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Ultraviolet Radiation upon Marine Ecosystems

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ASSESSMENT OF THE IMPACT OF INCREASED SOLAR ULTRAVIOLET RADIATION UPON MARINE ECOSYSTEMS

Space Shuttle operations through the stratosphere may lead to a small reduction of the earth's ozone layer, with a resultant increase in transmission of solar ultraviolet radiation in the 290-320 nm waveband (UV-B). Studies indicated under the Climatic Impact Assessment Program (CIAP) of the U. S. Department of Transportation demonstrate that simulated solar ultraviolet radiation can, under experimental conditions, detrimentally affect the marine organisms (algae and small invertebrates) that form the base of the food web of oceanic and estuarine ecosystems. These organisms survive in nature by a combination of mechanisms for tolerating the detrimental effects of UV-B radiation. The key question is whether a small increase in biologically harmful ultraviolet radiation might overwhelm these mechanisms and produce changes that will have damaging consequences to the biosphere.

Although UV-B radiation penetrates a relatively short distance into highly productive estuarine waters when the organic content of oceanic waters is low, a pathlength of nearly 15 meters may be required to reduce the UV-B radiation to the 10% level. The behavioral responses of marine organisms which tend to protect them from excessive exposure to biologically harmful ultraviolet radiation are cued, most likely, by long-wavelength UV and visible radiation, a waveband which would be unaffected by a partial depletion of stratospheric ozone. Therefore, marine forms which are capable of tolerating present levels of UV-B radiation would lack the cue for a protective response to enhanced levels of UV-B radiation. On the other hand, if organisms do respond to UV-B radiation by a downward migration in the water column, they would move into a region of different ecological, chemical, and physical properties, including decreased photosynthetically active radiation. Thus, there is a potential for an adverse effect of increased UV-B radiation in either case.

The objective of the present study is to provide data to assess the potential impact upon marine ecosystems if Space Shuttle operations contribute to a reduction of the stratospheric ozone layer.

Specifically, the study has addressed the following two questions:

1. Is there potential for irreversible damage to the productivity, structure and/or functioning of a model estuarine ecosystem by increased UV-B radiation or are these ecosystems highly stable or amenable to adaptive change?
2. What is the sensitivity of key community components (the primary producers and consumers) to increased UV-B radiation?

Two areas of study were examined during the past year:

1. A pilot study utilizing flow-through ecosystem tanks enclosed in a small, outdoor greenhouse located at the Oregon State University Marine Science Center, Newport, Oregon.
2. Sensitivity studies of representative primary producers and consumers.

UV SENSITIVITY OF AN ESTUARINE ECOSYSTEM

Materials and Methods

In 1977, a pilot study was initiated to determine whether natural levels of photoreactivating radiation longer than 380 nm would mitigate the potential damage to marine organisms caused by exposure to UV-B radiation. A small, Fiberglas greenhouse was constructed on the property of the Oregon State University Marine Science Center, Newport, Oregon. Utilizing three 800-liter tanks supplied with a common, continuous flow of seawater from Yaquina Estuary, three different UV-B exposure conditions were established by the use of sunlamp/filter systems. The developed ecosystems in each tank received natural levels of solar radiation in the 380-800 nm waveband (380 nm is the short-wavelength cut-off of the Fiberglas). The initial results from an analysis of planktonic chlorophyll α concentration, numbers of bacterial colony-forming units, proportion of chromogenic bacteria, and phytoplanktonic community diversity indicated a potential effect of UV-B radiation for these parameters under the high visible light conditions. Further studies were not conclusive due to the lack of replication within the experimental conditions. To obviate this deficiency, during the fall of 1978 the experimental apparatus was modified. Into each 800-liter tank four 15-liter containers were immersed to within 4 cm of their tops. The 15-liter containers were supported on a rotating turntable (0.25 revolutions/minute) (Fig. 1). The inflow and overflow ports for the containers were designed to allow approximately 15 liters of fresh seawater to enter each container daily from the continuous seawater supply. The temperature of the seawater supply was recorded continuously. Each of the four containers within a single, large tank was covered with a different UV-B filter: (1) a 0.18 mm thickness of Mylar 'D', (2) a 0.25 mm thickness of cellulose acetate (CA), (3) a 0.19 mm thickness of CA, and (4) a 0.13 mm thickness of CA. The CA was partially photodegraded prior to use and all of the filters were changed weekly. In addition to solar radiation the naturally recruited ecosystems developing within the containers were exposed to 6 h of radiation daily from Westinghouse FS40 sunlamps. The daily surface fluence in the 290-320 nm waveband under the Mylar was $1.15 \times 10^2 \text{ J/m}^2$ ($1.3 \text{ Eff}_{\text{DNA}} \text{ J/m}^2$), and under the 0.25, 0.19, and 0.13 mm thicknesses of CA was $1.03 \times 10^4 \text{ J/m}^2$ ($84.4 \text{ Eff}_{\text{DNA}} \text{ J/m}^2$), $1.09 \times 10^4 \text{ J/m}^2$ ($98.6 \text{ Eff}_{\text{DNA}} \text{ J/m}^2$), and $1.26 \times 10^4 \text{ J/m}^2$ ($145.4 \text{ Eff}_{\text{DNA}} \text{ J/m}^2$), respectively. Fluence rate measurements were made with a modified Gamma Scientific 2900SR Spectroradiometer System which has been characterized by the U.S. National Bureau of Standards (Worrest et al., 1978). The four filters for each of the three large tanks were randomly assigned to the 15-l containers, constituting a randomized complete block design with three replications.

Due to the severity of the cold and the low concentration of essential nutrients within the seawater during the fall and winter, the ecosystems developed very slowly. Samples of planktonic and attached algae were collected for taxonomic identification after a period of twelve weeks. At this time the estimation of planktonic radiocarbon uptake was determined according to the procedure of Strickland and Parsons (1972). Planktonic samples were also collected for chlorophyll α analysis and processed by the SCOR/UNESCO technique (Strickland and Parsons, 1972). A Coleman Model 124D Double Beam Recording Spectrophotometer with a 10 cm cell-path-length capability was utilized.

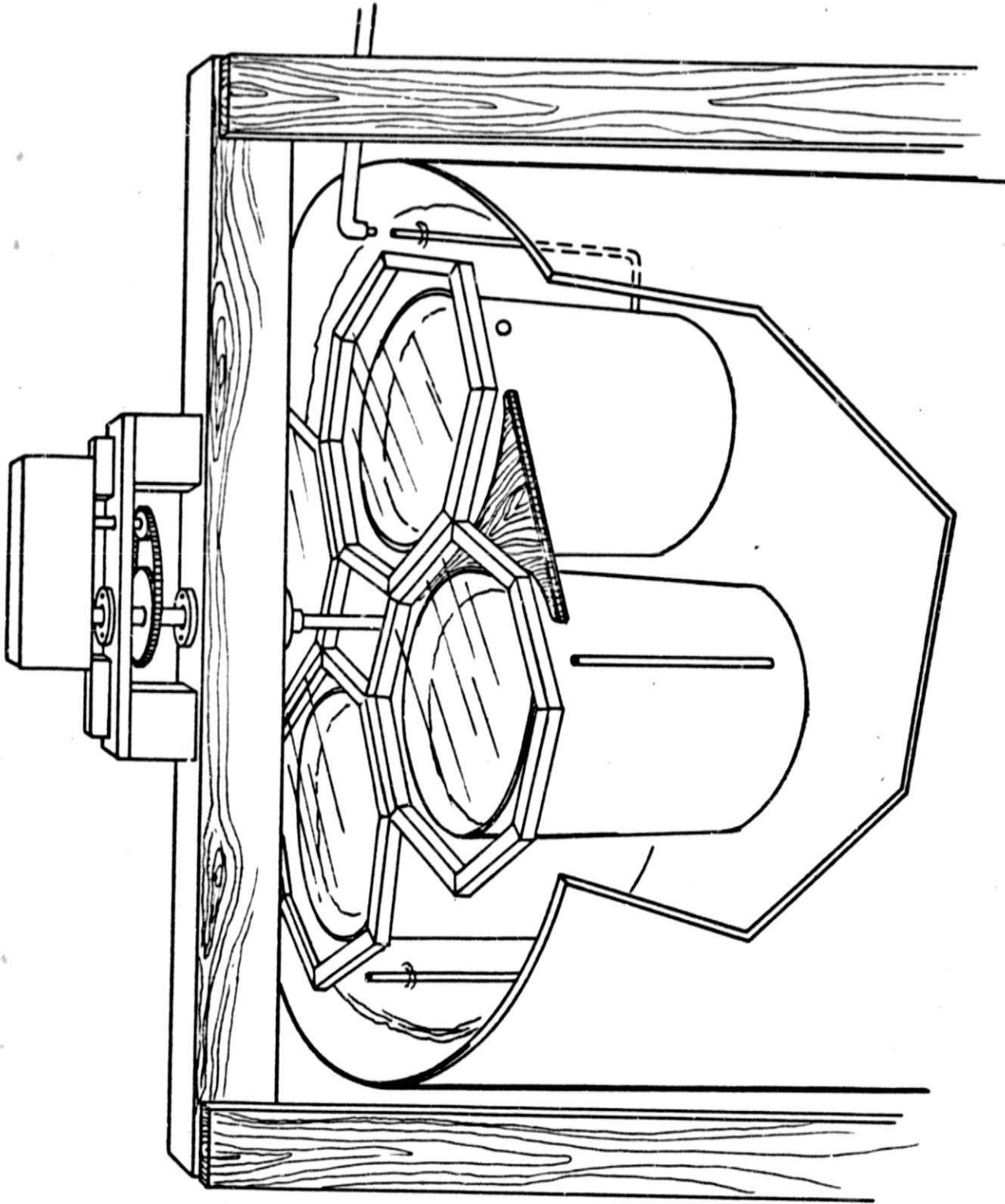


Fig. 1. Rotating 15-liter ecosystem chambers.

Preparation of samples and analysis of community composition were performed as described previously by Worrest et al. (1978). An estimate of the Shannon-Weiner Information measure (H'') expressed as nats per individual, and a measure of Redundancy (R') were determined. H'' ranges from 0 ($\log_e 1$), if all of the individuals in the assemblage are of one taxon, to $\log_e N$, if the number of taxa equals the number of individuals. The more taxa there are and the more nearly equal their proportions, the greater the uncertainty of predicting the taxon of the next individual to be observed and, therefore, the greater the diversity. The Redundancy index expresses the degree of dominance in a given sample relative to the partitioning of individuals among the taxa. Values of R' range from 0, when the individuals are equally distributed among the taxa, to 1, when all but one taxon are represented by a single individual.

Results

Microscopic study revealed diatom species to be, by far, the dominant autotrophic component of the samples analyzed. Comparative analyses of planktonic species diversity within the containers indicated that exposure to UV-B radiation resulted in a less diverse community structure, but that the communities developing under the three different thicknesses of CA (three different supplementary UV-B fluence rates) were not significantly different (Tables 1 and 2). Analysis of samples of attached diatoms, however, indicated that increasingly higher levels of UV-B radiation resulted in less diverse community structure (Tables 3 and 4). This is illustrated by the Information index, the measure of Redundancy, and the list of dominant, attached diatoms.

Planktonic chlorophyll concentrations and radiocarbon uptake analyses reflect the results of the planktonic community structures (Table 5). The ecosystems developing within Mylar-filtered containers (UV-B deficient) had significantly greater chlorophyll α concentration and radiocarbon uptake than those from the CA-filtered containers (UV-B enhanced). However, the differences among the ecosystems exposed to the three different levels of UV-B enhancement were not significant.

Discussion

Recruitment for the ecosystems developing within the experimental containers was drawn from Yaquina Estuary at a time of year when the natural UV-B fluence was low (typically, $< 10 \text{ Eff}_{\text{DNA}} \text{ J/m}^2/\text{d}$). The relative enhancement for those experimental systems developing under CA filters was at least one or two orders of magnitude greater than found in the estuary at that time. The significant differences between the UV-B deficient systems and the UV-B enhanced systems might not be as relevant as at another, more productive, time of year. Results of studies during the spring, summer, and fall are anticipated and should be of interest.

Table 1. Analysis of community composition of phytoplankton samples. N is the number of diatoms in the sample, S is the number of species represented in the sample, H'' is the estimator for the common Information index, and R' is a measure of Redundancy.

Filter	N	S	H''(nats)	R'
Mylar	503	72	3.013	0.242
0.25 mm CA	501	29	2.315	0.363
0.19 mm CA	512	32	2.441	0.361
0.13 mm CA	509	25	2.189	0.397

Table 2. A list of the dominant diatom species ($\geq 10\%$ of the total number of enumerated, planktonic diatoms) and the percent composition of those species.

Filter	UV-B Fluence (Eff _{DNA} J/m ² /d)	Species	Percent Composition
Mylar	1.3	<i>Chaetoceros radicans</i>	26
		<i>C. debilis</i>	18
		<i>Melosira sulcata</i>	13
		<i>C. didymus</i>	11
0.25 mm CA	84.4	<i>Chaetoceros debilis</i>	38
		<i>C. radicans</i>	31
		<i>Melosira sulcata</i>	12
0.19 mm CA	98.6	<i>Chaetoceros debilis</i>	37
		<i>C. radicans</i>	33
		<i>C. socialis</i>	10
		<i>C. didymus</i>	10
0.13 mm CA	145.4	<i>Melosira sulcata</i>	47
		<i>Chaetoceros radicans</i>	24
		<i>C. socialis</i>	12

Table 3. Analysis of community composition of attached assemblages of diatoms. N is the number of diatoms in the sample, S is the number of species represented in the sample, H'' is the estimator for the common Information index, and R' is a measure of Redundancy.

Filter	N	S	H''	R'
Mylar	506	41	2.713	0.287
0.25 mm CA	502	28	1.902	0.495
0.19 mm CA	510	30	1.738	0.513
0.13 mm CA	502	25	1.659	0.591

Table 4. A list of the dominant diatom species (>5% of the total number of enumerated, attached diatoms) and the percent composition of those species within each assemblage.

