To the Graduate Council:

I am submitting herewith a thesis written by Janet G. Packard entitled "Effects of Ultraviolet-B Radiation on the Productivity and Pigmentation of Gracilaria tikvahiae (Rhodophyta)." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology.

Walker O. Smith, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:

The Graduate School

EFFECTS OF ULTRAVIOLET-B RADIATION ON THE PRODUCTIVITY AND PIGMENTATION OF *GRACILARIA TIKVAHIAE* (RHODOPHYTA)

A Thesis

Presented for the

Master of Science

Degree

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Janet G. Packard

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ABSTRACT

The effects of ultraviolet-B radiation (280 to 320 nm) on the photosynthetic capacity and light-harvesting pigment levels of Gracilaria tikvahiae, an important primary producer in many eastern North American coastal ecosystems, were determined under carefully defined conditions. Effects on productivity and chlorophyll and phycoerythrin concentrations of G. tikvahiae were measured on UV-B- and non-UV-B-irradiated algae over 96 h. Three UV-B dose rate treatments were conducted: low = < 0.18 W m^{-2}, medium = < 0.43 W m^{-2} and high = 0.43 - 0.79 W m^{-2}.

During the first 12 h of the medium UV-B dose rate treatment, UV-B exposure stimulated productivity by 66% from the mean control productivity of 2.16 mg C (g dry wt)$^{-1}$ h^{-1}. At the same time, light-harvesting pigment concentrations were increased. During the first 24 h, UV-B exposure increased chlorophyll concentrations by 38% from the mean control concentration of 1.85 mg (g dry wt)$^{-1}$ and increased phycoerythrin concentrations by 50% from the mean control concentration of 29.78 mg (g dry wt)$^{-1}$. There were no effects on productivity or pigment concentrations at low doses (dose rate x exposure time) in the low or high UV-B dose rate treatments which may indicate the existence of a threshold dose rate for beneficial effects of UV-B.

UV-B radiation decreased primary production in all treatments, but only after a 12-h lag period in the low and high UV-B dose rate treatments and after the 12-h period of increased productivity in the medium UV-B dose rate treatment. Since increasing the UV-B dose rate did not accelerate the occurrence of decreased productivity, it

appeared that UV-B damage had to accumulate for 12 h before producing damaging effects. High doses of UV-B also decreased chlorophyll and phycoerythrin concentrations and increasing the UV-B dose rate accelerated the occurrence of the decreased pigment concentrations. In the low UV-B dose rate treatment, there were no effects on pigment concentrations by 72 h, while in the high UV-B dose rate treatment, UV-B exposure decreased chlorophyll concentrations by 72 h and phycoerythrin concentrations by 48 h ($p < 0.05$). Since the pigment concentration decreases occurred more gradually than the observed productivity decreases, initial productivity decreases were probably the result of UV-B effects on components of the photosynthetic system other than the light-harvesting pigments.

Statistically significant decreases in phycoerythrin concentration in UV-B-irradiated G. tikvahiae coincided with color change in the algae from purple to yellow-green. The yellow-green color, decreased pigment concentrations and decreased productivity in the UV-B-irradiated algae were maintained throughout the experiments and were not reversed by removal from the UV-B radiation and exposure to 48 h of visible light. The results indicate the potential for damage to these components of coastal ecosystems by elevated UV-B radiation and the need for an accurate assessment of ultraviolet radiation effects on nearshore systems.

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I. INTRODUCTION

Radiation of the electromagnetic spectrum with wavelengths from 280 to 320 nm is classified as ultraviolet-B radiation (UV-B). The other classes of ultraviolet radiation are ultraviolet-C radiation (UV-C) consisting of wavelengths from 200 to 280 nm and ultraviolet-A radiation (UV-A) consisting of wavelengths from 320 to 400 nm. Stratospheric ozone attenuates UV-B radiation from 320 to 295 nm and absorbs all ultraviolet radiation below approximately 295 nm. Theoretically, UV-B irradiance levels reaching the earth's surface are greatest when the solar pathlength through the ozone layer is at a minimum, e.g. at solar noon, on the summer solstice in mid-latitudes and at the equator. However, solar UV-B levels are also a function of altitude and cloud cover (National Academy of Sciences, 1982).

The amount of UV-B radiation reaching the earth's surface will increase if depletion of the ozone layer occurs as a result of the reduction of ozone concentration by chemical reactions with gaseous chlorinated compounds (National Academy of Sciences, 1982). A 5 to 9% decrease in the ozone layer is currently predicted as a result of this anthropogenic input, which would result in a 1% increase in UV-B radiation (National Academy of Sciences, 1982). Any increase in the solar UV-B reaching the earth's surface is of concern because it could cause increased numbers of cases of human skin cancer as well as disturbance of marine and terrestrial ecosystems (National Academy of Sciences, 1982).

