1.37×10^{-2}	1.74×10^{-2}
302	0.00	1.95×10^{-2}	2.49×10^{-2}
303	0.00	2.53×10^{-2}	3.24×10^{-2}
304	0.00	3.20×10^{-2}	4.11×10^{-2}
305	0.00	3.87×10^{-2}	5.08×10^{-2}
306	0.00	4.65×10^{-2}	6.03×10^{-2}
307	0.00	5.41×10^{-2}	7.03×10^{-2}
308	0.00	6.07×10^{-2}	7.99×10^{-2}
309	0.00	6.77×10^{-2}	8.84×10^{-2}
310	0.00	7.35×10^{-2}	9.62×10^{-2}
311	1.28×10^{-5}	8.16×10^{-2}	1.07×10^{-1}
312	6.95×10^{-5}	1.04×10^{-1}	1.36×10^{-1}
313	1.60×10^{-4}	1.25×10^{-1}	1.58×10^{-1}
314	3.23×10^{-4}	1.10×10^{-1}	1.42×10^{-1}
315	6.60×10^{-4}	9.73×10^{-2}	1.28×10^{-1}
316	1.23×10^{-3}	9.61×10^{-2}	1.27×10^{-1}
317	2.24×10^{-3}	9.69×10^{-2}	1.28×10^{-1}
318	3.79×10^{-3}	9.73×10^{-2}	1.28×10^{-1}
319	5.65×10^{-3}	9.65×10^{-2}	1.27×10^{-1}
320	8.34×10^{-3}	9.59×10^{-2}	1.27×10^{-1}
322	1.47×10^{-2}	9.37×10^{-2}	1.24×10^{-1}
324	2.18×10^{-2}	8.89×10^{-2}	1.18×10^{-1}
326	2.95×10^{-2}	8.49×10^{-2}	1.12×10^{-1}
328	3.62×10^{-2}	7.95×10^{-2}	1.05×10^{-1}
330	4.11×10^{-2}	7.32×10^{-2}	9.71×10^{-2}
332	4.39×10^{-2}	6.64×10^{-2}	8.83×10^{-2}
334	4.76×10^{-2}	6.37×10^{-2}	8.40×10^{-2}
336	4.39×10^{-2}	5.33×10^{-2}	7.09×10^{-2}
338	4.17×10^{-2}	4.68×10^{-2}	6.23×10^{-2}
340	3.90×10^{-2}	4.14×10^{-2}	5.51×10^{-2}
345	3.00×10^{-2}	2.93×10^{-2}	3.87×10^{-2}
350	2.13×10^{-2}	1.96×10^{-2}	2.59×10^{-2}
355	1.49×10^{-2}	1.34×10^{-2}	1.77×10^{-2}
360	1.03×10^{-2}	9.30×10^{-3}	1.22×10^{-2}

the stock cultures. After sufficient diatom production was apparent, an appropriate dilution scheme was employed to adjust relative biomass of the two diatoms and the culture medium was decanted to the 200-ml mark of each beaker.

Temperature uniformity within the chamber was maintained with a convection fan located at one end of the chamber. Prior to beginning the first experiment and before the lights came on for the day temperature readings were taken from beakers filled with water to the 200-ml mark using a VWR glass mercury thermometer. For the duration of each experimental replicate, temperature cycling in the chamber was continuously monitored with a Cole-Parmer 50-100^{o}F range thermograph (Appendix Figure A.2), and individual beaker temperatures were measured at 10 am, 1 pm, and 4 pm on the last day of each experiment to check for uniformity of temperature change rates between beakers. The temperatures were uniform to within 0.5 ^{o}C. The thermograph and glass thermometer were referenced against a Cole-Parmer NBS-traceable thermometer. Combined measurements from the three experimental replicates showed the culture seawater maximum temperature to be 15.7 +/- 0.4^{o}C after daylight thermal equilibration (4 pm reading).

The mixed cultures contained roughly equivalent biomasses of each species at the start of the

experiments. Biomass was determined using cell volume statistics and cell counts obtained with a Particle Data 80XY electrostatic particle counter calibrated with standard latex spheres, interfaced to a Particle Data TP50 microprocessor/printer. The periodic calibrations were done using standard methods of quality assurance in accordance to accepted U. S. Environmental Protection Agency procedures.