Filter	Species	Percent Composition
Mylar	<i>Fragilaria striatula</i>	19
	<i>Navicula salinicola</i>	17
	<i>Synedra fasciculata</i>	17
	<i>Amphora</i> sp. No. 2	8
	<i>A. tenerrima</i>	7
	<i>Melosira nummuloides</i>	6
	<i>Nitzschia frustulum</i> v. <i>perpusilla</i>	6
0.25 mm CA	<i>Stauroneis constricta</i>	47
	<i>Amphora</i> sp. No. 2	12
	<i>Nitzschia</i> sp. No. 5	11
	<i>Melosira nummuloides</i>	7
	<i>Amphora tenerrima</i>	7
0.19 mm CA	<i>Stauroneis constricta</i>	48
	<i>Amphora tenerrima</i>	27
	<i>Melosira nummuloides</i>	17
0.13 mm CA	<i>Amphora tenerrima</i>	71
	<i>Stauroneis constricta</i>	12
	<i>Navicula salinicola</i>	7
	<i>Amphora</i> sp. No. 2	5

Table 5. Planktonic chlorophyll α concentrations and radiocarbon uptake determined after 12 weeks of ecosystem development.

Filter	UV-B fluence (Eff_{DNA} $\text{J}/\text{m}^2/\text{d}$)	Chlorophyll α concentration ($\text{mg Chl. } \alpha/\text{m}^3$)	Radiocarbon uptake ($\text{mg C}/\text{m}^3/\text{h}$)
Mylar	1.3	1.91 ± 0.25 *	3.44 ± 0.29 ***
0.25 mm CA	84.4	1.25 ± 0.16	1.38 ± 0.21
0.19 mm CA	98.6	1.31 ± 0.19	1.38 ± 0.13
0.13 mm CA	145.4	1.23 ± 0.23	1.24 ± 0.19

* $p < 0.05$

*** $p < 0.001$

The doubling time for organisms at non-productive times of year generally exceeds two days. For a population to increase in numbers within a homogeneously mixed flow-through container the doubling time of the organisms must be less than the "flushing half-life" of that container. This assumes no new recruitment into the system. In other words, more than half of the organisms must reproduce (binary) before half of the original population is flushed through the outflow. The flushing half-life ($t_{1/2}$) can be calculated for a homogeneously mixed flow-through container from the following:

$$t_{1/2} = \log_e \frac{V}{R} ,$$

where V represents the volume of the container and R represents the flow rate into the container (inflow = outflow). The flushing half-life for the containers described in this report was about 16.8 h. This is far less than the doubling time for the representative organisms within the system, but is typical of the flushing half-life for Yaquina Bay as a whole. Approximately 70% of the water in Yaquina Bay is replaced with each tidal cycle, resulting in about a 90% replacement per day. If the experimental containers had been homogeneously mixed, they would have had 63% of their water replaced per day. Actually, the flushing of the containers could be represented by a model somewhere between a homogeneous mixing flow-through and a linear (non-mixed) flow-through model. At a flow rate of 0.6 l/h linear flow-through would result in 96% of the water being replaced each day. Therefore, if the recruited organisms had neutral density, and did not exhibit any tactic or kinetic responses, and were not preyed upon, and were non-reproducing, somewhere between 63 and 96% of the organisms entering the system would leave the system within 24 h. At the time of the current experiment the reproductive rate of the organisms was not sufficiently high to cause a significant increase in the size of the planktonic community, nor to allow the majority of the organisms to remain in the experimental containers for several generations. If organisms are more sensitive to UV-B stress during DNA synthesis, an organism with a long doubling time would be less likely to incur damage from a series of acute exposures to UV-B radiation than one with a shorter doubling time. From previous studies it has been shown that during the warmer, more productive periods the doubling time of representative recruited organisms is close to the flushing half-life of the experimental containers. This should provide more information regarding the effect of UV-B radiation upon a "typical" estuarine ecosystem.

UV-B SENSITIVITY OF *MELOSIRA NUMMULOIDES*

Introduction

M. nummuloides is a centric chain-forming marine littoral diatom which, in nature, can be found in the water column as well as attached to surfaces (Lebour, 1930). This species has cosmopolitan distribution ranging from the Arctic Ocean to the coastal waters around Capetown, South Africa (McIntire and Moore, 1971). It is among the thirty-five most abundant species of attached diatoms found in Yaquina Estuary located at Newport, Oregon (44°37'N) (Wulff and McIntire, 1972). *M. nummuloides* was listed among the seven most abundant species of marine littoral diatoms during a year-long study of epilithic assemblages of an intertidal substrate (Castenholz, 1963). Among other parameters, Castenholz discusses light intensity and ultraviolet radiation as factors determining the vertical distribution of attached diatoms.

From geographical data and from studies of vertical distribution which list *M. nummuloides*, it is clear that this species is adaptable to a wide range of radiant energy conditions. While past studies have discussed the role of ultraviolet radiation as an environmental parameter (Castenholz, 1963; Steemann Nielsen, 1964; Jitts et al., 1976), the present study was designed to characterize the response of one species of a common marine diatom to radiation in the UV-B region (specifically, 290-320 nm). This waveband is the portion of the natural solar spectrum that would be altered by a decrease in stratospheric ozone.

Materials and Methods

Melosira nummuloides was isolated from algal mats near the surface of 700-liter ecosystem chambers exposed to a simulated solar spectrum as described by Worrest et al. (1978). A stock culture of this isolate was subcultured every 10-14 days using f/2 enriched seawater (Guillard and Ryther, 1962) and incubated at 13°C. The stock culture was exposed to a daily 11L:13D photocycle which utilized deluxe fluorescent lamps (Duro-Test 40W Vita-Lite) and 5 h of supplemental ultraviolet radiation (Westinghouse FS40) centered in the light cycle. A 0.25 mm thickness of partially photodegraded cellulose acetate (CA) film served as a 290 nm cut-off filter. Photodegradation of the filter is exponential and, therefore, prior to use the film was photodegraded through the initial, rapid phase to create a filter whose transmittance was far more stable. The CA filter was changed every ten days. The result was a fairly constant fluence rate at the surface of the stock culture. The surface fluence rate in the 290-320 nm waveband was 0.84 W/m² and the fluence rate in the 320-700 nm region was 12.64 W/m². Fluence rate measurements in both the UV and visible wavebands were made with a modified Gamma Scientific 2900SR Spectroradiometer System which has been characterized by the U.S. National Bureau of Standards (Worrest et al., 1978). Short chains of *M. nummuloides* (4-10 cells/chain) from the stock culture were irradiated at 14°C on f/2 seawater agar plates (10 x 10 cm). The covers of the plates were modified to accommodate two pieces of partially photodegraded filter material (9.5 x 5 cm) so that different areas of each agar plate could simultaneously be exposed to two levels of UV-B radiation.

Prior to each experimental exposure the stock culture was mixed well and a portion was withdrawn and diluted in an erlenmeyer flask containing sterile f/2 medium. This inoculum was acid-shocked to lyse bacterial cells. Previous studies indicated that the bacteria present in the stock culture had a significant effect, over time, on the UV-response of the diatom grown on agar. The acid treatment consisted of a reduction of the pH of the inoculum to 2.8 for 10 minutes using 0.5N HCl followed by a titration back to the original pH of the inoculum using 0.5N KOH. Based on the statistical analysis of subsequent growth studies, this treatment did not appear to damage the diatom cells and it removed a variable from the interpretation of results. Portions of the acid-shocked inoculum were transferred to agar plates. These agar plates were not irradiated prior to inoculation since previous studies indicated such treatment had no significant effect on the growth of the diatom. The inoculum was spotted (0.05 ml/spot) on each plate such that each half-plate contained a row of three spots. All chains containing more than 10 cells were removed from each spot leaving approximately two chains per spot. In some cases long chains were cut to size using the point of a fine gauge hypodermic needle. Greater than 95% of all chains survived this transfer technique. The end result was ten plates with approximately twelve chains per plate. The number of cells in each chain was counted and the plates were covered with a 0.13 mm thickness of Mylar 'S' and placed on a turntable (16 RPM).

Daily exposure on the turntable began on the next morning following a recount of the chains spotted onto the agar plates. Visible radiation (Duro-Test 20W Vita-Lite) was 'on' from 0800 h to 1900 h. Supplemental UV radiation (Westinghouse FS20) was 'on' from 1000 h to 1400 h. The peak fluence rate at the surface of the agar through Mylar was 4.48 W/m² in the 320-700 nm region and approximately 0.1 W/m² in the 290-320 nm waveband. The chains were counted after two days under Mylar and the average growth rate (k) in divisions per day was calculated from

$$k = \frac{1}{t \log_e 2} \log_e \frac{N_1}{N_0}$$

where N_1 is the number of cells at the end of the time period, N_0 is the number of cells at the beginning of the experimental exposure, and t is the time in days.

The mean growth rates for the chains on each half plate were calculated. The 20 half-plates were then organized into four groups such that there was no significant difference among the four groups in the mean of the mean growth rates. One of these groups of half-plates was again covered with Mylar and placed on the turntable. The other groups were covered with one of three thicknesses of partially photodegraded cellulose acetate (0.13 mm, 0.25 mm, or 0.50 mm) before being returned to the turntable. The employment of Mylar plus the three thicknesses of cellulose acetate, along with two different distances between the source of UV-B radiation and the surface of the agar, provided eight levels of UV-B fluence rates ranging from 3.12×10^{-2} W/m² to 2.05 W/m². The fluence rate in the 320-700 nm waveband was 4.48 W/m² in all cases. After an additional three days of exposure to the simulated solar spectrum, the chains were counted a fourth time and the growth rate for each chain was calculated. Analysis of variance was used to test the significance of the various levels of UV-B treatment.

Johnson et al. (1976) have calculated values for the daily average global UV-B fluence at the earth's surface as a function of wavelength, latitude, and season, for clear sky and seasonally- and latitudinally-averaged ozone concentrations. These tabulations can be utilized to estimate the UV-B radiant energy incident on selected surface locations for a clear sky at any time of year. Average corrections for the degree of cloudiness are referenced and easily introduced. More recent calculations have also been made available by A. E. S. Green, K. R. Cross, and L. A. Smith (personal communication).

However, the global UV-B fluence estimate still does not permit the direct comparison of the experimental UV-B exposure to that actually occurring in nature. Expressing the radiation fluence in terms of total energy for a defined waveband does not specify the fluence present for each wavelength within the waveband, which is a characteristic dependent upon the radiation source or other factors. The energy distribution within the UV-B waveband will differ when the filtered sunlamp and solar spectrum are compared (Fig. 2). At 290 nm there may be a greater fluence rate under the sunlamps than for solar radiation at 45°N latitude. For UV-B hazard analysis, the nonequivalent distribution of energy between radiation sources must be carefully considered since each wavelength within the UV-B waveband may differ in its degree of effectiveness in producing a specific biological endpoint. Weighting the fluence rate at a particular wavelength by its relative effectiveness over all UV-B wavelengths yields the effective surface fluence rates within the UV-B waveband and it permits comparisons of the two radiation regimes. To compare the effectiveness of the experimental radiation to natural sunlight a DNA action spectrum (Setlow, 1974) was used as the biological weighting function. Using a least squares method, a regression of growth rate on the biologically weighted UV-B fluence was used to determine the overall significance of UV treatment.

Results

Analysis of variance indicates differences in growth rates of *M. rotunda-loides* to be significant ($p < 0.001$) for all exposure levels. With increased exposure to UV-B radiation a significant depression of the growth rate occurred (Fig. 3). Least squares regression analysis resulted in the following relationship,

$$\log_e (\text{GROWTH RATE [DIV./DAY]}) =$$

$$-0.544 - 0.00389 (\text{WEIGHTED DAILY FLUENCE [EFF}_{\text{DNA}} \text{ J/m}^2/\text{d}]),$$

with a slope significantly different from zero ($p < 0.001$).

Discussion

UV-B radiation (e.g. 310 nm) penetrates approximately the upper 10% of the marine euphotic zone before it is reduced to 1% of its surface fluence rate (Jerlov, 1976). For clear oceanic waters this may be to a depth of approximately 30 meters. Any increase in the surface fluence resulting from a diminution of the stratospheric ozone concentration would result in a comparable increase in the UV-B fluence throughout the entire water column. As stated by Steemann Nielsen (1964) and Jitts et al. (1976), there is good evidence that present

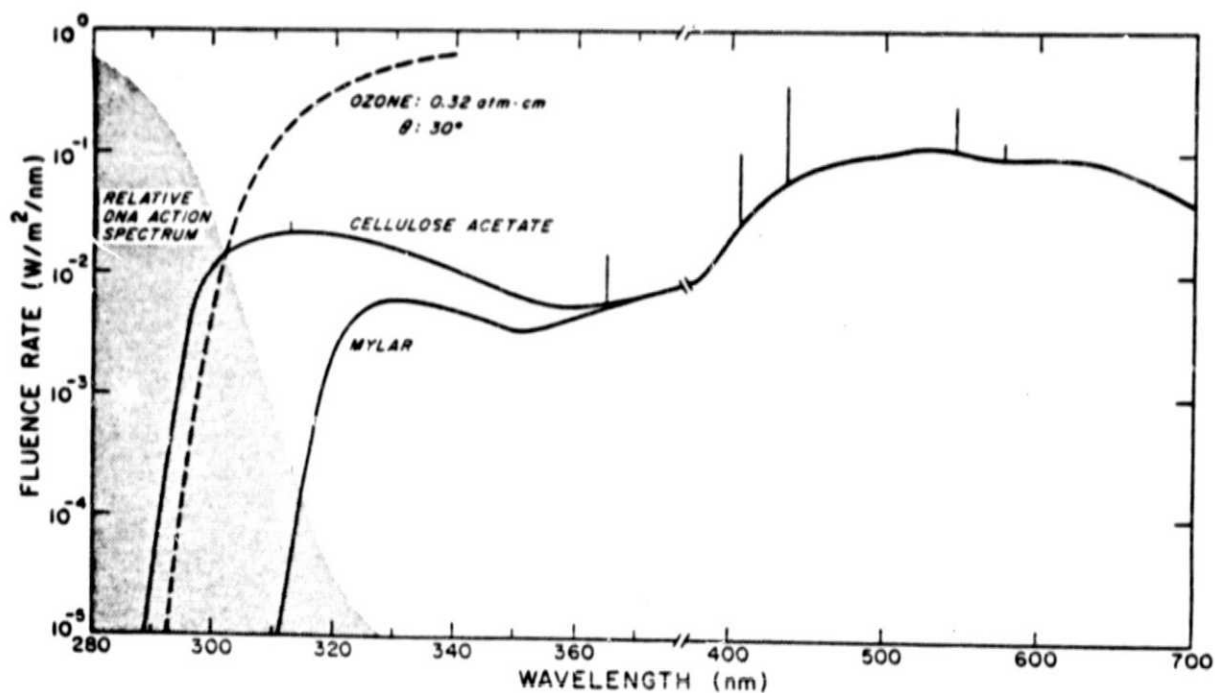


Fig. 2. Sample spectra of deluxe-white fluorescent lamps plus sunlamp/filter systems utilizing two different filters: (1) a 0.25 mm thickness of cellulose acetate, and (2) a 0.18 mm thickness of Mylar polyester film. The dashed line represents a solar global spectrum as calculated by Green et al. (1974). The shaded area represents an analytic representation of the long-wavelength tail of a DNA action spectrum as calculated by Green and Miller (1975).