Action spectra for effects of ultraviolet light on nucleic acids and proteins are well known (see Caldwell, 1982). Nucleic acids and proteins absorb ultraviolet radiation maximally at 260 and 280 nm, respectively, and absorption occurs in the UV-B waveband, although to a lesser extent than in the UV-C waveband. Molecular repair mechanisms repair most of the DNA damage at present ambient levels of UV-B, but higher doses could result in cellular damage in sensitive organisms resulting from disruption of DNA replication, denaturing of proteins and the interruption of the synthesis of macromolecules. A 5 to 9% decrease in the ozone layer would cause a 19% increase in DNA-damaging UV-B (UV-B radiation weighted by the DNA-damage action spectrum; National Academy of Sciences, 1982); furthermore, certain species may exhibit extreme sensitivity to UV-B radiation. For example, the developmental stages of aquatic and amphibious species appeared to be particularly vulnerable to solar UV-B radiation because of their lack of protection from UV-B while the sensitive processes of organogenesis were taking place (see review by Worrest, 1982).

In experimental studies on higher plants, enhanced UV-B radiation lowered biomass, growth rate and productivity according to the sensitivity of the species (see review by Caldwell, 1982). Reduced growth rate in higher plants in response to UV-B enhancement resulted from disruption of mitotic processes (Dickson and Caldwell, 1978). In addition, Teramura and Caldwell (1981) observed that in soybean leaves "relative changes in net photosynthesis in leaves irradiated early in leaf ontogeny . . ., suggested that leaves were more sensitive to UV-B radiation damage prior to leaf expansion."

Reduced productivity resulting from enhanced UV-B irradiation may be partly due to chloroplast membrane disruption and the subsequent reduction of light and dark reactions of photosynthesis (Brandle et al., 1977). Photosystem II, besides being indirectly damaged by chloroplast degradation, is also directly affected by UV-B radiation (Brandle et al., 1977). Decreased chlorophyll concentration accompanying decreased productivity has been found in only a few UV-B studies on higher plants (See review by Caldwell, 1982). In a study of UV-B-irradiated Rumex patientia, Sisson and Caldwell (1976) found no effect on chlorophyll concentration although productivity was decreased and concluded that decreased productivity was not a secondary effect of decreased chlorophyll concentration. Recently, enhanced UV-B radiation has been shown to inhibit the activity of photosynthetic enzymes of soybean (Glycine max), pea (Pisum sativum), tomato (Lycopersicon esculentum) and sweet corn (Zea mays; Vu et al., 1982).

Stimulation of productivity by low doses of UV-B radiation compared to controls receiving no UV-B has been observed in some species of higher plants. For example, in sweet corn (Zea mays) irradiated with low doses of UV-B radiation, increased productivity occurred and was correlated with an increased concentration of PEP carboxylase, the primary carbon fixation enzyme in C_4 plants. Also, in fully expanded soybean leaves irradiated with low doses of UV-B, productivity and chlorophyll concentrations were increased compared to controls (Teramura and Caldwell, 1981).

UV-B radiation at present ambient levels is an important environmental parameter that is partly responsible for photoinhibition

of phytoplankton primary production (see review by Worrest, 1982). Photoinhibition can be defined as the decrease in photosynthetic rate at or near the water surface compared to rates found deeper in the water column and is due to the damaging effects of excess light. Quantitative evaluation of photoinhibition of phytoplankton showed that 25% of the photoinhibition-weighted dose is due to radiation below 340 nm; 50% of this dose is due to radiation below 390 nm and the remainder is due to wavelengths longer than 390 nm (Smith et al., 1980).

The photoinhibitory effects of UV-B radiation on phytoplankton photosynthesis and productivity are usually not observed due to the use of glass bottles in most productivity studies which are opaque to UV-B radiation (Smith and Baker, 1980; Worrest et al., 1980). As a result, it may be that most reports of primary production are overestimates. In coastal waters where the euphotic zone (depth to which 1% of the surface radiation penetrates) was 5 m deep, differences in ^{14}C-uptake between quartz tubes (which are transparent to UV) and tubes shielded from UV-B were detected at 1.2 to 1.5 m (Lorenzen, 1979). In open ocean waters, a larger fraction of the euphotic zone should be similarly affected. In seawater, UV-B radiation is attenuated by water molecules, dissolved organic material and "chlorophyll-like pigments" that covary with chlorophyll (Baker and Smith, 1982). Open ocean waters are more transparent than coastal waters; for example, UV-B radiation penetrates the upper 15 m of open ocean waters but only the upper 1 to 2 m in productive coastal waters. Because UV-B is rapidly attenuated with depth and phytoplankton biomass usually exhibit subsurface maxima, total

water column productivity figures may only be overestimates of about 2%, although some surface values may be overestimates of up to 40% (Lorenzen, 1979).