There were two beakers of mixed culture per region (A-F), and one beaker of each separate species per region. This arrangement yielded two sample populations of T. pseudonana per irradiance treatment, two sample populations of T. weisflogii per treatment, and four sample populations of mixed culture per treatment for the experiment. Three experimental replicates were run, each of 4-day duration. The visible light:dark cycle in the chamber was 18 h:6 h. The daily UV-B exposure period was 6.5 h, administered during the middle of the 18 h light cycle. The 6.5 h daily exposure duration was determined by dividing the previously-defined total daily DNA effective UV-B irradiance by the hourly normal dose rate.

Each day before the UV-B lights came on, the cultures were agitated by stirring the medium in each beaker fifty times with a glass rod, and 45 minutes later 100-ml samples were taken from each beaker. Levels in the beakers were then restored to the 200-ml mark by adding

100 ml of fresh nutrient medium. Cell counts, including cell size distributions and their associated descriptive statistics computations (mean, median and mode) were carried out on each sample using the particle counter.

Dilution-corrected cell quantities, doubling times, and biomass ratios were computed for each replicate. Doubling times were computed from data obtained over day 2 through day 4 of each replicate; whereas analysis of cell numbers was done only on data obtained on the last day (day 4). Doubling time (t_2) for this analysis was computed using the exponential growth equation

$$r = \ln[N(4)/N(2)]/t$$

and

$$t_2 = \ln(2)/r$$

where N(2) is the population size at day 2, N(4) is the population size at day 4, r is the growth rate constant, and time (t) = 2 days.

Biomass ratios were computed for the mixed cultures by dividing the total biomass per unit volume of T. pseudonana by the total biomass per unit volume of T. weisflogii, where total biomass is equivalent to the product of the modal volume/cell (assumed proportional to the cell's specific gravity) and the cell concentration.

A two-way analysis of variance (Ingram, 1974) was used to analyse the data from the mixed cultures to determine if there were any position effects influencing cell quantity and biomass ratio.

RESULTS

Two-treatment Analysis: UV/No UV

Normalized percentage differences of cell numbers, biomass ratios, and doubling times from the three experimental replicates were computed and combined for the two-treatment analysis. Table 2 provides the percentage differences for cell quantity for day 4 (the last day). Unialgal cultures of Thalassiosira pseudonana receiving the daily UV-B irradiation showed a 35% depression in numbers relative to those receiving negligible UV-B exposure, and T. pseudonana in the mixed cultures showed a 32% depression. The p-values show a strong relationship (p=0.008-0.014) between cell quantity and UV-B exposure, with lower cell numbers resulting from UV-B exposure. Exposed cultures of Thalassiosira weisflogii showed a 19% and a 16% depression of cell numbers in the unialgal and mixed cultures, respectively. No statistically significant difference in cell numbers was noted for the unialgal cultures of T. weisflogii, but a strong difference (p=0.007) was found for T. weisflogii in the mixed cultures.

The p-values from t-tests of doubling times showed significance in the unialgal and mixed cultures with T. pseudonana. Statistically significant differences in doubling times for the two treatments were found in the

Table 2. Cell numbers, doubling times (t_2), and relative biomass differences between UV-B treated and UV-B deficient cultures expressed as percentage differences with 95% confidence intervals. A negative difference indicates lower cell numbers or smaller biomass ratios for the UV-B enhanced cultures. A positive difference indicates longer doubling times for the UV-B enhanced cultures. '*' indicates a significant difference at the p = 0.05 level. Two treatment analysis.

Culture	Measured Parameter	Percentage Differences with 95% C.I.
Thalassiosira pseudonana Unialgal	Cell #'s	-35 +/- 14*
	t_2	+30 +/- 12*
Thalassiosira pseudonana Mixed	Cell #'s	-32 +/- 16*
	t_2	+21 +/- 5*
Thalassiosira weisflogii Unialgal	Cell #'s	-19 +/- 74
	t_2	+58 +/- 113
Thalassiosira weisflogii Mixed	Cell #'s	-16 +/- 6*
	t_2	+ 1 +/- 32
	Biomass	-18 +/- 31

mixed cultures with T. pseudonana (UV-irradiated cultures showed a 21% longer doubling time than no-UV cultures, p=0.003), and in the unialgal cultures of T. pseudonana (30% difference, p=0.009). In the cultures with T. weisflogii the percentage differences do suggest possible UV-B sensitivity, although not statistically significant.