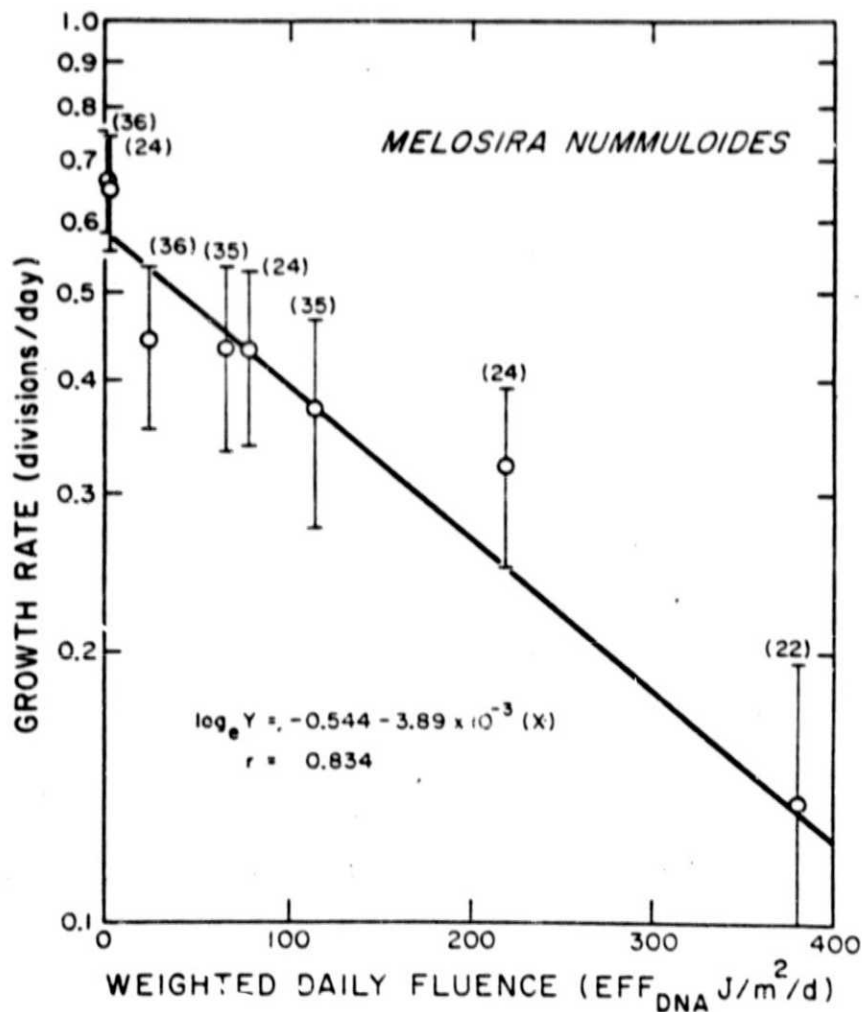


Fig. 3. Regression curve representing the effect of UV-B radiation upon the growth rate of *Melosira nummuloides*. The UV-B fluence was weighted at each wavelength in the 290-320 nm region by a relative DNA action spectrum (Setlow, 1974). Mean growth rates (\pm st. dev.) are indicated at each experimental daily fluence. Figures in parentheses represent total number of chains exposed at each fluence.

levels of ultraviolet radiation depress the primary productivity in natural marine waters and this must be taken into consideration in productivity and growth rate studies.

The solar spectrum at the earth's surface (30° zenith angle) contains approximately three orders of magnitude less energy at 295 nm when compared to the quantity of energy at 320 nm (Fig. 2). However, the biological effectiveness of 295 nm photons, based on DNA damage, is approximately four orders of magnitude more effective than 320 nm photons. When these two considerations are accounted for, in addition to the fact that radiation with wavelengths longer than 320 nm would not be greatly affected by an ozone decrease, the importance of the UV-B waveband becomes apparent.

World-wide average, natural fluence rates have been calculated by Green, Cross, and Smith (personal communication). Table 6 provides both the daily absolute fluence and the daily biologically weighted (effective) fluence of the natural spectrum at selected latitudes and times. This table was compiled by applying an analytic representation, as calculated by Green and Miller (1975), of the long-wavelength tail of a DNA action spectrum (Setlow, 1974) to the data of Green, Cross, and Smith. Most of the UV-B fluences used in the experimental exposures were representative of the effective surface fluences, in nature, over the distributional range of this species of diatom.

As stated earlier, *M. nummuloides* is a common marine littoral diatom. Its world-wide distribution is an indication of its tolerance to a wide variety of environmental conditions. It was noted to be highly tolerant of salinity and temperature fluctuations by Wulff and McIntire (1972). Also, Moore and McIntire (1977) attributed a relatively wide niche breadth to this species. The present investigation defines the growth response of *M. nummuloides* through a three-order magnitude increase in the effective UV-B fluence.

An example of the effect of a diminution of the stratospheric ozone layer upon the growth rate of *M. nummuloides* can be determined from Figure 3. A calculated daily UV-B surface fluence for 30°N latitude in June is approximately 141 EFFDNA J/m². At this level of exposure a 15% increase in effective fluence (NAS, 1976) would result in an 8% decrease in the growth rate of *M. nummuloides*. A 32% increase in effective fluence (from a recent scenario presented at the Symposium on the Geophysical Aspects of Consequences in the Composition of Stratosphere, World Meteorological Organization, 1978) would result in a 16% decrease in the growth rate.

Using the diffuse attenuation coefficient calculated by Smith and Baker (1979) at 310 nm ($K_{310} = 0.86$) for moderately productive waters (0.5 mg Chl a · m⁻³) with "abnormally high" concentrations of dissolved organic material relative to Chl a (water type 4) and those coefficients described by Jerlov (1976) at 310 nm for water type III ($K_{310} = 0.65$) and type 1 ($K_{310} = 1.77$) we may assume that water type 4 described by Smith and Baker has optical properties in the photosynthetically active region which lie somewhere between Jerlov types III and 1. The depth of the euphotic zone for these water types is approximately 39 m. Based on an article by Small et al. (1972), Zaneveld (1975) has presented data which shows that approximately 12.9% of the productivity off the coast of Oregon (water type II or III) is found in the upper two meters. The biologically effective attenuation coefficient (K_{DNA}) for water type 4 is approximately

Table 6. Representative daily, absolute and biologically weighted, fluences for the distributional range of *Melosira nummuloides*, based on calculations by A.E.S. Green, K.R. Cross, and L.A. Smith (personal communication) and D.S. Nachtwey (personal communication).

Latitude	Month	Daily fluence [290-320 nm]	Weighted daily fluence
		(J/m ²)	(EFF _{DNA} J/m ²)
0° N	Jun	1.27 x 10 ⁵	125.7
	Sep	1.56 x 10 ⁵	189.9
30° N	Jun	1.56 x 10 ⁵	141.1
	Sep	1.09 x 10 ⁵	90.9
60° N	Jun	1.07 x 10 ⁵	53.2
	Sep	2.88 x 10 ⁴	9.22

0.92 m^{-1} for present ozone concentrations (Smith and Baker, 1979). Based on the current productivity study with *M. nummuloides*, integration of the percent reduction in productivity for all depths throughout the upper two meters of typical coastal waters, resulting from a 15% ($K_{DNA} = 0.94$) and 32% ($K_{DNA} = 0.96$) increase in surface UV-B fluence, yielded an average decrease of 3.3% and 6.8%, respectively. To determine the *total* effect of enhanced levels of UV-B radiation upon phytoplankton productivity more data must be gathered relating UV-B penetration to the partitioning of phytoplankton within the euphotic zone.

This study has shown that the growth of this diatom, a tolerant species by many ecological definitions, is significantly affected by UV-B radiation. It seems quite likely, therefore, that many other organisms would be similarly affected and that UV-B radiation should be taken into consideration as a naturally occurring environmental stress factor.

UV-B SENSITIVITY OF *ACARTIA CLAUSII*

Introduction

Copepods identified as *Acartia clausii* Giesbrecht have been documented as a dominant form in coastal and estuarine zooplankton communities throughout the northern hemisphere (Riley 1967; Carillo et al. 1974). A recent revision of the *Acartia* subgenus *Acartiura* distinguishes between *A. clausii* found on the North American west coast and the organism originally described by Giesbrecht (Bradford 1976). Due to large variation in the organisms examined, West Coast forms have been collectively called *Acartia* sp. Until this species is more clearly identified, organisms used in this study will be referred to as *A. clausii*. It is nonetheless representative of the genus *Acartia*, which has a worldwide distribution (Bradford 1976). *Acartia* has also been documented as an important food source for a number of pelagic fishes and invertebrate predators (Petipa 1958; Brodskii 1967; Heinle 1974; Landry 1976). Due to its large population size and relative importance as a converter of phytoplankton into food suitable for higher organisms, the impact of both current and enhanced levels of UV-B radiation upon this organism have been assessed.

Methods

A. clausii was collected from Yaquina Bay, Oregon (44°37'N, 124°02'W). Cultures were maintained in sterilized three-liter glass containers in a controlled temperature regime of 15°C (\pm 2°C SD), under a diel photoperiod of 8L:16D, with a visible (380-700 nm) fluence rate of 0.06 Wm⁻². The organisms were cultured and irradiated in sterilized seawater, salinity varying from 25 to 29‰. At two-day intervals the copepods were fed a mixture of *Isochrysis galbana*, *Thalassiosira pseudonana*, and *Platymonas suecica*, in a concentration of about 400,000 cells ml⁻¹. On every eighth day the animals were transferred to fresh sterilized seawater. A detritus- and bacteria-feeding ciliate, *Euplotes* sp., similar to the organism mentioned by Zillioux (1969), was also present in all stock cultures. All glassware was originally leached, washed after use with distilled water only, and sterilized before reuse.

The twelve instars of the *Acartia* life history were divided into five groups: nauplii 1 and 2 (N1-N2); nauplii 3 and 4 (N3-N4); nauplii 5 and 6 (N5-N6); copepodites 1, 2 and 3 (C1-C3); and copepodites 4, 5 and adults (C4-adult). It was the intent of this investigation to determine the sensitivity of these five developmental phases to UV-B irradiation.

Adequate numbers of unhatched fertilized eggs were obtained by introducing stock cultures acclimated to 15 \pm 2°C to warmer conditions (19 \pm 1°C), allowing the water to fully adjust to the new higher temperature, separating a substantial number of adults (\approx 200), placing them in sterile seawater at the higher temperature, and providing ample food (200,000 cells ml⁻¹ of *I. galbana*). At the end of a 12-h period, the bottom of the beaker containing the adults was frequently littered with eggs. At this time many eggs

were at different physiological stages of development, and hatching would not have been simultaneous. Simultaneous hatching was essential to ensure a large number of copepods of the same developmental stage. Landry (1975a) demonstrated that development of *A. clausii* eggs in the dark is arrested just prior to hatching. Subsequent exposure to light permits hatching in a near-synchronous burst. Following a pattern which permitted full development (Landry 1975b), experimental eggs were placed in a light-secure container and incubated at 19°C for 42 h.

Soon after being returned to the light, N1 nauplii were separated from unhatched eggs. These animals were reared to the desired stage for all naupliar age groups. Large numbers of the copepodite age groups (C1-C3, C4-adult), easier to sort by developmental stage, were selected directly from cold-acclimated stock cultures. The water temperature for these animals was adjusted to $19 \pm 1^\circ\text{C}$ over a 24-h period. Equal numbers of males and females were separated for the C4-adult age group. Prior to irradiation, a representative sample (30) of each group of copepods was preserved using 10% buffered formaldehyde solution. Samples were examined microscopically to confirm the use of the desired age group for the planned irradiation.

The radiation source consisted of four fluorescent Westinghouse FS40 sunlamps, with a peak emission at 310 nm, interspersed with four "deluxe white" fluorescent lamps (Vita-Lite, Duro-Test Corp., North Bergen, NJ). Since the power output of new sunlamps declines rapidly before attaining a slower rate of change, the tubes were aged prior to use for this investigation. Organisms to be exposed to the enhanced UV-B levels were placed under photooxidized 0.25 mm cellulose triacetate sheets (Kodacel-TA 401). The Kodacel reduces transmission of wavelengths shorter than 290 nm to a non-detectable level, providing a spectrum enriched with UV-B radiation. The organisms to be shielded from UV-B were placed under 0.25 mm thick polyester sheets (Mylar 'S') which detectably transmit only wavelengths longer than 135 nm. Fluence rate measurements at the water surface of the exposure chambers were made with a modified Gamma Scientific 2900 SR Spectroradiometer System (Worrest et al. 1978). The fluence rate at the surface of the water in the 290-320 nm waveband was 0.02 Wm^{-2} under the Mylar and $1.42 \pm 0.09 \text{ Wm}^{-2}$ under Kodacel (Fig. 4).