In experiments with natural phytoplankton populations exposed for 6-h incubations to low UV-B dose rates which were less than the ambient UV-B levels, Modert et al. (1982) found that the low exposures had higher productivity than the controls receiving no UV-B. Phytoplankton populations exposed to a higher dose rate, but one which was still below the ambient UV-B level experienced decreased productivity compared to the controls. Therefore, it was concluded that UV-B radiation stimulated productivity up to a threshold level and that this threshold level was less than the ambient level of UV-B (Modert et al., 1982). Phytoplankton species have been shown to exhibit differential sensitivity to UV-B radiation (Worrest et al., 1981a). Some species exhibited increased ^{14}C-uptake compared to controls at low doses of UV-B while more sensitive species exhibited decreased ^{14}C-uptake compared to controls at the same dose level. The diatom Thalassiosira pseudonana was the most sensitive of the group investigated, whereas the flagellated green algae Dunaliella tertiolecta was the most resistant.

Growth rates in phytoplankton may also be affected by enhanced UV-B radiation. For example, the growth rate of the diatom Melosira nummuloides was depressed by enhanced UV-B radiation (Thomson et al., 1980). Differential sensitivity of phytoplankton in UV-B-irradiated estuarine microcosms resulted in an alteration of community structure (Worrest et al., 1981b). Daily exposure to enhanced levels of UV-B radiation also depressed biomass, chlorophyll a concentration and radio-

carbon uptake of samples from the systems. The entire marine ecosystem could be affected by altered community composition and/or lowered primary production at the base of the food web.

The effect of UV-B radiation on macroalgae remains largely unstudied. Since macroalgae are a significant group of primary producers in coastal marine ecosystems, this study was designed to measure the potential effect of UV-B radiation on their productivity. Gracilaria tikvahiae (Rhodophyta, Gigartinales, Gracilariaceae; McLachlan, 1979), a red algae formerly assigned to G. foliifera, was chosen as the test organism due to its presence in shallow estuarine waters and its apparent importance as a primary producer. Thalli of G. tikvahiae are in the form of a branched subterete axis with a cortex and a medulla and have a surface area to volume ratio of about 30:1 (Rosenberg and Ramus, 1982). Since decreased concentrations of light-harvesting pigments could contribute to decreased productivity, the potential effects of UV-B radiation on the two major pigments in red algae, chlorophyll a and phycoerythrin (a red phycobiliprotein), were also measured.

The hypotheses tested were:

1. Whether any stimulation of productivity or increase in pigment concentrations occurred at low UV-B doses.
2. Whether any damaging effects to productivity or pigment concentrations occurred at high UV-B doses.
3. Whether UV-B radiation affected productivity before affecting pigment concentrations.
4. Whether UV-B effects were permanent or reversible.

II. METHODS

Collection and Maintenance of Gracilaria tikvahiae

Gracilaria tikvahiae and seawater were collected from the Fort Macon jetty in Beaufort Inlet near Beaufort, North Carolina and the algae and seawater were transported immediately by car to The University of Tennessee. Seawater was filtered through .22 μ Millipore filters and stored in polyethylene carboys in the dark at 2° C until use. The seawater used in the experiments was enriched with 7.5% W/V $NaNO_3$ and 0.5% W/V KH_2PO_4, the "f/2" medium described by Guillard and Ryther (1962). Algae were kept in two aerated aquaria in filtered enriched seawater at 25° C. Visible light was provided on a daily 12 h light:12 h dark photocycle by two Sylvania 40 W fluorescent lamps (approximate irradiance = 6.2 W m^{-2}). The algae were used for experiments within 8 weeks of collection.

Experimental Apparatus

All experiments were conducted under defined laboratory systems with known amounts of UV-B and visible radiation. Two Westinghouse FS-40 fluorescent sunlamps provided ultraviolet radiation and two Sylvania 40 W cool white fluorescent lamps provided supplemental visible radiation. These lamps were mounted in light banks and the experimental area was enclosed with black felt curtains. Algal segments were placed in open plastic containers filled with filtered seawater and positioned 50 cm below the lamps. The sunlamps were covered with cellulose acetate (0.13 mm thickness; Transilwrap Corp., Atlanta, Ga.) which limited ultraviolet radiation reaching the algae to UV-B radiation above

approximately 290 nm, providing an approximation of the solar UV-B spectrum. The control group of containers was covered with Mylar (0.13 mm thickness, Transilwrap Corp.) which filtered out more than 99% of the UV-B radiation. Transmission in the UV-A and visible wavebands was similar in the UV-B radiation and control treatments (Sisson and Caldwell, 1975).

Measurement of Irradiance

Visible irradiance in all treatments was approximately 4.1 W m^{-2} as measured by a LI-COR radiometer Model LI-185A. UV-B dose rates were measured with a spectroradiometer (Optronics Laboratories Inc. Model 742) which had been calibrated against a radiometric standard traceable to the National Bureau of Standards. The spectroradiometer was interfaced with a Hewlett Packard 85 computer system for data collection. Dose rates (mW m^{-2} nm^{-1}) were recorded in the UV-B waveband from 290 to 320 nm at 1 nm intervals. The total UV-B dose rate (mW m^{-2}) from 290 to 320 nm was summed by a computer program using trapezoidal integration. Spectral irradiance measurements of solar UV-B were also conducted (Table 1).