The mean percent difference in the biomass ratio of the two diatoms indicates a possible community shift toward T. weisflogii under UV stress, although it is not statistically meaningful.

Three-treatment Analysis:
UV-Ambient/UV-Enhanced/No UV

Table 3 shows percentage differences of cell numbers with their confidence intervals from t-tests for cell quantity on the last day of the experiment. Thalassiosira pseudonana populations in the mixed and unialgal cultures exhibited significantly lower cell numbers from exposure to 1.58 Wm^{-2} (high level exposure) than populations exposed to 1.26 Wm^{-2} (intermediate level exposure) ($p=0.023$ and $p=0.012$, respectively), with the populations exposed to 0.02 Wm^{-2} (low level exposure) showing the highest cell numbers. However, t-tests for doubling times of T. pseudonana cultures yielded statistically significant differences between the low and high exposure regimens for the unialgal cultures only (Table 4). As noted previously, doubling times were computed from data obtained over the last two days of each replicate (days 2 through 4), whereas analysis of cell numbers was done only on data obtained on the last day (day 4). This would account for the apparent discrepancy between significances of cell quantity and doubling time data. Significant differences in cell quantity were found only between high and intermediate exposure for unialgal (fewer cells and more cells, respectively with $p=0.029$) cultures of Thalassiosira weisflogii, although statistical testing of doubling times revealed no

Table 3. Cell number differences expressed as percentage differences with 95% confidence intervals. Treatment comparisons: UV-B intermediate/UV-B enhanced (N/+), UV-B deficient/UV-B intermediate (-/N), and UV-B deficient/UV-B enhanced (-/+). A negative difference indicates lower cell numbers for the second treatment in each comparison. '*' indicates a significant difference at the p = 0.05 level. Three treatment analysis.

Culture	Treatment Comparison	Percentage Differences with 95% C.I.
Thalassiosira pseudonana Unialgal	N/+	-42 +/- 20*
	-/N	-23 +/- 26
	-/+	-65 +/- 28*
Thalassiosira pseudonana Mixed	N/+	-28 +/- 18*
	-/N	-28 +/- 43
	-/+	-56 +/- 28*
Thalassiosira weisflogii Unialgal	N/+	-28 +/- 21*
	-/N	-15 +/- 84
	-/+	-43 +/- 95
Thalassiosira weisflogii Mixed	N/+	-14 +/- 51
	-/N	-11 +/- 25
	-/+	-25 +/- 26

Table 4. Doubling time differences expressed as percentage differences with 95% confidence intervals. Treatment comparisons: UV-B intermediate/UV-B enhanced (N/+), UV-B deficient/UV-B intermediate (-/N), and UV-B deficient/UV-B enhanced (-/+). A positive difference indicates longer doubling times for the second treatment in each comparison. '*' indicates a significant difference at the p = 0.05 level. Three treatment analysis.

Culture	Treatment Comparison	Percentage Differences with 95% C.I.
Thalassiosira pseudonana Unialgal	N/+	+41 +/- 53
	-/N	+8 +/- 52
	-/+	+49 +/- 41*
Thalassiosira pseudonana Mixed	N/+	+16 +/- 44
	-/N	+11 +/- 15
	-/+	+27 +/- 29
Thalassiosira weisflogii Unialgal	N/+	+103 +/- 153
	-/N	-6 +/- 136
	-/+	+97 +/- 85*
Thalassiosira weisflogii Mixed	N/+	+196 +/- 893
	-/N	-2 +/- 38
	-/+	+194 +/- 880

differences in treatment comparisons among the mixed cultures or between high/intermediate exposure ($p=0.102$) and low/intermediate exposure ($p=0.872$) among the unialgal cultures. A significant position effect on cell quantity was noted in replicate 3.

In general, cultures exposed to low level UV-B radiation had the highest cell counts and the shortest doubling times, those exposed to intermediate levels yielded intermediate numbers and intermediate doubling times, and the cultures exposed to high levels showed the greatest depression in numbers with the longest doubling times.

When biomass ratios for day 4 were compared no statistically meaningful contrasts were revealed for the remaining treatment comparisons (Table 5). Moreover, interaction effects between position and irradiance were evident in replicate 2, and a position effect was found in replicate 3.