Each experiment employed copepods of a specific age group. Individuals to be irradiated were pipetted into a circular glass exposure chamber (5 cm diameter, 1.5 cm depth) containing 20 ml of sterile seawater and *I. galbana* ($50,000 \text{ cells ml}^{-1}$). All organisms were irradiated on a rotating turntable (16 rpm) for a predetermined time corresponding to the fluence they were to receive (using the known fluence rate beneath the Kodacel). The UV-B fluence rate was measured prior to each experiment and subsequent treatment times calculated accordingly. The controls, also on the turntable, were shielded by Mylar, preventing any significant UV-B irradiation. All experimental chambers, except those receiving the maximum treatment for that age group, were initially covered with Mylar filters. These were removed at appropriate intervals during irradiation and replaced with Kodacel until the end of the experiment. Irradiation time necessary for a desired fluence could be predicted using the known UV-B fluence rate under Kodacel. With the exception of the C4-adult age group, all treatment groups and controls remained on the turntable for the duration of the longest exposure time for that age group.

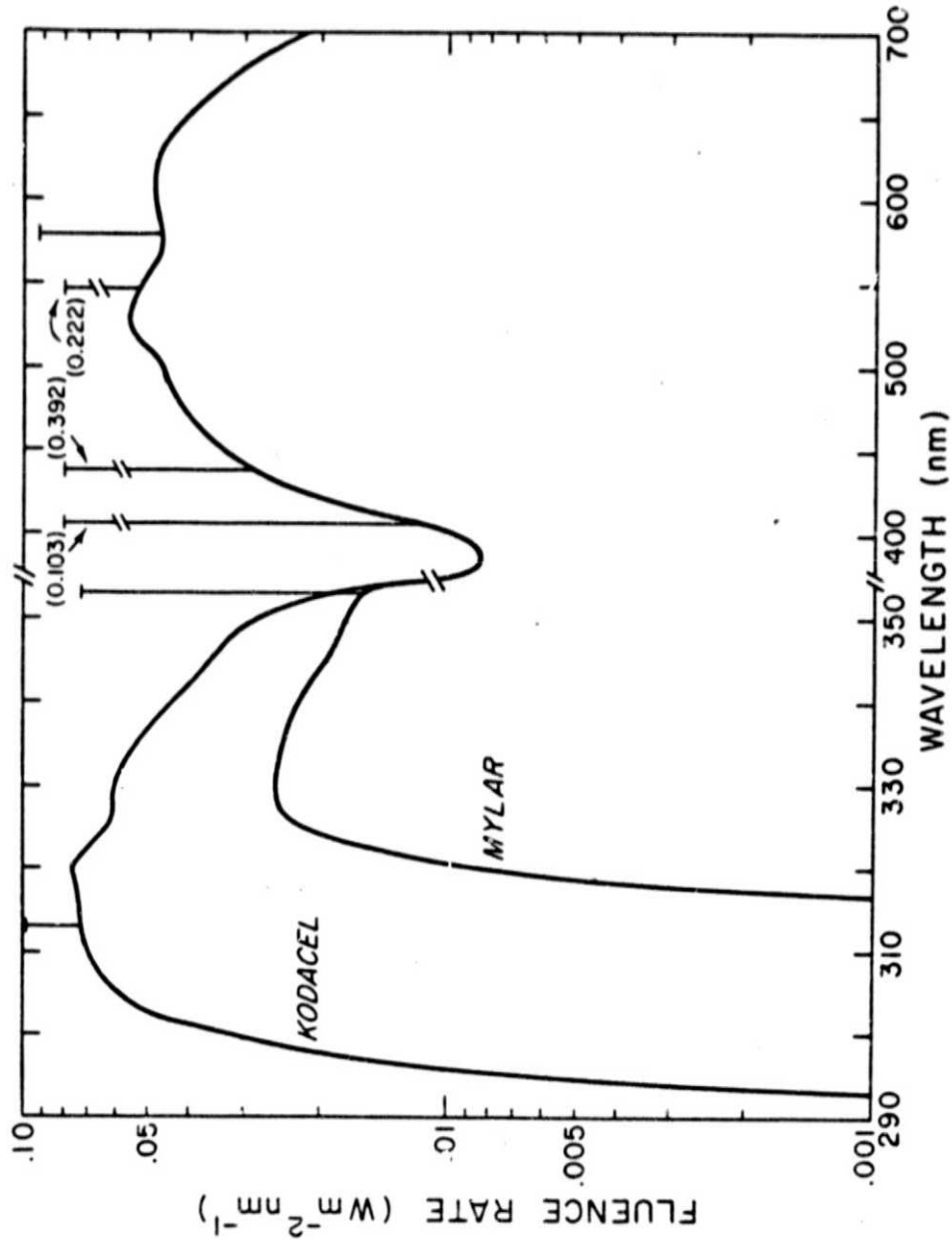


Fig. 4. Fluence rate spectra of experimental conditions. Fluence rate was measured at the water surface of the exposure chambers by a modified Gamma Scientific 2900 SR Spectroradiometer System. Spectral fluence rate in the 290-320 nm waveband under Mylar was 0.02 Wm^{-2} and under Kodacel was $1.42 \pm 0.09 \text{ Wm}^{-2}$. Characteristic emission lines of fluorescent lamps used are illustrated.

Those organisms of the C4-adult age group receiving 26.89 kJm^{-2} of UV-B were irradiated at a later date, and with a separate set of controls. This later experiment was performed to provide survival data in the high lethality region not ascertained by the first series of experiments (see C4-adult data -- Table 7). Control survival did not differ significantly between the two series ($p > 0.05$). All other members of this age group were on the turntable for the length of time necessary for the 16.46 kJm^{-2} treatment.

Exposure chambers were not placed randomly on the turntable but were distributed equidistant from the center among either two or three sections of equivalent area. One representative dish for each treatment to be administered to the copepods was placed in each section. In this way, position of the exposure chamber on the turntable was introduced as a "block variable" (three sections would designate three levels of block). The number of blocks per age group ranged from three to five. The copepods within a block were randomly assigned to treatments, constituting a randomized complete block for a two-factor design. Survival data are presented in Table 7.

The water temperature within the exposure chambers prior to irradiation was $18 \pm 0.5^\circ\text{C}$. Once animals were placed beneath the lamps, the rate of temperature increase was approximately $1.35^\circ\text{C h}^{-1}$ (essentially the same under both filters: Yellow Springs Inc. Teletherometer and Thermistor). As the maximum exposure time differed between age groups, so did the thermal range to which experimental animals were exposed. The maximum treatment for the N1-N2 age group, 7.25 kJm^{-2} (Table 7), required an exposure time of 80 minutes at the fluence rate listed for that age group (Table 8). Thus, N1-N2 nauplii were subjected to a temperature regime of $18-20^\circ\text{C}$ during irradiation. The thermal range experienced by each age group was as follows: N3-N4, $18-21^\circ\text{C}$; N5-N6, $18-20^\circ\text{C}$; C1-C3, $18-22^\circ\text{C}$; C4-adult (16.46 kJm^{-2} max), $18-22^\circ\text{C}$; C4-adult (26.89 kJm^{-2}), $18-25^\circ\text{C}$.

A fact which must be considered during review of the discussion is that *A. clausii* is a cold-water, spring species primarily found in systems at $10-15^\circ\text{C}$. Although surface water temperatures of some regions in which *A. clausii* distribution is reviewed approach 20°C while the animal is still abundant, the major portion of the population is not likely to receive significant amounts of incident UV-B at the water temperatures recorded for this experiment. Several references have described both the optimal and maximal thermal range of *A. clausii* for a wide variety of environmental and laboratory conditions (Conover 1956; Deevey 1960; Jeffries 1962; Anraku 1964; McLaren et al. 1969; Gonzalez 1974; Landry 1975b). Most authors concur that the upper limit of the organisms's optimal thermal range is about 20°C . Anraku, however, suggests metabolic stress may begin as low as 18°C .

It should be noted that the thermal range to which all age groups were subjected during irradiation is in the region of the upper thermal limit of *A. clausii*. Although the critical thermal maxima and upper lethal temperature for *A. clausii* acclimated to recorded temperatures, as determined by González (1974), were not encountered, and though the controls were exposed to the same thermal range as experimentals, the possibility of a synergistic action may have existed for organisms receiving UV-B irradiation.

Table 7. Survival data in block form with the mean surviving proportion of, and total fluence (290-320 nm) for, each treatment group.

Age group	Sample size	Total fluence (kJm ⁻²)	Survivor blocks					Mean surviving proportion
N1-N2	30	0.10	27	23	18	22	24	0.76
		1.82	14	16	10	16	9	0.43
		3.61	13	10	7	10	7	0.31
		5.43	3	5	4	5	4	0.14
		7.25	4	2	2	2	4	0.09
N3-N4	40	0.14	33	35	35			0.86
		2.55	33	28	29			0.75
		5.10	30	26	25			0.68
		7.66	15	16	18			0.41
		10.21	11	10	14			0.29
N5-N6	22	0.10	19	18	17	19		0.83
		1.62	17	12	13	14		0.64
		3.24	15	11	9	9		0.50
		4.86	6	9	5	7		0.31
		6.47	9	6	3	3		0.24
C1-C3	30	0.22	28	24	22			0.82
		3.61	14	19	18			0.57
		7.22	16	11	11			0.42
		10.81	10	11	8			0.32
		14.42	6	5	4			0.17
C4-adult female	15	0.22	6	8	9	10	10	0.57
		4.11	8	8	6	7	10	0.52
		8.24	6	5	7	7	7	0.43
		12.35	7	7	4	5	6	0.39
		16.46	6	5	7	3	6	0.36
		0.36	10	10	5	9	8	0.56
		26.89	0	1	0	1	1	0.04
		0.22	6	7	10	11	8	0.56
		4.11	4	5	5	6	6	0.35
		8.24	4	3	7	5	6	0.33
12.35	3	3	1	2	4	0.17		
16.46	3	3	1	3	3	0.17		
0.36	10	9	8	12	6	0.60		
26.89	0	1	0	0	0	0.01		
C4-adult male	15	0.22	6	7	10	11	8	0.56
		4.11	4	5	5	6	6	0.35
		8.24	4	3	7	5	6	0.33
		12.35	3	3	1	2	4	0.17
		16.46	3	3	1	3	3	0.17
		0.36	10	9	8	12	6	0.60
		26.89	0	1	0	0	0	0.01

Table 8. Comparison between the daily effective Setlow (Eff_{DNA}) or Caldwell (Eff_{GEN}) fluence (290-320 nm) for a typical spring-summer day at 45°N latitude for current (0.32 cm) and depleted (0.28, 0.24 cm) ozone concentrations (Shettle et al. 1975) and the effective fluence required to elicit a 50% reduction of laboratory populations.

Age group	Absolute LD50 fluence (kJm^{-2})	Absolute fluence rate (Wm^{-2})	LD50 time (s)	LD50 Eff_{DNA} fluence (Jm^{-2})	LD50 Eff_{GEN} fluence (Jm^{-2})
N1-N2	3.75	1.51	2483	28.06	235.64
N3-N4	7.71	1.42	5430	61.36	515.31
N5-N6	4.32	1.33	3248	36.70	308.24
C1-C3	8.90	1.34	6642	75.05	630.33
C4-ad	14.31	1.52	9414	106.38	893.39

Daily surface fluence -- Average clear spring-summer day at 45°N latitude

Ozone (atm-cm)	Daily Eff_{DNA} surface fluence (Jm^{-2})	Daily Eff_{GEN} surface fluence (Jm^{-2})
0.32	55.9	963
0.28	79.4	1250
0.24	113	1610

Following irradiation the animals were placed in sterilized 600 ml beakers containing sterile seawater and maintained at $19 \pm 1^\circ\text{C}$ under a diel light cycle of 14L:10D (0.06 Wm^{-2} , 380-700 nm). Each beaker contained an initial concentration of 200,000 cells ml^{-1} of *I. galbana* and was aerated by a micropipette. Organisms were then reared to reproductive maturity, the survivors filtered to a smaller vessel and counted. Postirradiated N1-N2 nauplii required 14 days to reach adulthood. Survivors of the C4-adult age group were counted one week after their estimated attainment of sexual maturity, permitting the production of offspring. With irradiation of equal numbers of males and females in this age group, appraisal of UV-B effects was extended to include an examination of fecundity. The number of offspring produced per exposure replicate was recorded along with the number of survivors. The reduced survival evident for the C4-adult controls requires consideration. Since organisms of this age group were counted one week after their estimated attainment of sexual maturity, they were thus subject to death from other causes for one week longer than were the controls of the other age groups. The copepods had approached their mean life expectancy by the time of counting.

Two-factor analysis of variance was performed, testing the equality of group means within treatments and blocks. Analysis indicated nonsignificant differences ($p > 0.05$) between replications (blocks) for all age groups except N5-N6. This permitted the combining of replicates and a more accurate estimate of surviving fractions. The indication of increased differences between blocks for N5-N6 age group may be a result of the reduced sample size used in those experiments ($n=22$ per replication of each treatment versus $n \geq 30$ per replication of each treatment). Nevertheless, the N5-N6 group received the same statistical treatment as all other age groups. Least-squares regression analysis was performed between surviving fraction and total fluence for all treatments administered to each age group and also between the offspring/survivor ratio and total fluence for treatments administered to the oldest age group. The surviving fractions of males and females for each treatment within the C4-adult age group were regressed separately with fluence to compare UV-B sensitivity between sexes.

Results

The survival curves depicted in Figs. 5 and 6 illustrate an inverse relationship between exposure to UV-B radiation and the surviving fraction of *A. clausii*. Points on each curve represent the ratio between total surviving number for each treatment (fluence) for all blocks within an age group and total number of controls surviving for all blocks within the same age group. Ninety-five percent confidence intervals were constructed for these surviving fractions based on random large samples of a binomial distribution (Ingram 1974).

Analysis of variance indicates differences in survival between treatments to be significant ($p < 0.001$) for all age groups. This indicates a significant difference in the response of copepods to various fluence levels. However, to identify differences between any two particular treatments within an age group would require further testing.