Cellulose acetate (CA) photodegrades upon exposure to radiation and therefore allows less UV-B radiation penetration over time (Table 2). Three dose rates were created using various stages of CA photodegradation. The low UV-B dose rate was created by allowing the CA to photodegrade for 48 h before starting the experiment. The medium UV-B dose rate was created by allowing the CA to photodegrade

TABLE 1. Spectral irradiance of UV-B radiation at solar noon under cloudless skies at Knoxville, Tennessee on indicated dates.

Wavelength (nm)	Spectral irradiance (W m^{-2} nm^{-1})		
	Dec. 20, 1982	March 15, 1983	May 4, 1983
290	ND*	ND	ND
292	ND	ND	ND
294	ND	ND	ND
296	8.36×10^{-7}	8.57×10^{-5}	5.44×10^{-5}
298	6.77×10^{-5}	4.72×10^{-4}	4.34×10^{-4}
300	3.15×10^{-4}	1.55×10^{-3}	1.80×10^{-3}
302	1.08×10^{-3}	4.18×10^{-3}	5.22×10^{-3}
304	3.36×10^{-3}	1.18×10^{-2}	1.53×10^{-2}
306	8.17×10^{-3}	2.20×10^{-2}	3.02×10^{-2}
308	1.48×10^{-2}	3.43×10^{-2}	4.82×10^{-2}
310	2.32×10^{-2}	4.56×10^{-2}	6.85×10^{-2}
312	4.31×10^{-2}	7.74×10^{-2}	1.06×10^{-1}
314	5.55×10^{-2}	9.55×10^{-2}	1.34×10^{-1}
316	6.73×10^{-2}	1.07×10^{-1}	1.65×10^{-1}
318	9.31×10^{-2}	1.42×10^{-1}	2.01×10^{-1}
320	1.10×10^{-1}	1.60×10^{-1}	2.26×10^{-1}
Total (W m^{-2})	0.73	1.24	1.80

*ND = not detectable. Irradiance was less than maximum sensitivity of instrument (10^{-8} W m^{-2}).

TABLE 2. Spectral irradiance of cellulose acetate transmittance of UV-B radiation as a function of time of exposure.

Wavelength (nm)	Spectral irradiance (W m^{-2} nm^{-1})				
	Mylar	Cellulose acetate			
		0 h	24 h	48 h	96 h
290	ND[a]	5.50×10^{-5}	2.60×10^{-5}	1.89×10^{-5}	1.22×10^{-5}
292	8.25×10^{-8}	3.10×10^{-4}	1.09×10^{-4}	5.50×10^{-5}	1.98×10^{-5}
294	ND	1.84×10^{-3}	6.43×10^{-4}	2.93×10^{-4}	1.73×10^{-4}
296	ND	5.80×10^{-3}	2.14×10^{-3}	9.17×10^{-4}	3.85×10^{-4}
298	ND	1.36×10^{-2}	5.48×10^{-3}	2.14×10^{-3}	1.05×10^{-3}
300	ND	2.07×10^{-2}	8.71×10^{-3}	3.32×10^{-3}	1.56×10^{-3}
302	ND	2.80×10^{-2}	1.25×10^{-2}	4.96×10^{-3}	2.44×10^{-3}
304	3.32×10^{-7}	3.35×10^{-2}	1.61×10^{-2}	6.14×10^{-3}	3.29×10^{-3}
306	ND	3.56×10^{-2}	1.78×10^{-2}	6.97×10^{-3}	3.75×10^{-3}
308	7.54×10^{-7}	3.74×10^{-2}	1.97×10^{-2}	7.84×10^{-3}	4.37×10^{-3}
310	9.01×10^{-7}	3.78×10^{-2}	2.08×10^{-2}	8.47×10^{-3}	4.84×10^{-3}
312	2.77×10^{-6}	3.90×10^{-2}	2.23×10^{-2}	9.79×10^{-3}	5.69×10^{-3}
314	1.51×10^{-5}	5.22×10^{-2}	3.34×10^{-2}	1.53×10^{-2}	1.18×10^{-2}
316	1.02×10^{-4}	3.82×10^{-2}	2.36×10^{-2}	9.95×10^{-3}	6.12×10^{-3}
318	7.86×10^{-4}	3.67×10^{-2}	2.33×10^{-2}	9.93×10^{-3}	6.60×10^{-3}
320	2.52×10^{-4}	3.52×10^{-2}	2.29×10^{-2}	9.86×10^{-3}	6.08×10^{-3}
Total (W m^{-2})	0.004[b]	0.79	0.43	0.18	0.14

[a]ND = not detectable. Irradiance was less than maximum sensitivity of instrument (10^{-8} W m^{-2}).