Table 5. Relative biomass differences expressed as percentage differences with 95% confidence intervals. Treatment comparisons: UV-B intermediate/UV-B enhanced (N/+), UV-B deficient/UV-B intermediate (-/N), and UV-B deficient/UV-B enhanced (-/+). A negative difference indicates smaller biomass ratios for the second treatment in each comparison. No significance was noted at the p = 0.05 level. Three treatment analysis.

Treatment Comparison	Percentage Differences with 95% C.I.
N/+	-21 +/- 56
-/N	-13 +/- 76
-/+	-34 +/- 45

DISCUSSION

Biological action spectra were used to weight the summed UV-B irradiance measurements. The product of the observed "relative biological effectivenes" of UV-B radiation on a chromophore at a specific wavelength and the absolute dose at that wavelength is evaluated, yielding a "relative effective irradiance". Such values determined at different wavelengths are then integrated over the spectral range in question to yield the "effective irradiance" for that range. Action spectra illustrate the wavelength-dependent nature of UV-B effects on biological systems. They are usually determined by observing the effect of monochromatic UV-B radiation on a particular organism. The effects determined at each wavelength are then compiled into an analytic representation of the relative effects over the spectral range of choice. In this manner the relative biological effectiveness of polychromatic radiation can be approximated (Caldwell et al., 1986).

Various experiments have estimated expected increases in DNA and plant weighted effectiveness for different ozone depletion scenarios (see Worrest, 1982 for review of the literature). The percentage increases in biological effectiveness appear to be non-linear for ozone decreases of 10-40% (Worrest, 1986). It is important to note that weighting functions are usually

based on relative effects determined using monochromatic radiation, and do not take possible spectral synergy into account. UV-A radiation drives photorepair of UV-B-induced thymine dimer formation, and low-level UV-B radiation can also indirectly repair UV-induced damage to a limited extent (Caldwell, 1986). Levels of PAR radiation ranging from ambient to high (20% above surface intensity) have been shown to have little or no photoinhibitory effect on the growth of six representative species of algae (Jokiel and York, 1984). Accordingly, the modest variations in PAR radiation in the experiments of the present study were not considered critical.

UV-B radiation is reduced to 1% of its surface irradiance at a depth of about 10% of the marine euphotic zone. This depth can range from approximately 6 meters for some coastal waters to 30 meters for clear oceanic water (Thomson et al., 1980). Natural, filtered estuarine sea water was used for the present study. It was assumed that the diatom cultures in each beaker were sustaining measured surface UV-B radiation doses throughout the 9 cm water column; however, the rate of attenuation of short wavelength and PAR radiation is strongly dependent on water type (Smith and Baker, 1979).

In a natural environment, direct UV radiation increases after sunrise and reaches peak irradiance at

solar noon, then decreases continuously toward sunset. The laboratory diatoms received a constant dose-rate of UV-B radiation equivalent to solar noon during the entire 6.5 h period each day to simulate the expected total dose that would be received in nature from sunrise to sunset. Reciprocity between total dose and dose-rate was assumed to hold over the ranges and time intervals of UV-B radiation exposure. The law of reciprocity states that the photo effect of a given total radiation dose is independent of the dose rate (Smith et al., 1980), i.e., brief doses of high-intensity UV radiation may produce effects not significantly different from those produced from longer UV radiation exposure at lower intensities, provided the range of intensities is not large (Damkaer et al., 1980). The biological effect would therefore only be a function of total dose, and not of the time over which this irradiation occurs (Jagger, 1967) (e.g., the effect will be the same if the dose-rate is doubled and the time of exposure is halved). The concept of reciprocity is of significance in this study because the effects on the diatoms receiving a constant dose-rate administered under the laboratory conditions were assumed to be equivalent to the effects expected from the variable dose-rates occurring in nature, for the same total dose. Reciprocity fails near the organism's mean dose-rate threshold, with the over-threshold dose-rate

effect causing irreversible damage to repair systems (Nachtwey, 1976). Studies of UV-B threshold levels of zooplankton have shown that dose-rates exceeding current natural mean threshold levels significantly depress activity, development, and survival of shrimp larvae, crab larvae, and euphausids (Damkaer,1980).