Figure 7 represents least-squares regression analysis between surviving fraction and total fluence for all age groups. All equations have significant

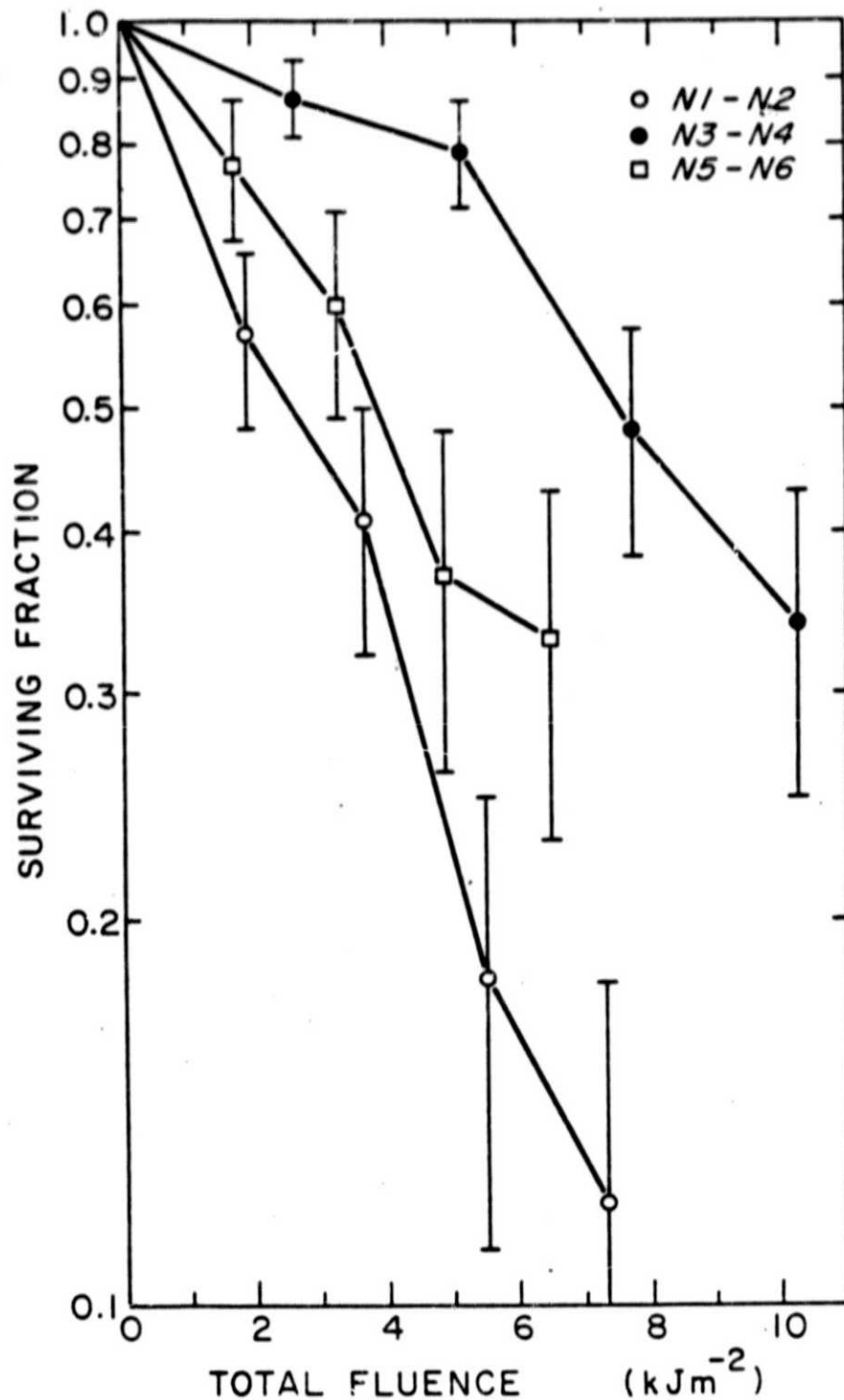


Fig. 5. Relative survival curves for the naupliar stages of development of *A. clausii*. Sample sizes and UV-B fluence rates were: N1-N2 (n = 30/replication of each treatment, 1.51 Wm⁻²; N3-N4 (n = 40/rep.), 1.42 Wm⁻²; N5-N6 (n = 22/rep., 1.33 Wm⁻². 95% confidence intervals of surviving fractions are shown.

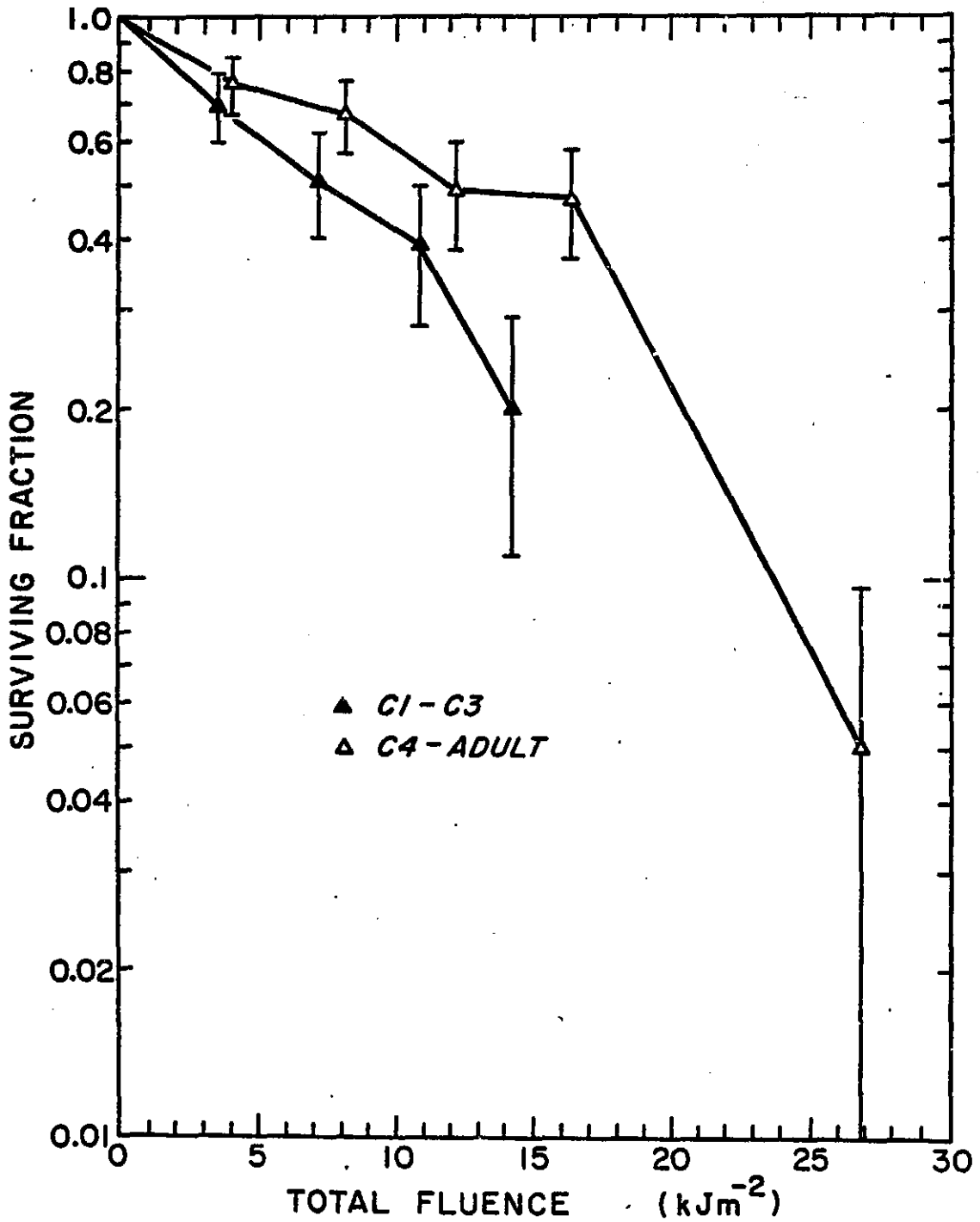


Fig. 6. Relative survival curves for copepodite age groups of *A. clausii*. Sample sizes and UV-B fluence rates were: C1-C3 (n = 30/rep.), 1.34 Wm⁻²; C4-adult (n = 30/rep.), 1.52 Wm⁻². Each C4-adult exposure group contained 15 males and 15 females. 95% confidence intervals of surviving fractions are illustrated.

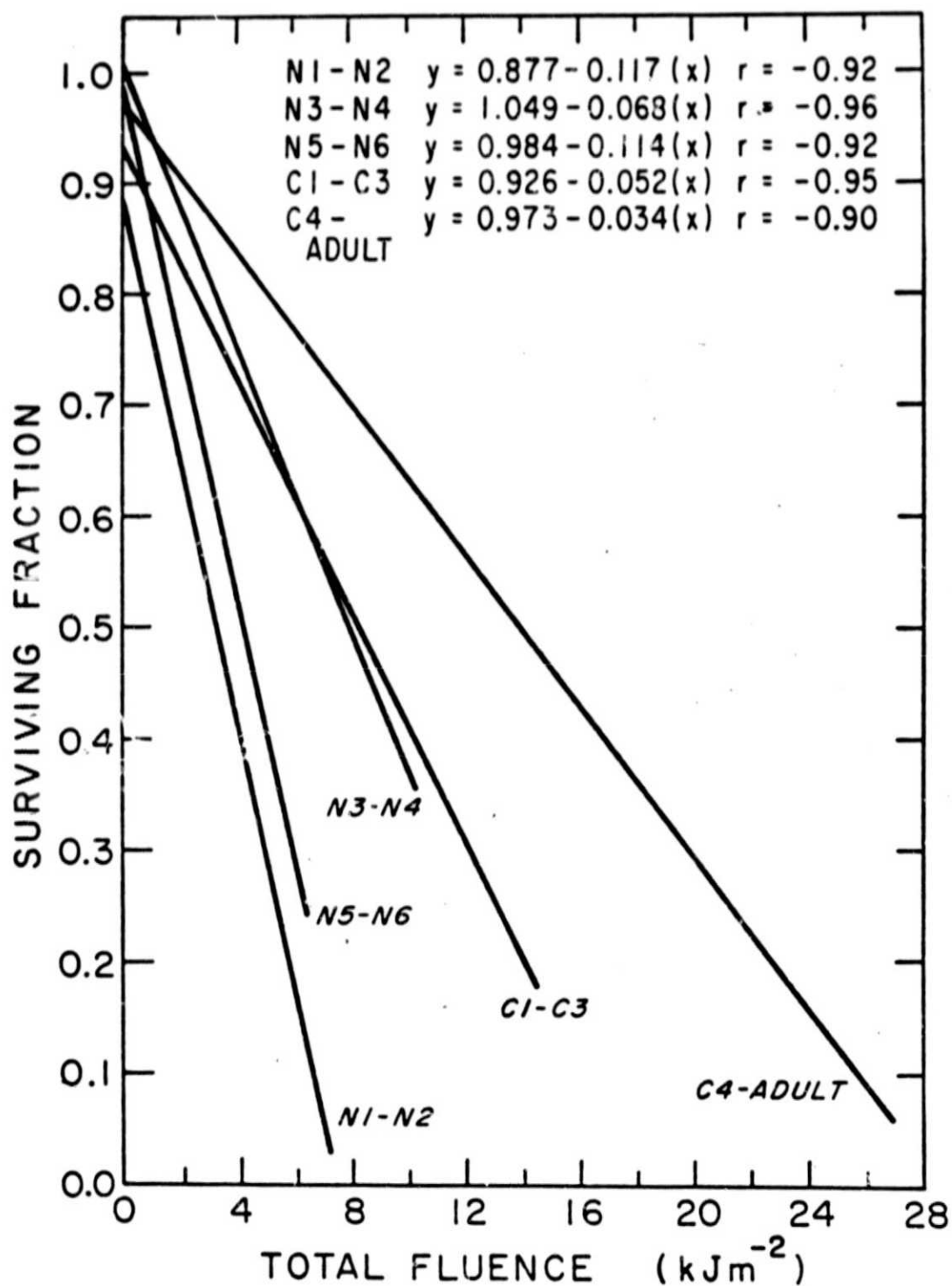


Fig. 7. Least-squares regressions between surviving fraction and fluence for five developmental phases of *A. clausii*. Regression model with correlation coefficient shown for each age group. Slopes of all regressions are significant ($p < 0.01$).

slopes ($p < 0.01$) indicating that, with increase in fluence, there was a significant decrease in the surviving fraction. Data was regressed in its original form, although transformations improved both the N1-N2 and N3-N4 models. Best fit for all other age groups was attained with untransformed data, so for the purpose of comparison the data for these five regressions were not transformed.

With one exception the comparison of slopes indicates significant differences ($p < 0.05$) between all combinations of any two of the five regressions. The slopes of N1-N2 and N5-N6 exhibit no statistical difference ($p > 0.05$).

Differing sensitivity can be most accurately assessed if the post-irradiation treatment of each age group is the same. However, since the copepods were cultured to maturity before counting, treatments were identical except for the duration of incubation, which varied depending on the age of the organism at the time of irradiation. Determination of the number of organisms reaching a stage capable of reproduction was the intended objective of the investigation, as it provides an ecologically relevant view of how the reproductive capacity of the species may be affected in nature. If incubation time before counting were indeed a relevant variable, one might expect the proportion of controls surviving for the older age groups to be higher than that of the earlier naupliar controls. As can be seen in Table 7, control survivorship did not differ significantly ($p > 0.05$) between any combination of naupliar and C1-C3 age groups. The lower survival of the C4-adult controls is discussed in the Methods section.

The irradiation of equal numbers of males and females for each treatment within the C4-adult age group provided the opportunity to test for differential sensitivity between sexes. The fraction of the original fifteen animals (male or female) surviving each exposure was regressed with total fluence. This provided the two least-squares regressions illustrated in Fig. 8:

$$\text{Male: } y = 0.506 - .023 (x)$$

$$\text{Female: } y = 0.566 - 0.014 (x)$$

Regression coefficients were compared to zero and to each other. Both slopes were significantly negative ($p < 0.01$). No significant difference existed between the regression intercepts ($p > 0.05$, the surviving fraction for zero fluence), indicating no significant difference in survival between males and females protected from the UV-B radiation. However, a statistical difference between the slopes of the two regressions was evident ($p < 0.05$), which may demonstrate that, per unit increase in UV-B fluence, the males are more sensitive than the females.