[b]Total Mylar transmittance after 24 h of exposure. Total Mylar transmittance ranged from 0.10 W m^{-2} at 0 h to 0.003 W m^{-2} at 96 h.

24 h before starting the experiment. The high UV-B dose rate was created by using new CA filters at the start of the experiment and changing the filters every 24 h. The resulting UV-B dose rate treatments were: low = < 0.18 W m^{-2}, medium = < 0.43 W m^{-2}, and high = 0.43 - 0.79 W m^{-2}.

The cellulose acetate UV-B transmission included wavelengths from 290 to 295 nm (Table 1), whereas solar UV-B reaching the earth's surface does not include wavelengths less than 295 nm (Table 2). Lower wavelengths of the UV-B spectrum, e.g. 290 to 295 nm, are more damaging to organisms than the higher UV-B wavelengths (see review by Caldwell, 1982). Therefore, the UV-B dose rates created in the laboratory are not directly comparable to solar UV-B dose rates.

Experimental Design

Experiments were conducted at three UV-B dose rates. Radiation was provided continuously to algal segments in the UV-B and control groups for 96 h. Productivity was measured every 3 h for the first 12 h and every 24 h thereafter. Chlorophyll and phycoerythrin concentrations were measured after the first 12 h of radiation and every 24 h after the initial determination. Each productivity and pigment concentration measurement was conducted on three replicate algal segments. To test for recovery of decreased productivity and pigment concentrations, UV-B-irradiated G. tikvahiae segments were removed from the UV-B radiation and placed under two Sylvania cool white fluorescent lamps (approximate irradiance = 6.2 W m^{-2}) for 48 h. Productivity and pigment concentrations were then remeasured.

Productivity Measurements

The ^{14}C-technique developed by Steemann Nielsen (1952) as modified for macroalgal photosynthesis (Kremer, 1981) was used for productivity measurements. All ^{14}C-incubations were conducted on approximately 2.5 cm algal segments (cut from mid-sections of fronds) in 200 ml filtered enriched seawater at 27 to 30° C. ^{14}C-incubations for the first 24 h were conducted for the length of time of exposure. After the first 24 h, all incubations were conducted for 24 h. At the beginning of each 24-h period, approximately 2 μCi of $NaH^{14}CO_3$ were added to each container of seawater and algal segments to be tested during that period. Blanks used for the ^{14}C-incubations were time zeros. The algal segments in the time zero ^{14}C-incubations were processed immediately after addition of carbon-14.

After the ^{14}C-incubations, the algal segments were removed from the seawater, blotted and weighed to 30 ± 10 mg and placed into individual scintillation vials. One-half ml Protosol (New England Nuclear) was added to each vial and the vials were left for 12 h during which the Protosol partially dissolved the segments. Then 10 ml Omnifluor (New England Nuclear) were added to each vial. Radioactivity of the samples was counted on a liquid scintillation counter (Tracor Analytic Model 6892) and counting efficiency of the Protosol-Omnifluor preparation was determined by addition of internal standards (^{14}C-toluene).

Total inorganic carbon-14 present in each container of seawater was determined by counting 0.5 ml of the ^{14}C-inoculated seawater in 10 ml Scinti Verse I (Fisher Scientific Co.) and corrected for counting

efficiency by the external standard ratio method. This method of measuring total carbon-14 was also used to test for loss of radioactivity due to atmospheric exchange during the 24-h incubations. The radioactivity in test containers of ^{14}C-inoculated seawater was measured at the time of inoculation and after 24 h. No loss of radioactivity was observed. Dry weight/wet weight conversion ratios were determined after algae were dried at 90° C; dry weight was found to be 13% wet weight (n = 11). Productivity was calculated (Strickland and Parsons, 1972) as:

$$\text{Productivity (mg C (g dry wt)}^{-1}\ \text{h}^{-1}) = \frac{(DPM/g_n - DPM/g_o)\ (W)\ (1.05)}{(R_o)\ (N)}$$

where:

DPM/g_n = disintegrations per minute per g dry wt of algal segment

DPM/g_o = disintegrations per minute per g dry wt of time zero

W = inorganic carbon concentration available in container of seawater (24 mg C l^{-1}; Strickland and Parsons, 1972)

1.05 = correction factor for isotope discrimination (Strickland and Parsons, 1972)

R_o = disintegrations per minute of total inorganic carbon-14 in container of seawater

N = number of hours of incubation

Pigment Concentration Measurements

Algal pigment concentrations were determined on 2.5 cm frond segments which were removed from the seawater, blotted and weighed to 30 ± 10 mg. The algal segments for chlorophyll measurements were ground by hand with a glass homogenizer in 90% acetone, centrifuged, and the absorbance of the supernatant was read at 664.3 nm on a spectrophotometer (Shimadzu UV-120-02 or Hitachi Model 100-20). The concentration of chlorophyll in the algal segments was calculated as:

$$\text{Chlorophyll (mg (g dry wt)}^{-1}) = \frac{(A_{664.3})\ (V_{ext})}{(87.67)\ (Wt)}$$

where:

$A_{664.3}$ = absorbance at the absorption maximum of chlorophyll a in 90% acetone (664.3 nm)

V_{ext} = extraction volume in ml

87.67 = extinction coefficient of chlorophyll a in 90% acetone in ml mg^{-1} cm^{-1} (Jeffrey and Humphrey, 1975)

Wt = g dry wt

The algal segments for phycoerythrin concentration determinations were ground by hand with a glass homogenizer in cold 0.05 M potassium phosphate buffer (ph 6.7) to extract the phycobiliproteins. The buffer was made by mixing equal solutions of 0.1 M KH_2PO_4 and 0.1 M K_2HPO_4 (Siegelman and Kycia, 1978). After centrifugation, the absorbance of the

supernatant was read at 565, 615, and 650 nm. The concentration of phycoerythrin in the supernatant was calculated from a trichromatic equation produced for Gracilaria tikvahiae which corrects for the spectral overlap of the phycobiliproteins (Rosenberg, 1981) as:

$$\text{R-phycoerythrin } (\mu g\ ml^{-1}) = 123.5\ A_{565} - 73.5\ A_{615} + 16.3\ A_{650}$$

where:

A_{565} = absorbance at the absorption maximum of R-phycoerythrin (565 nm)

A_{615} = absorbance at the absorption maximum of R-phycocyanin (615 nm)

A_{650} = absorbance at the absorption maximum of allophycocyanin (650 nm)

The concentration of phycoerythrin in the algal segments was then calculated as:

$$\text{Phycoerythrin } (mg\ (g\ dry\ wt)^{-1}) = \frac{(C_{RPE})\ (V_{ext})}{Wt}$$

where:

C_{RPE} = concentration of phycoerythrin in mg ml^{-1} calculated by the trichromatic equation

V_{ext} = extraction volume in ml

Wt = g dry wt

Statistical Tests

One-way Analyses of Variance (ANOVAs), Student-Newman-Keuls tests and Student's t-tests were conducted on the data on The University of Tennessee, Knoxville computer system (IBM 360/5-370/148) using the Statistical Analyses System package (SAS; Helwig and Council, 1979). The level of significance at which the null hypothesis of the statistical tests was rejected was set a priori at alpha = 0.05.

ANOVAs were used to determine if change in productivity or pigment concentrations occurred in the UV-B-irradiated or control algae over time. When change was found to occur, the Student-Newman-Keuls test was used to determine at which sampling times change occurred. Because of the diel rhythms, ANOVAs and Student-Newman-Keuls tests for pigment concentrations were conducted only on values occurring at 24-h intervals. Student's t-tests were used to determine if significant decrease or increase in productivity or pigment concentrations occurred compared to the control at each sampling time. No statistical tests were made between dose rate treatments because the low and medium UV-B dose rates were conducted on algae used within 2 weeks of collection, whereas the high dose rate treatment was conducted on algae used 7 weeks after collection.

III. RESULTS

Stability of Control Measurements over Time

The productivity of the control in the low and high UV-B dose rate treatments changed over time (Figure 1A and 1C, Table 3), whereas the productivity of the control in the medium UV-B dose rate treatment remained relatively constant (Figure 1B, Table 3). The mean productivity of the control in the medium UV-B dose rate treatment was 2.16 ± 0.21 mg C (g dry wt)$^{-1}$ h^{-1} (n = 8) which falls within the reported range of productivity for macroalgae (Littler, 1980). The mean assimilation number (carbon fixed per unit chlorophyll) for the control algae at 6 h in the three treatments was 2.05 ± 0.85 mg C (mg chlorophyll)$^{-1}$ (n = 3).

Control pigment concentrations did not change significantly over time in any of the treatments (Figures 2 and 3, Table 3). Chlorophyll was determined every 12 h in the medium UV-B dose rate treatment (Figure 2B). Over the four days of treatment, higher chlorophyll concentrations occurred at midday than at night in both the control and the UV-B-irradiated algae, indicating that a diel (circadian) rhythm in chlorophyll concentration occurrred. In all other treatments, pigment concentration measurements were taken every 24 h (with the exception of the measurement after the first 12 h) to avoid the variation in concentrations due to the diel rhythms.

Mean chlorophyll concentration of the control in the three dose rate treatments was 1.85 ± 0.32 mg (g dry wt)$^{-1}$ (n = 16) which was similar to the chlorophyll concentrations for G. tikvahiae reported by Ramus and van der Meer (1983). Mean phycoerythrin concentration of the

Figure 1. Effects of UV-B radiation on productivity of Gracilaria tikvahiae over 96 h at low, medium and high UV-B dose rates. (A) Low UV-B dose rate = < 0.18 W m^{-2}. (B) Medium UV-B dose rate = < 0.43 W m^{-2}. (C) High UV-B dose rate = 0.43 - 0.79 W m^{-2}. The low and medium UV-B dose rate treatments were conducted on algae used within 2 weeks of collection. The high UV-B dose rate treatment was conducted on algae used 7 weeks after collection.