As is the case with most experiments to date using artificial sources of UV and visible radiation, the flourescent lamps employed generally do not approximate the relative radiation intensities characteristic of the solar spectral distribution, particularly with respect to the UV-A (320-400 nm) and visible components. UV-A and visible radiation of flourescent lamps are generally more than an order of magnitude less than that of solar radiation (Nachtwey and Rundel, 1982). Photoreactivation effects may therefore not be as pronounced as in nature, leading any UV-B effects observed under laboratory conditions to possibly overestimate those occurring from sunlight. The situation is reversed when comparing the short wavelength radiation of the two sources. The ratio of short wavelength to long wavelength UV radiation is much higher for flourescent light than for sunlight (Maxwell and Elwood, 1983). However, the total UV-B dose can be made equivalent to any dose encountered in nature, as was done for the present study.

Solar and flourescent radiation differences considered, UV-B measurements of sun and lamps can be compared in terms of total dose, but not in terms of biologically effective irradiance because the spectral differences are considerable within the DNA effective waveband (Damkaer et al., 1980). Still, a reasonable degree of comparability is possible between many studies using flourescent lamps as well as between experiments conducted in the field, and laboratory and field studies can be compared to evaluate the differences in magnitude of the effects of varying levels of photoreactivating radiation in the presence of enhanced UV-B radiation.

A number of studies have been done using marine diatoms to determine the effects of enhanced levels of UV-B radiation on biomass production, and on interspecies competition. In one such study, seven species of phytoplankton all exhibited significant depression of primary productivity as measured by radiocarbon uptake when exposed to UV-B radiation equivalent to what would be expected from a 16% reduction of stratospheric ozone (Worrest et al., 1981). An alteration of community composition was also noted. No significant community shift was demonstrated in the present study, as seen from the biomass ratios on day 4 (two- and three-treatment studies. In Worrest's experiment, the relative sensitivities of the seven species to UV-B radiation

ranged from least sensitive (Dunaliella tertiolecta) to most sensitive (Thalassiosira pseudonana). At least with respect to the sizes of these two species representing the relative sensitivity extremes, the smaller diatom T. pseudonana was shown to be nearly four times as sensitive as the flagellate D. tertiolecta but is only about half the diameter of the latter. This tends to show consistency with the notion that one would expect the cytoplasm of the larger organism to absorb more UV quanta before a critical nuclear chromophore is damaged because larger cells have a greater cytoplasmic volume relative to the nucleus. This apparent relationship of size with sensitivity to UV-B radiation is supported by the present study, where Thalssiosira pseudonana demonstrated a greater sensitivity than Thalassiosira weisflogii as measured by observed relative changes of cell numbers of the two species (two- and three-treatment studies).

Variables other than organism size have been shown to influence sensitivity of marine diatoms to UV-B stress. Several species of marine diatoms grown at two different temperatures exhibited species-dependent responses to supplemental UV-B radiation, with growth and primary productivity depression and decreases in protein and pigment content common to all under enhanced exposure (Dohler, 1984). Temperature effects on UV-B stress varied widely between species, with some diatom species showing

greater UV-B sensitivity under the lower temperature while other species showed greater sensitivity at the higher temperature. These studies demonstrate the species-dependent aspect of UV-B radiation effects on primary producers.

Greenhouse experiments have been undertaken in efforts to more closely approximate the wavelength-dependent solar radiant energies and variable irradiance encountered by marine organisms in nature. In one such study where estuarine microcosms in flow-through tanks were exposed to four levels of supplemental UV-B radiation, depression of biomass, chlorophyll-a concentration, and primary productivity was demonstrated at UV-B levels simulating a 15% decrease in stratospheric ozone (Worrest, Thomson, and Van Dyke, 1981). A shift in community composition was also demonstrated. Although natural visible solar radiation is employed, the use of greenhouses in such studies entail some of the problems inherent to experiments using only artificial UV radiant sources. Among these are the previously discussed spectral differences between natural and artificial UV-B radiation, and attenuation of photoreactivating UV-A by the greenhouse structure. The structural fiberglass of the greenhouse used by Worrest filters out UV radiation with wavelengths shorter than 360-380 nm, effectively eliminating a large portion of the photoreactivating UV-A

spectrum. UV-A radiation (320-400 nm) comprises more than 90% of the total energy of the UV spectrum (Caldwell, 1971), and therefore might be considered a significant source of photoreactivating influence. Intermittent shading of the microcosms by the lamp fixtures could also be a problem.