Another negative response that occurred with supplemental UV-B irradiation involved reduced offspring production. Figure 9 illustrates a least-squares regression between the offspring/survivor ratio and fluence for the C4-adult age group. Two-factor analysis of variance was performed, testing the equality of group means within blocks and treatments. The analysis indicated nonsignificant differences ($p > 0.05$) between replications (blocks) and significant differences ($p < 0.001$) between treatments. The best fit of the regression was with a natural log transformation of the original data, for which the slope was significantly different from zero ($p < 0.01$). As described in the Methods section, the C4-adult organisms receiving 26.89 kJm^{-2} of UV-B were irradiated at a later date than other organisms for that age group. Due to low survival and zero offspring production for this treatment group, as well as the paucity of data in the high lethality

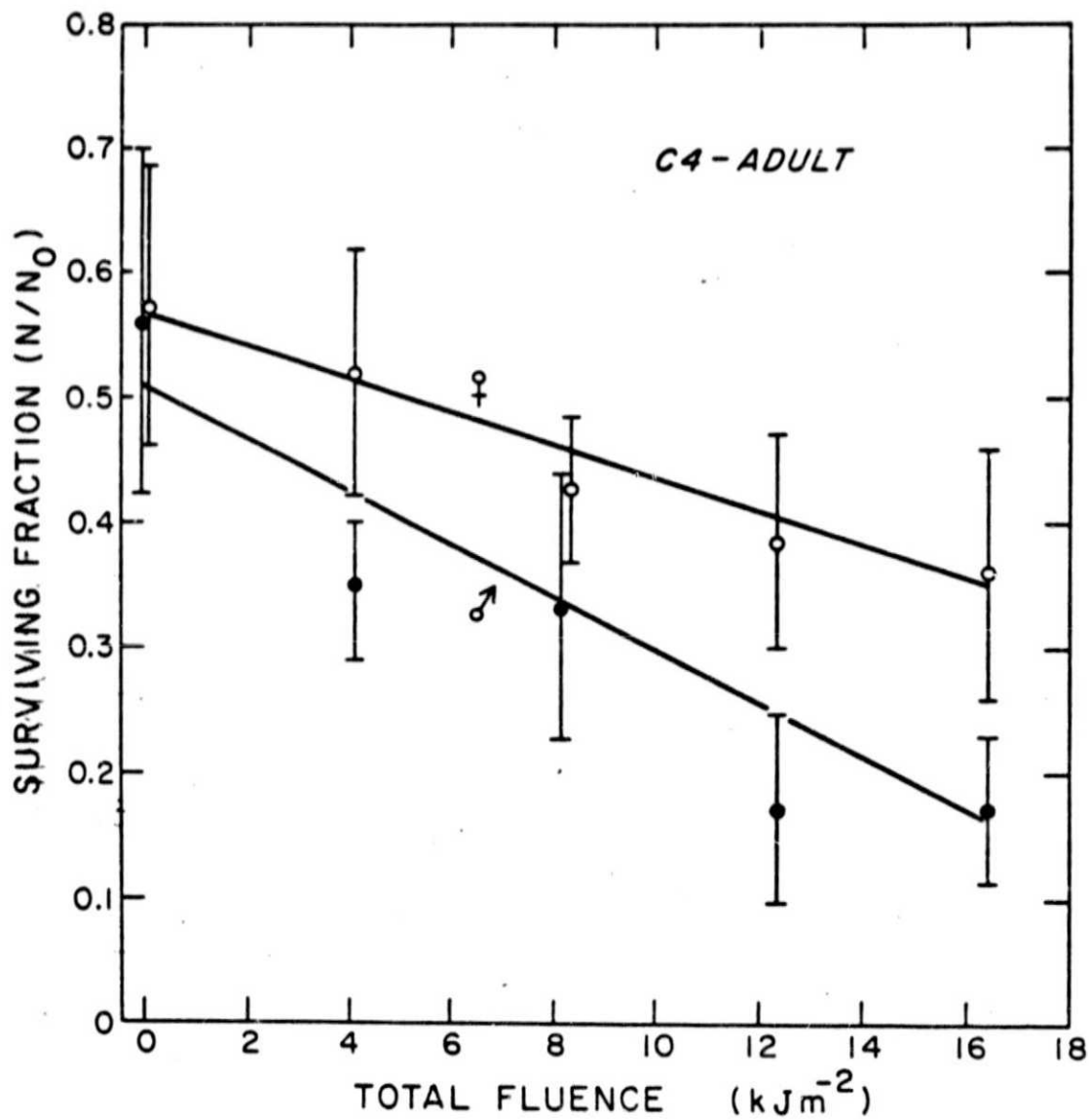


Fig. 8. Least-squares regressions between male or female surviving fractions and fluence after exposure to the C4-adult age group. Regression intercepts are not significantly different ($p > 0.05$). Slopes of the regressions (males = -0.023 , females = -0.014) differ significantly ($p < 0.05$). Mean and standard deviation of original data are shown. UV-B fluence rate, 1.52 Wm^{-2} .

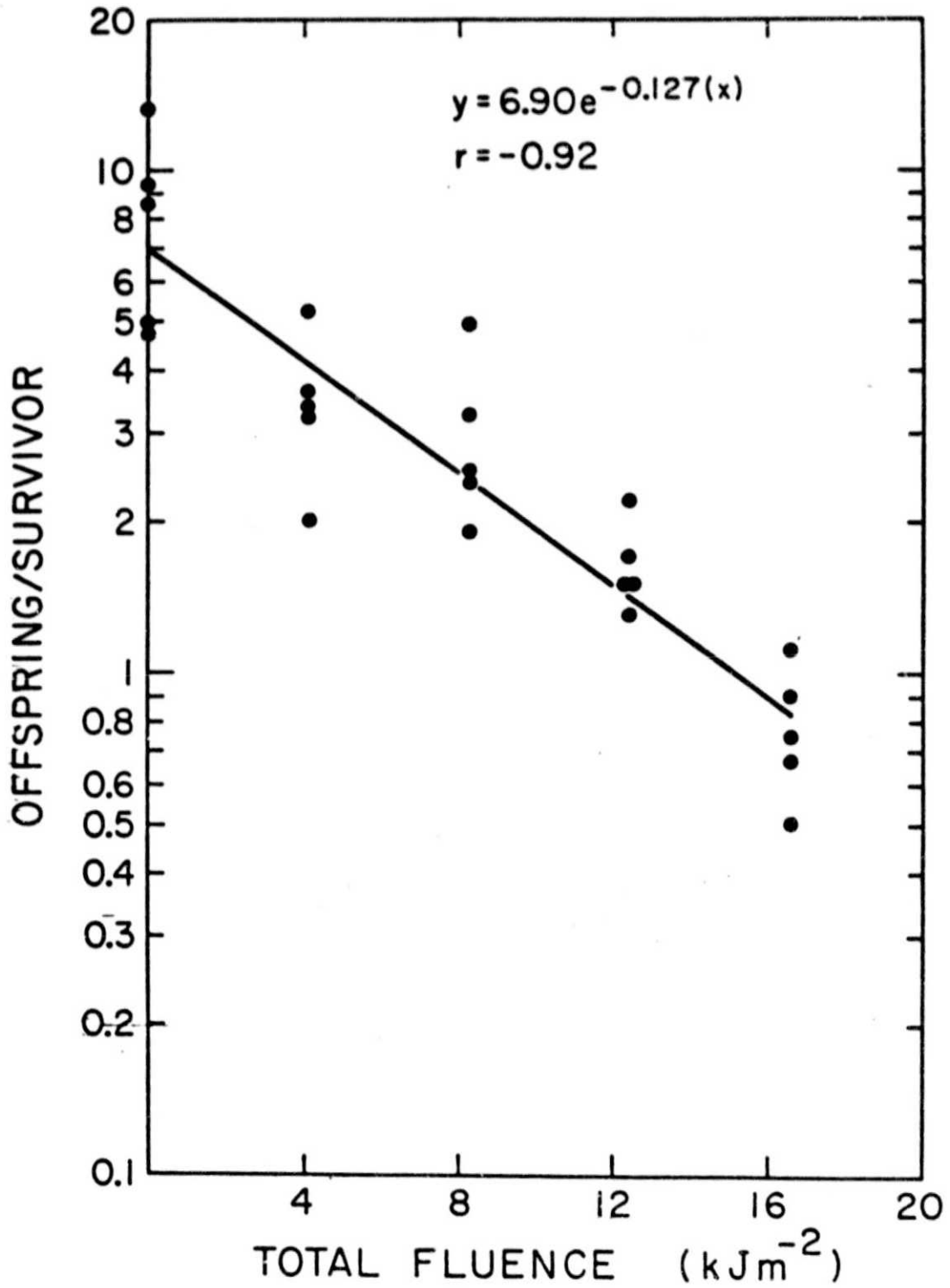


Fig. 9. Relationship between the number of offspring produced per survivor of the irradiated C4-adult age group and fluence as fitted by a least-squares regression. Original data are shown.

region for this age group, results for the $26,89 \text{ kJm}^{-2}$ treatment were omitted from regressions in Figs. 8 and 9.

Discussion

Results indicate that UV-B exposure, for the experimental conditions described, negatively affects both the survival and the reproduction of *A. clausii*. Among the groups of copepods exposed to UV-B there was a marked decrease in the number of second generation organisms produced per surviving adult (Fig. 9). This suggests that even when a copepod survives exposure to UV-B radiation, the ability to reproduce suffers. The combination of reduced survival and decreased reproduction indicates a potential for an adverse effect on this integral part of the marine ecosystem.

Results of this investigation also indicate a direct relationship between age of the organism and ability to tolerate UV-B irradiation. The only exception to this is the N5-N6 age group. The sensitivity exhibited by this age group may be a result of the physiological state of the sixth nauplius prior to molting. The only major morphological transformation in the life of *A. clausii* occurs between the last naupliar and first copepodite stage, the preparatory phase of which may place a greater strain on the organism than do all other molts. This may sensitize animals of this stage to environmental stress to a greater degree, accounting for increased mortality with the N5-N6 group under UV-B exposure. Gehrs and Robertson (1975) observed high mortality occurring for the sixth naupliar stage of laboratory populations of *Diaptomus clavipes*, a freshwater calanoid copepod with a life history similar to that of *A. clausii*. Gehrs and Robertson speculated that the increase in mortality at this stage of the life cycle may be associated with the morphological changes and stresses occurring when the organism molts from naupliar to copepodite form. A similar mortality pattern has been documented for the sixth nauplius of the pelagic harpacticoid copepod *Euterpina acutifrons* (D'Apollito 1975).

Evaluating the impact of solar UV-B upon a single component of a marine ecosystem, such as *Acartia*, leads to many difficulties. The foremost of these is that of providing an adequate comparison between the radiant energy necessary to produce the detrimental effects observed under laboratory conditions and the quantities of similar radiation incident upon the organisms of interest under natural conditions. The combined effects of absorption and scattering due to suspended and dissolved biogenous materials, particularly phytoplankton pigments and associated degradation products, play a significant role in determining the optical properties of natural waters (Smith and Baker 1978a). The total diffuse attenuation coefficient for irradiance, $K(\lambda)$, is the optical parameter that relates the spectral fluence rate just beneath the ocean surface to the downward spectral fluence rate at depth Z (Smith and Tyler 1976). $K(\lambda)$ has component parts which contribute differentially to the underwater spectral attenuation of radiant energy. Given a quantitative relationship between the attenuation of selected wavelengths and the concentration of primary absorbing material(s) in the water column, it is possible that a surface fluence measurement would allow an accurate estimate of underwater spectral fluence in the waveband of interest. Such a relationship has been determined for the visible wavelengths with respect to total chlorophyll-like pigment concentration (Smith and Baker 1978a, b).

Dissolved organic material (DOM) or yellow substance begins to contribute significantly to the spectral attenuation for wavelengths below 400 nm (Jerlov 1976). Though the specific attenuation of middle ultraviolet has not as yet been quantitatively related to the concentration of DOM in natural waters, the diffuse attenuation coefficient for the 280-340 nm waveband has been determined for four sets of aquatic conditions of widely varying optical properties through direct measurement with an underwater UV-spectroradiometer (Smith and Baker, 1979). These figures coupled with surface fluence values can provide a first order estimate of UV-B penetration.

This estimate still does not permit, however, the comparison of treatments administered under laboratory conditions to radiation actually received under natural conditions, even though the quantities of radiation have been determined in comparable absolute terms (Jm^{-2}). Expressing the fluence in terms of total energy for a defined waveband does not specify the fluence for each wavelength within the waveband, which is a characteristic dependent upon the radiation source. The energy distribution within the UV-B waveband differs when the FS40 and solar spectra are compared. In the 290 nm region there was a greater fluence rate under the FS40 sunlamps than for noontime solar radiation at 45°N latitude. For UV-B hazard analysis, this nonequivalent distribution of energy between radiation sources must be carefully considered, since each wavelength within the UV-B waveband differs in its degree of effectiveness in producing specific biological endpoints (in the case of this investigation, the mortality and the decreased fecundity of *A. clausii*).

The sensitivity of *A. clausii* to the individual wavelengths comprising the UV-B region can be described by an action spectrum (specific to the biological endpoint desired). Photobiological action spectra demonstrate the relationship between biological effectiveness and wavelength of monochromatic radiation. The biological effectiveness is usually expressed as the reciprocal of the photon fluence required to elicit a particular quantitative biological response (Jagger, 1977). Analytic representations of action spectra can then be used to weight the spectral fluence rates for the sun and the experimental radiation source. Weighting the fluence rate at a particular wavelength by its relative effectiveness over all UV-B wavelengths yields the effective surface fluence rates and permits comparison of the two radiation regimes.