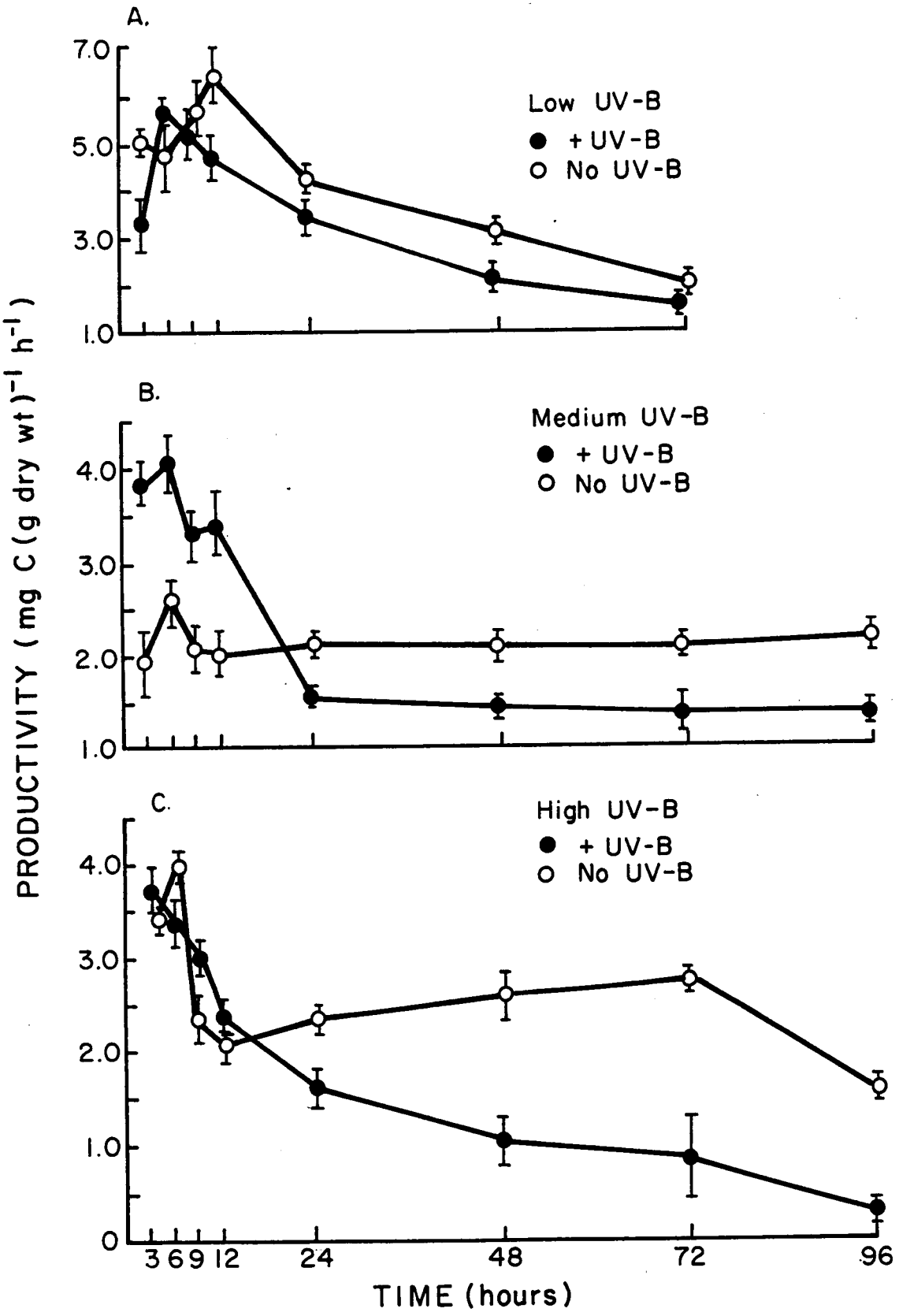
A.
7.0
5.0
3.0
1.0
Low UV-B
+ UV-B
No UV-B
B.
4.0
3.0
2.0
1.0
Medium UV-B
+ UV-B
No UV-B
C.
4.0
3.0
2.0
1.0
0
High UV-B
+ UV-B
No UV-B
3 6 9 12
24
48
72
96
TIME (hours)
PRODUCTIVITY (mg C (g dry wt)$^{-1}$ h^{-1})

Figure 1

Figure 2. Effects of UV-B radiation on chlorophyll concentrations of Gracilaria tikvahiae over 96 h at low, medium and high UV-B dose rates. (A) Low UV-B dose rate = < 0.18 W m^{-2}. (B) Medium UV-B dose rate = < 0.43 W m^{-2}. (C) High UV-B dose rate = 0.43 - 0.79 W m^{-2}. The low and medium UV-B dose rate treatments were conducted on algae used within 2 weeks of collection. The high UV-B dose rate treatment was conducted on algae used 7 weeks after collection.