With the problems of artificial radiation considered, the greenhouse experiments of Worrest et al. (1981) were designed utilizing continuous temperature monitoring and control and fresh estuarine water flow-through, as well as an automated vertically moveable system of UV-B lamp banks, the distance from the tanks (irradiance) of which was controlled by a computerized feedback algorithm coordinated with cloud cover and time of day. Such experiments effectively reduce the number of remaining environmental variables (e.g., mixing, turbidity, etc.) that need consideration when attempting to predict the biological effects of increased UV-B radiation on marine primary producers.

CONCLUSION

The results of the present study demonstrate that UV-B radiation depresses the growth of diatom populations, and could possibly cause a competitive shift toward the larger organism in the community structure. This was demonstrated in the two-treatment study, where UV-B radiation resulted in a statistically significant depression of the cell numbers of unialgal and mixed cultures of Thalassiosira pseudonana and in the mixed cultures of Thalassiosira weisflogii, as determined from analysis of cell counts taken on the last day of each replicate. Perceptible UV-B sensitivity was still evident where results lacked statistical significance. Doubling time data analysed over the last two days of each replicate revealed depression of growth rate which was significant for the unialgal and mixed cultures of T. pseudonana. Again, a suggestion of UV-B effects on growth rate was noted for the other cultures, although inconclusive. These analyses of cell numbers and doubling times suggest differential sensitivity between the two diatoms, with T. weisflogii exhibiting greater resistance to UV-B stress, however biomass ratios from the two- and three-treatment studies show no significant community shift toward the larger diatom T. weisflogii.

In general, cell numbers decreased with increasing UV-B stress in the three-treatment analysis, with T.

pseudonana in the unialgal and mixed cultures exhibiting the greatest sensitivity. This is in general agreement with the doubling time data. Most cultures with T. weisflogii demonstrated perceptible UV-B sensitivity, but this was for the most part not statistically significant. Changes in relative biomass with UV-B stress were also not significant, although the mean values of biomass ratios suggested a possible community shift in favor of T. weisflogii.

A continuing reduction of stratospheric ozone as a result of ongoing human perturbation will result in a steady increase in the amount of biologically damaging UV-B radiation reaching the earth's surface. If the enhanced UV-B stress results in a competitive shift toward larger species of primary producers in marine and freshwater ecosystems, grazing efficiency of the primary consumers may be adversely affected. Having to expend more metabolic energy in catching, ingesting, and digesting larger and/or fewer phytoplankton (Nachtwey, 1976) would almost certainly affect the quality and quantity of nutrients passed on to the higher trophic levels of the food web.

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APPENDIX

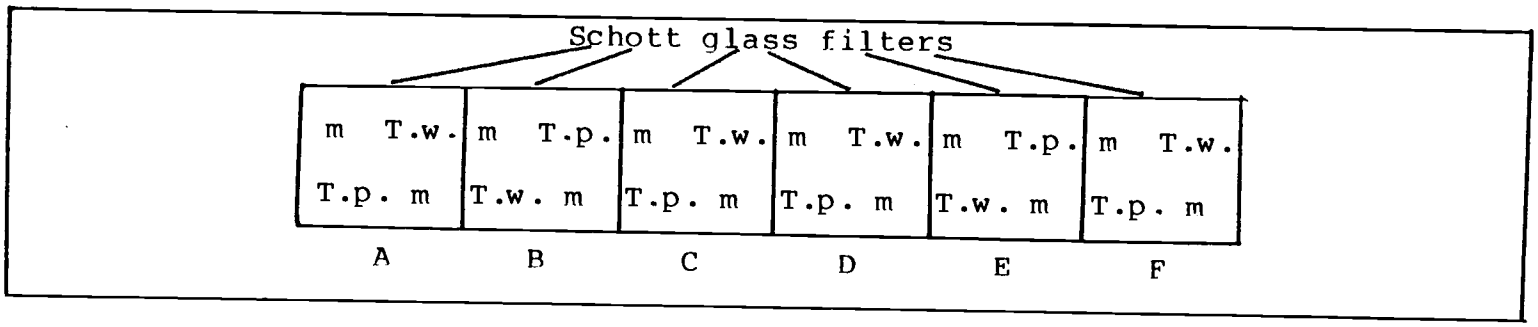

Fig. A.1. Arrangement of irradiance regions (A-F) as viewed from chamber ceiling. T.p. = beaker with unialgal culture of Thalassiosira pseudonana, T.w. = beaker with unialgal culture of Thalassiosira weisflogii, and m = beaker containing mixed culture of the two diatoms. Groups of four beakers are under each glass filter.