This approach presupposes knowledge of the action spectrum of interest, which in the case of *A. clausii*, is not known. Green and Miller (1975) have calculated analytic representations of two action spectra: (1) the long-wavelength tail of a DNA action spectrum compiled by Setlow (1974), and (2) a generalized action spectrum developed by Caldwell (1968). Caldwell compiled data on inhibition of photosynthesis, mutation in spores and a fungus, frequency of endosperm deficiencies, a germicidal action spectrum, epidermal cell damage, and induction of chromosomal aberrations. Though the Caldwell action spectrum is generally used to weight radiation affecting plant material, it provides more of an estimate of the sensitivity of the whole organism than the DNA action spectrum, which is specific for that target molecule.

The analytic equations of Green and Miller were used to obtain a weighting value for the fluence at each UV-B wavelength for both the sun on a typical, clear spring-summer day at 45°N latitude and the experimental FS40 sunlamps used for laboratory irradiation of copepods. Shettle et al. (1975) tabulated

global UV fluence rates for each wavelength within the 280-340 nm band as a function of ozone thickness and solar angle. Values for a 30° solar zenith angle were weighted with both the Caldwell and the Setlow action spectra for current (0.32 cm), 13% and 25% reduced (0.28, 0.24 cm) ozone concentrations. Effective (i.e., weighted) values were combined to provide two estimates (Setlow and Caldwell) of the daily global UV-B effective surface fluence for present and depleted ozone concentrations.

Comparisons were made between the amount of effective simulated solar radiation required to kill 50% of the laboratory population for each of the five age groups and the effective solar fluence incident upon the surface of the water at 45°N latitude on a typical spring-summer day (Table 8). The experimental effective fluence rate for the 290-320 nm waveband using the Setlow weighting function was $1.13 \times 10^{-2} \text{ Eff}_{\text{DNA}} \text{ Wm}^{-2}$. It was $9.49 \times 10^{-2} \text{ Eff}_{\text{GEN}} \text{ Wm}^{-2}$ for the Caldwell model (290-313 nm).

Further, Smith and Baker (1979) discuss the penetration of biologically weighted UV-B into various ocean waters. This work produces an effective diffuse attenuation coefficient weighted by both the Setlow (DNA) and Caldwell (GEN) action spectra for the water types discussed. From this an "effective depth" can be determined for an arbitrary percent reduction of laboratory populations for each of the five developmental phases of *Acartia*. Both Caldwell and Setlow daily LD₅₀ depths are presented (Table 9) for current and reduced ozone concentrations for the water types (see Legend--Table 9) discussed by Smith and Baker.

It appears, from Table 8, that more than enough UV-B radiation is presently striking the surface of the water in one day to do considerable damage to population levels. Table 9 illustrates that in moderately productive waters with a high DOM concentration (water type 4), under present ozone concentrations (0.32 cm), enough effective radiation penetrates to a depth of approximately one meter during every clear summer day to eliminate about 50% of the early and late naupliar (N1N2, N5N6) developmental phases. Population reductions of less than 50% would consequently have deeper effective depths. Therefore sufficient UV-B radiation may be present in the upper meters of this water type to inhibit the development of *A. clausii*.

To predict environmental effects, this information must be compared with the natural vertical distribution of *Acartia*. A variety of accounts of *Acartia* distribution are available in the literature. Barnes and Marshall (1951) correlate a natural vertical distribution of salinity, which varies with depth and changes over time, with distinct population patterns for the nauplius larvae of several species of copepods, including *A. clausii*. Pump samples were taken at 1, 5, 8, and 10 m depths southeast of Little Cumbrae Island (4°57' W, 55°41' N) from 1000-1500 hours on 3 May 1949. The bottom depth of the station was recorded as being 60 m. *Acartia* nauplii density ranged from 6,800 individuals m⁻³ to 11,730 m⁻³ at the 1 meter depth for the 5-h sampling period. Though the sky was reported overcast, which would reduce UV-B fluence levels, a similar naupliar distribution may occur on days when the sky is clear. This could locate the organisms in a zone of potential UV-B stress depending on the attenuating properties of the water. Unfortunately, no information regarding water transparency, productivity, DOM concentration, or any other hydrological parameters relevant to predicting effective UV-B penetration was included.

Table 9. Predicted effective (Setlow or Caldwell) LD₅₀ depths of *Acartia clausii* under current, 13%, and 25% reduced ozone concentrations in water types 1-4 discussed by Smith and Baker (1979). K_{DNA} and K_{GEN} obtained from Smith and Baker (1979). K values for the 0.28 atm-cm ozone concentration (13% reduction) obtained by interpolation (R. Smith pers. comm.).

Water types can be physically described as:

- 1 - clearest open ocean water containing minimum amounts of Chl α (0.025 mgm⁻³) and DOM (Sargasso Sea 65°41'W, 25°43'N)
- 2 - clear ocean water with low chlorophyll concentration (0.05 mg Chl α m⁻³) from the middle of the Gulf of Mexico (89°W, 25°N)
- 3 - moderately productive water (0.5 mg Chl α m⁻³ from the model of Smith and Baker (1978 b)
- 4 - moderately productive water (0.5 mg Chl α m⁻³) containing abnormally high concentrations of DOM relative to chlorophyll from coastal waters northwest of Tampa, Florida, (83°06'W, 28°38.5'N).

The effective depth for an arbitrary percent reduction of a laboratory population (Z_{LD50} in this case) can be determined using Beer's Law:

$$E(Z) = E_0 e^{-KZ},$$

where E(Z) is the effective fluence required to produce the desired population reduction, E₀ is the effective surface fluence for the location and ozone concentration of interest, K is the overall effective attenuation coefficient of UV-B waveband for the water type and ozone concentration of interest, and Z is the effective depth where the effective fluence required to produce the measured effect can be expected to occur. Determination of LD₅₀ depths do not take into consideration the negligible attenuation at the air-water interface. As an example, the Setlow LD₅₀ depth in water type 4 under current ozone concentrations of the N1-N2 age group is:

$$28.06 = 55.9 e^{-(0.92) Z}$$

$$Z_{LD50} = 0.75m$$

Where no effective LD₅₀ depth is given, an exposure equivalent to an LD₅₀ fluence would require more than one day at the surface.

A. Setlow (DNA)

Water type	Ozone (atm-cm)	K _{DNA}	Daily effective _{DNA} LD ₅₀ depth (m)				
			N1-N2	N3-N4	N5-N6	C1-C3	C4-ad
1	0.32	0.163	4.23	-	2.58	-	-
	0.28	0.165	6.30	1.56	4.68	0.34	-
	0.24	0.167	8.34	3.66	6.73	2.45	0.36
2	0.32	0.197	3.50	-	2.14	-	-
	0.28	0.2005	5.19	1.29	3.85	0.28	-
	0.24	0.204	6.83	2.99	5.51	2.01	0.30
3	0.32	0.377	1.83	-	1.12	-	-
	0.28	0.3805	2.73	0.68	2.03	0.15	-
	0.24	0.384	3.63	1.59	2.93	1.07	0.16
4	0.32	0.92	0.75	-	0.46	-	-
	0.28	0.94	1.11	0.27	0.82	0.06	-
	0.24	0.96	1.45	0.64	1.17	0.43	0.06

B. Caldwell (GEN)

Water type	Ozone (atm-cm)	K _{GEN}	Daily effective _{GEN} LD ₅₀ depth (m)				
			N1-N2	N3-N4	N5-N6	C1-C3	C4-ad
1	0.32	0.161	8.74	3.88	7.08	2.63	0.47
	0.28	0.1615	10.33	5.49	8.67	4.24	2.08
	0.24	0.162	11.86	7.03	10.20	5.79	3.64
2	0.32	0.195	7.22	3.21	5.84	2.17	0.38
	0.28	0.196	8.51	4.52	7.14	3.49	1.71
	0.24	0.197	9.75	5.78	8.39	4.76	2.99
3	0.32	0.378	3.72	1.65	3.01	1.12	0.20
	0.28	0.3795	4.40	2.33	3.69	1.80	0.89
	0.24	0.381	5.04	2.99	4.34	2.46	1.55
4	0.32	0.90	1.56	0.69	1.27	0.47	0.08
	0.28	0.91	1.83	0.97	1.54	0.75	0.37
	0.24	0.92	2.09	1.24	1.80	1.02	0.64

While investigating both *A. clausii* and *A. tonsa* in Long Island Sound, New York (73°40'W, 40°55'N), Conover (1956) recorded their vertical distribution for intervals from April 1953 through July 1954. Tows were made at two depths, one in the upper five meters, the other near the bottom. Throughout the spring of 1953, adults as well as copepodites were more frequently observed in the upper meters regardless of the time of day. In the summer of 1953, daytime surface tows almost always contained more *A. tonsa* than deep tows. Observational record depicts *A. tonsa* "struggling" against the surface film on a bright day with no wave action. Conover speculated that with calm water conditions *A. tonsa* may display a positive photoactive response to direct sunlight, and that if the summer sun is fairly bright and the water surface relatively smooth, significant numbers of *A. tonsa* might collect in surface waters during the day.

The attenuation of incident mid-ultraviolet radiation was not a parameter investigated by Conover. However, the average chlorophyll concentration observed in the Sound was recorded for the period November 1953 - August 1954. Though the attenuation of middle ultraviolet is more dependent upon the concentration of dissolved organic material, the relative productivity of the water encountered by Conover can be compared with that of the Smith and Baker water types. Concentration ranged from 1.0 mg Chl m⁻³ in December to a maximum of about 19.0 mgm⁻³ in late February to early March. This decreased steadily to summertime (May to August) value of 4-8 mgm⁻³. *Acartia clausii* appeared in early winter, reached maximum abundance in May and disappeared by August. *Acartia tonsa* appeared in June, rose to maximum abundance in August and declined steadily through fall and winter.

Cameron (1957), also in the summer of 1953, investigated the horizontal and vertical distribution of many pelagic copepods in the Queen Charlotte Islands area (132°W, 53°N). *Acartia clausii* was described as a species living near the surface, occurring immediately below the surface in most areas. It was, however, absent from surface waters of deeper stations where water clarity generally improves, deepening effective UV-B penetration. A description of the vertical distribution encountered at Masset Inlet placed *A. clausii* entirely in the surface three meters. No physical data relevant to UV-B penetration were presented, but the depth range of occurrence at Masset Inlet, as well as off Little Cumbrae Island (Barnes and Marshall 1951) finds *A. clausii* in regions of potential UV-B impact.

Stickney and Knowles (1975) recorded the summer zooplankton distribution from 29 July to 9 August 1974 for two estuarine stations near Savannah, Georgia, (81°W, 32°N). Distribution polygons of the overall zooplankton and *A. tonsa* populations demonstrated fairly homogeneous vertical distribution irrespective of time of day and tide stage. *Acartia tonsa* population densities of approximately 20,000 m⁻³ were recorded for surface values. Copepod nauplii showed a distribution pattern throughout the day of consistently greater numbers at the surface than at the bottom. It is brought to attention that high loads of suspended solids are typical of Georgia estuaries and light intensity is rapidly reduced with depth, though the mean suspended solid level is lower at the surface than at the bottom (0.9 ratio).

Anraku (1975) described the microdistribution of several copepods, including *A. clausii*, from 23 August to 3 September 1958 at different

localities in Oshoro Bay, Japan (141°E, 43°N). No statement is made of naupliar distribution; reference is made only to copepodites and adults. At station 6 (depth = 2.2 m) the day and twilight vertical distributions of *A. clausii* were compared. In this shallow depth, *Acartia* displayed epibenthic characteristics in distribution. During the day this benthic affinity was most pronounced, only small numbers of *Acartia* copepodites and adults (500 m^{-3}) were present in the surface matter. Though hydrological factors relevant to prediction of UV-B penetration are not included, the statement is made that simultaneous measurements of certain physical, chemical and biological parameters are necessary to understand the microdistribution of copepods in shallow waters. In a situation such as this, UV-B attenuation seems a relevant parameter to assess.

Furuhashi (1976) describes both the vertical distribution and the evident migration of *A. clausii* in Maizuru Bay, Japan, (135°E, 35°30'N), on 15-16 October 1974. A rather stable occurrence of copepod nauplii (including *Acartia*) was found in the 0-2 m layer, a region of possible UV-B stress, throughout the day. Though water transparency to UV-B was not indicated, the total surface irradiance presented for noon on 16 October ($50\text{ cal cm}^{-2}\text{ h}^{-1}$ -- Fig. 11) indicates clear weather.

Landry (1976) describes the vertical migration of *A. clausii* for two 24-h periods in Jakle's Lagoon, Washington (123°W, 48°30'N). On 25-26 June 1973, under clear skies, nauplii (N1-N6) were distributed primarily in the upper two meters throughout the day. On 11-12 June 1974, with reduced sunlight, the early nauplii (N2-N4) were still located primarily in the top 2 m. N5-N6 nauplii, however, maintained a closer association with the bottom during the day and migrated up at night. The older stages in both studies maintained a close association with the bottom during the day and migrated to the surface at night.

The seasonal variation of chlorophyll α concentration in Jakle's lagoon is presented for March 1973 to September 1974. May, June, and July 1973 values (peak months for *A. clausii* abundance) ranged from 10-30 mg Chl $\alpha\text{ m}^{-3}$. In 1974 the range was 10-45 mg m^{-3} for the same time period. Though DOM concentration is not indicated, productivity is substantially higher in Jakle's lagoon than in water type 4 (0.5 mg Chl $\alpha\text{ m}^{-3}$) of Smith and Baker (1979). UV-B transmission in this system would, more than likely, be severely limited.

Bakker et al. (1977) described the copepod biomass in two differing water masses. Data include species abundance (*A. tonsa* included) at 0, 5, 10, 15, and 20 m depths for Lake Veere, a stagnant brackish environment, and Westershelde estuary (3°30'E, 51°30'N--Lake Veere is 20 km NE) in southwestern Netherlands. The summer Secchi disk depth of Lake Veere approached 5.5 m, while in Westershelde it was never greater than 0.7 m owing to high content of silt and detritus.

Maximum copepod densities for Lake Veere were found at different depths during the summer of 1974. Large concentrations of animals were sampled in the top layers of three stations in July, but for the most part, numbers increased with depth. In Westershelde, copepod numbers in near-bottom samples were larger than surface values. For both localities, older stages were commonly found in deeper layers than younger stages.

For Lake Veere on 18 July 1974, at all three sampling locations, high numbers of *A. tonsa* were sampled at the surface (0 m depth). Concentrations as dense as $322,000 \text{ m}^{-3}$ were recorded for stations 2. Unfortunately, weather conditions were not indicated for any of the sampling dates. This magnitude of surface density was not recorded for any day other than 18 July, though for all sampling days, some percentage of the sample population was always at the surface. For months June-August, this percentage ranged from 5-42%, indicating that significant portions of the population sampled were often found in a region where detrimental levels of UV-B occur (regardless of the clarity of the water). Naupliar concentration was determined only in Westerschelde where the abundance was always far greater than that of older stages. Yearly naupliar average was $120,000 \text{ m}^{-3}$ (71% of total numerical density) as compared to the copepodite and adults yearly average of $50,000 \text{ m}^{-3}$. Sixty-three percent of the naupliar population was found near the surface in June.

In addition to an investigation of the literature concerning the natural vertical distribution of *Acartia*, it is necessary to further evaluate the data available for estimating the penetration of UV-B in marine waters where this organism is found. Schemes for the optical classification of ocean water, other than Smith and Baker (1979), exist. Jerlov (1950) obtained penetration data for 310 nm radiation for a broad scheme of water types, some of which may be more relevant to *A. clausii* distribution than those discussed by Smith and Baker. The knowledge of the attenuation of one wavelength in the waveband of interest, however, does not provide a basis for adequate extrapolation to the attenuation of other wavelengths. In addition, the Jerlov water types are specified by optical transmissivity only. No other relevant physical description (e.g. Chl α , DOM content) is provided.

The attenuation of the 310 nm wavelength for both the Jerlov and the Smith and Baker schemes can nonetheless provide a basis of comparison between the two classifications. Smith and Baker water type 4 ($K_{310} = 0.86$) describes coastal water which is clearer than Jerlov type 1 ($K_{310} = 1.83$). Smith and Baker type 4 can be described by $0.5 \text{ mg Chl } \alpha \text{ m}^{-3}$ and high DOM concentrations. Smith and Tyler (1976--Fig. 8) have presented the K_{310} and K_{375} of Jerlov coastal water type 1 with $K(\lambda)$ values $\geq 375 \text{ nm}$ of other water types measured by other investigations where Chl α concentration was determined. It appears that Jerlov water type 1 could have a Chl α concentration ranging from 2.2 mgm^{-3} (Fl1-15 cruise) to 10 mgm^{-3} (Fl1-5 cruise). No indication of DOM content was presented.

Lenoble (as cited in Smith and Tyler 1976) presented an adequate spread of diffuse attenuation coefficients for wavelengths in the UV range of interest to predict the downwelling fluence rate at several depths. However, these values are for the relatively clear water from the eastern Mediterranean Sea. *A. clausii* is not usually found in water of this clarity. Smith and Calkins (1976) have used data from Lenoble to predict the downwelling fluence rate at several depths and have then folded in the weighted response of the Robertson UV-B meter (a weighting function similar to the human erythemal action spectrum: Berger 1976) to determine the erythemal effective attenuation coefficient for this water type. Neither the water type nor the weighting function used is considered applicable to the situation of *A. clausii*.

Recently H. Jerslev (1978) compared the use of the Robertson meter (R-meter) and the Jerlov meter for measuring UV-B penetration into coastal waters. The Jerlov meter provides a more accurate estimate of penetration into coastal waters high in yellow substance than does the R-meter. Like the R-meter, it is a broad-band detector, although its weighted response peaks at approximately 312 nm. Again, this weighted response is considered inapplicable to *Acartia*.

From a review of the literature it appears that the most accurate data assessing the penetration of UV-B into natural waters is that of Smith and Baker (1979). The submersible spectroradiometer used by Smith and Baker provides absolute and weighted fluence rates at each wavelength within the waveband of interest. Although diffuse attenuation coefficients for wavelengths below 310 nm must at this time be extrapolated, they provide the best estimate of $K(\lambda)$ between 280 and 340 nm, given current data. Therefore, if *A. clausii* is located in the upper meters of moderately productive coastal waters with a high DOM concentration (Smith and Baker type 4), results indicate they may be subject to significant UV-B stress when the sky is clear (though UV-B incidence still occurs, at a reduced rate, during cloudy weather). Effective depths would consequently be lessened if one were to consider the other coastal water types discussed by Jerlov. While these waters are not described by any physically relevant parameters, effective depths can be crudely estimated. Smith and Baker (1979) mention that the DNA effective attenuation coefficient for the UV-B waveband approximates the diffuse attenuation coefficient for 305 nm ($K_{DNA} \approx K_{305}$). Therefore, the K_{DNA} will always be greater than the K_{310} determined by Jerlov. Proceeding as in Table 9 using Jerlov K_{310} values (realizing this to be an underestimate of K_{DNA}), the N1-N2 DNA effective LD50 depths for Jerlov coastal water types 1, 3 and 5 under present ozone concentrations would be: $Z_1 < 0.38$ m, $Z_3 < 0.29$ m, $Z_5 < 0.20$ m.

Whether solar UV-B presently affects *A. clausii* in nature has not been determined at this time. Its potential effects, however, should not be ignored. Though data quantitatively predicting the penetration of UV-B into waters where *Acartia* is found are sparse, several citations in the literature locate species of *Acartia* in the surface meter and in some cases immediately below the surface. Organisms in this region certainly must be receiving significant levels of UV-B irrespective of water clarity. If the sensitivity of *A. clausii* is at all representative of that of other copepods, many pelagic species may currently be receiving detrimental levels of UV-B radiation.

What is most needed at this time is an in situ investigation of zooplankton sensitivity to current UV-B fluence levels, accompanied by precise radiometric determination of the attenuating properties of the water. In the past, data determining the UV-B attenuation by water has not been obtained when natural distribution studies have been performed or evaluated. Without accurate assessment, sufficient radiation could be assumed present in the upper meters of various water columns to inhibit the development of *A. clausii*, indicating solar UV-B radiation as an ecological parameter which possibly limits natural populations.

CONTINUOUS RECIRCULATING CULTURE SYSTEM

The continuous recirculating culture system for planktonic copepods has been constructed and tested. Although the sensitivity of various marine forms to acute exposure of UV-B radiation has been studied, information on the less obvious effects of chronic exposure is lacking. At exposure levels that do not necessarily produce a significant increase in mortality there may be significant changes in various physiological and metabolic aspects when organisms are exposed over an extended time-period. One such parameter is the uptake and assimilation of phytoplanktonic fixed carbon by marine copepods - the transfer of energy from a primary producer to a primary consumer. This type of energy transfer at the base of the marine food web is vital to the survival of the entire ecosystem.

In the past, laboratory cultures of marine copepods used in energy transfer studies have required frequent changes of media to raise sufficient numbers through several generations. The success rate of this technique has been very low for many dominant pelagic forms. To study the long-term effects of UV-B exposure on copepods, these organisms will be reared in large culture tanks supplied with conditioned seawater. In the laboratory the medium can be reused if appropriate "cleansing" takes place (Zillioux, 1969). The proposed study will make use of the recirculating apparatus described by Zillioux to assess the impact of increased levels of chronic UV-B exposure upon the trophodynamic relationship between phytoplankton and zooplankton.

Materials and Methods

The attached figure (Fig. 10) is a schematic representation of the apparatus designed by Zillioux (1969). Eight culture tanks were constructed of 6.5 mm thick acrylic plastic (35 cm long x 20 cm wide x 40 cm deep: 25-liter capacity). The culture medium is circulated through the system flowing from the "head" tank into the culture tanks, where it is mixed by gentle aeration within the tanks. From the tanks it passes through removable screens into troughs located along a side of the tank, and flows out through a gravity drain. The flow rate of 35 ml/min/tank is sufficiently low so as not to concentrate the copepods at the screen. The screen has a porosity of approximately 75 μ m which restricts the loss of even the naupliar stages of copepod development, but does allow the passage of the phytoplankton. The medium then circulates through a foam tower where dissolved organic wastes are removed. The tower effluent flows by gravity through a glass-wool filter and into a lower reservoir. From the lower reservoir the medium is forced through a 15 μ m cellulose acetate filter cartridge in series with a 3 μ m and a 0.45 μ m pleated membrane cartridge and into the "head" tank.

Natural seawater will be used in this system. The seawater will be collected from Yaquina Bay, Newport, Oregon, at times when the salinity will not exceed the range of 28 -33‰. The seawater will then be autoclaved.

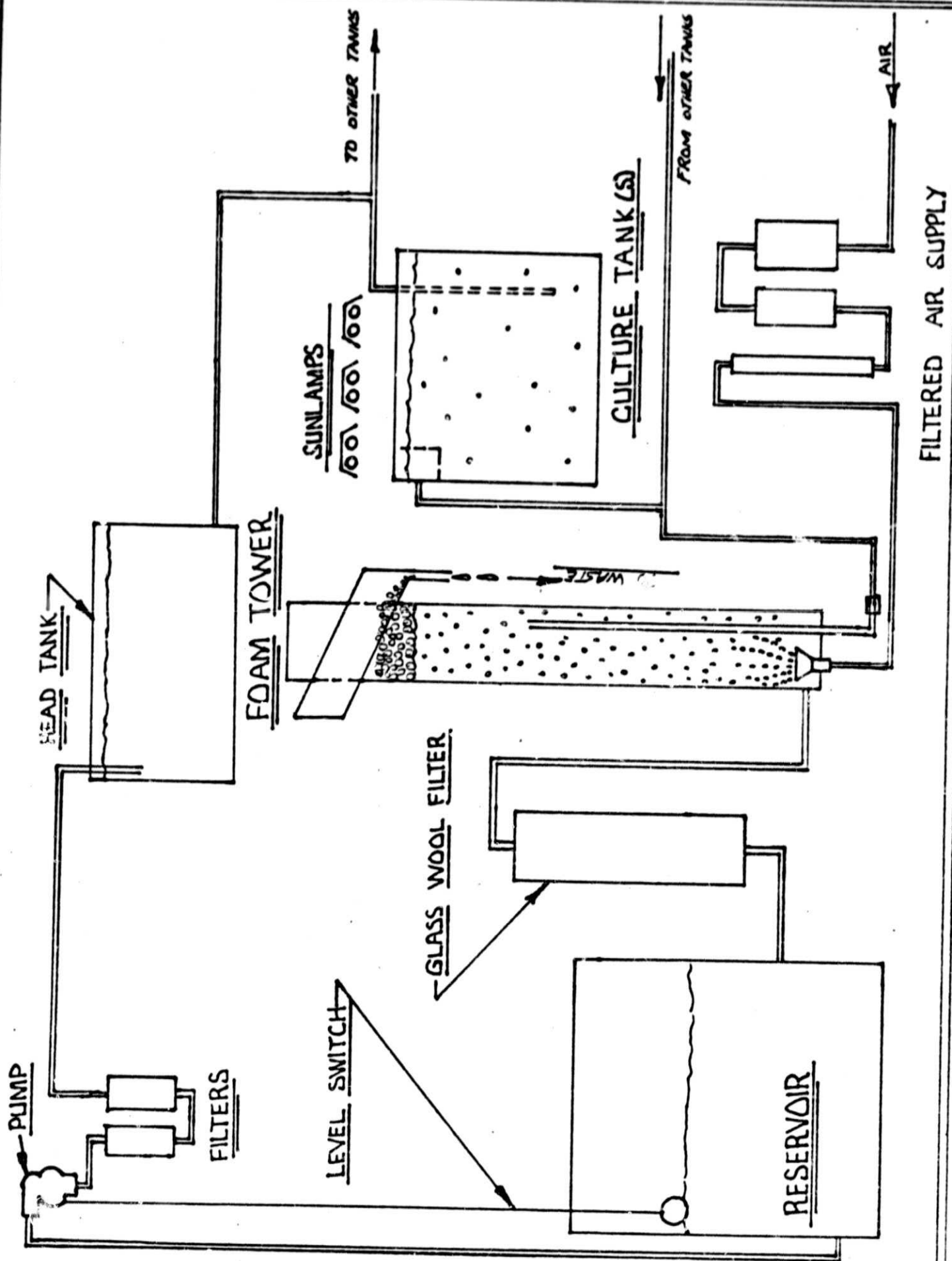


Fig. 10. Continuous recirculating culture system.

Labeled *Dunaliella tertiolecta* will be used as food to assess the impact of UV-B radiation on the assimilation rates of copepods. The *D. tertiolecta* will be reared in a medium containing ^{14}C -sodium bicarbonate. Following successive washes by centrifugation, the algae will be resuspended in sterile seawater. Aliquots will be measured for carbon-14 content, total organic carbon, and cell concentration. All radiocarbon determinations will utilize liquid scintillation techniques.

To determine the energy transfer, predetermined numbers of adult copepods of the same sex from the culture tanks will be introduced into bottles containing equal concentrations of labeled *P. suecica*. The copepods will be incubated for a period of time with the labeled food and then transferred to a medium containing non-radioactive food. A bottle of labeled algae with no copepods will be incubated to determine the respiratory contribution of the algae. The measurement of ^{14}C -activity in the animals when defecation of labeled food ceases allows one to calculate the assimilation rate of carbon (Sorokin, 1966). Respiratory rates can be calculated from successive determinations of $^{14}\text{CO}_2$ content in the culture medium.

The rates of energy transfer between the first and second trophic levels are highly critical. Utilizing the equation from a study by Schaefer (1965):

$$P = BE^n,$$

where P represents the production at the top of a marine food web, B is the annual biomass at the primary trophic level, E is the ecological efficiency (the ratio of energy extracted from a trophic level to the energy supplied to a trophic level) and n is the number to trophic level exchanges, a 5% decrease in the average ecological efficiency would result in approximately a 19% decrease in fish productivity. For example, if the annual production of phytoplankton is 1.9×10^{13} kg of carbon and the average ecological efficiency were reduced from 10% to 9.5%, the production of fish at the fifth trophic level would be reduced from 1.90×10^9 kg carbon to 1.55×10^9 kg carbon. The exponential amplification of slightly reduced efficiencies at lower trophic levels could produce highly significant consequences at higher trophic levels. The object of this proposed study is to determine whether a reduction of ecological efficiency would occur at slightly enhanced UV-B fluence levels.

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