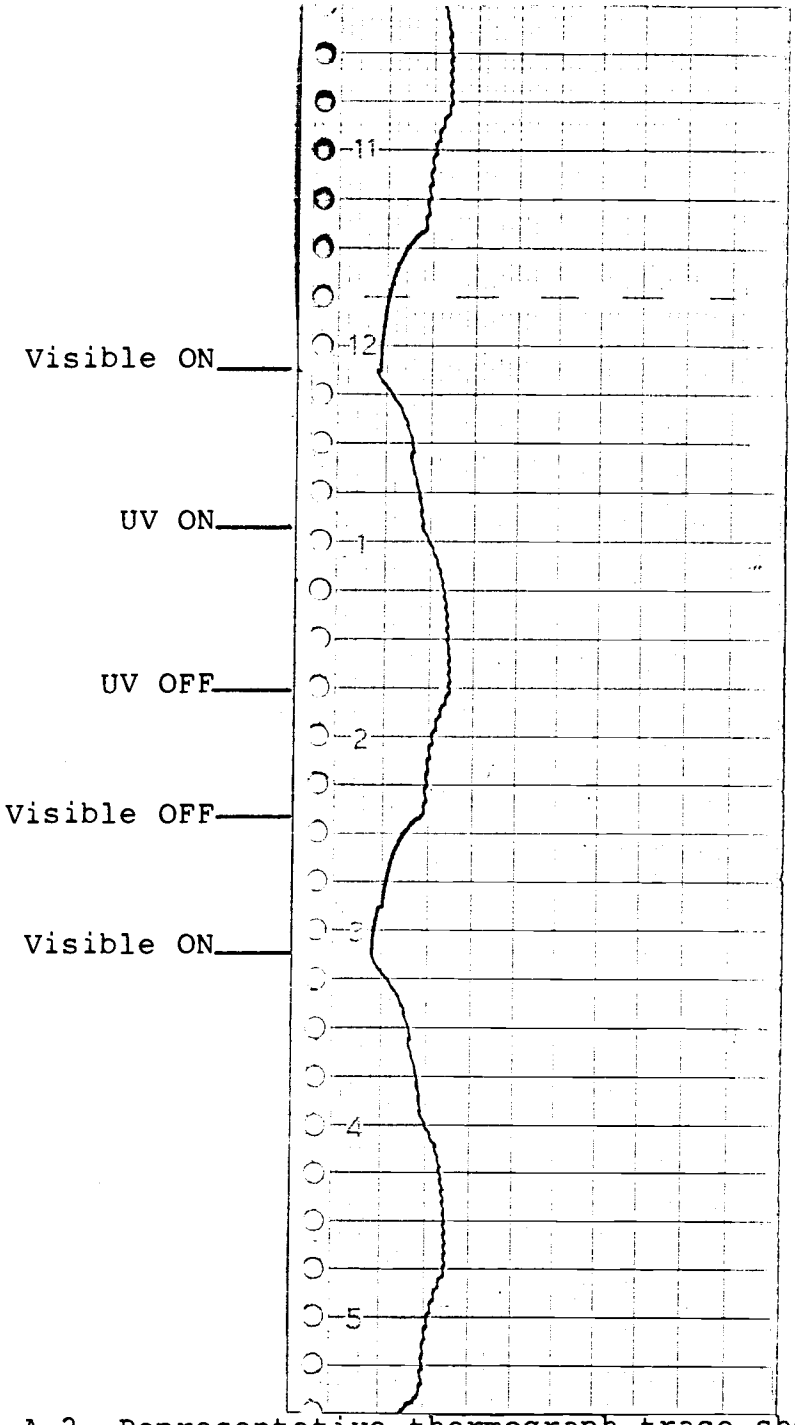

Fig. A.2. Representative thermograph trace showing temperature cycling during a portion of one experimental replicate. Temperature probe was immersed in a 250-ml beaker filled with water and placed at one end of bank of beakers containing the experimental cultures. Range of culture seawater temperature was 54-62.5°F over one light-dark cycle.