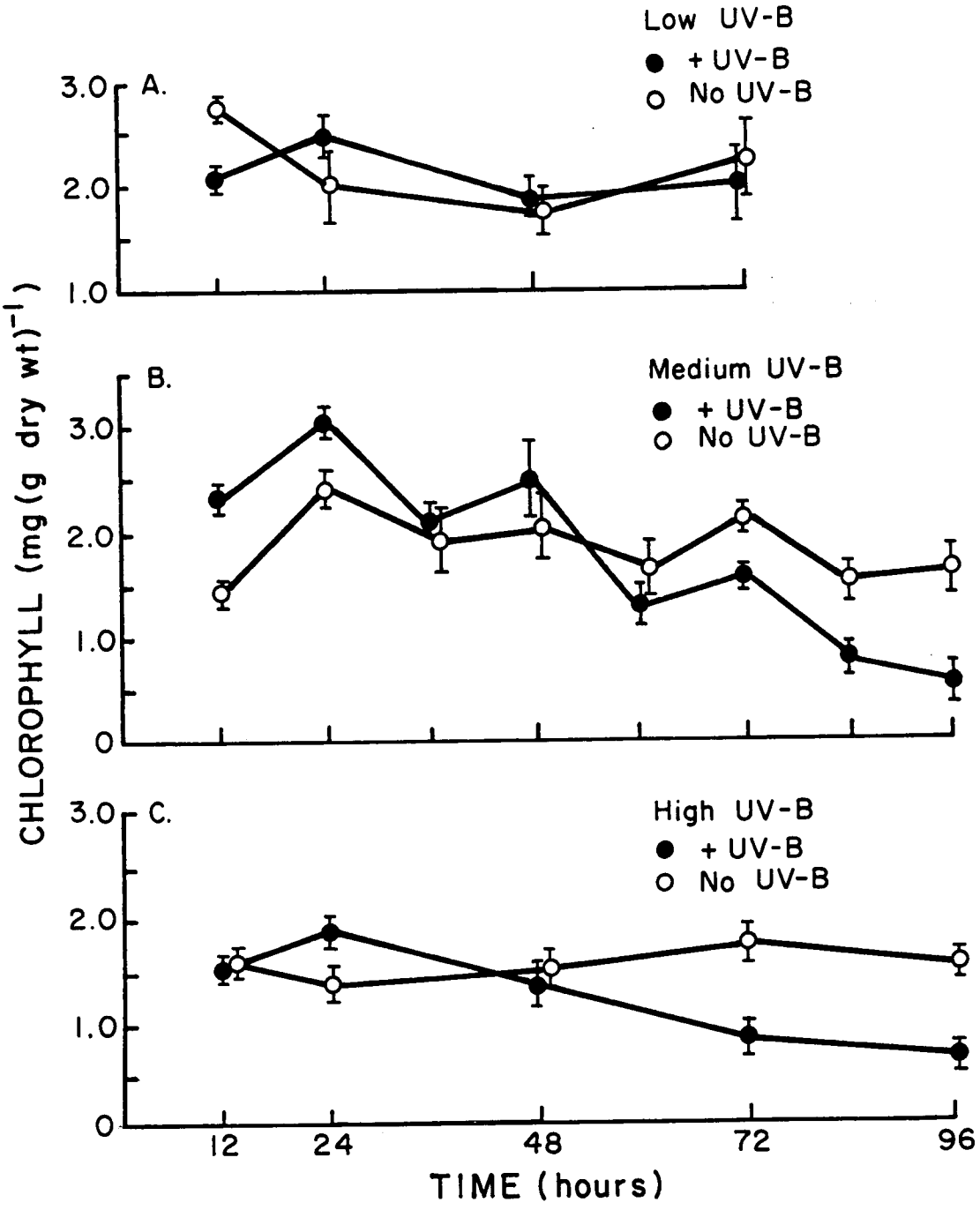
Low UV-B
+ UV-B
No UV-B
A.
3.0
2.0
1.0
Medium UV-B
+ UV-B
No UV-B
B.
3.0
2.0
1.0
0
High UV-B
+ UV-B
No UV-B
C.
3.0
2.0
1.0
0
12
24
48
72
96
TIME (hours)
CHLOROPHYLL (mg (g dry wt)$^{-1}$)

Figure 2

Figure 3. Effects of UV-B radiation on phycoerythrin concentrations of Gracilaria tikvahiae over 96 h at low, medium and high UV-B dose rates. (A) Low UV-B dose rate = < 0.18 W m^{-2}. (B) Medium UV-B dose rate = < 0.43 W m^{-2}. (C) High UV-B dose rate = 0.43 - 0.79 W m^{-2}. The low and medium UV-B dose rate treatments were conducted on algae used within 2 weeks after collection. The high UV-B dose rate treatment was conducted on algae used 7 weeks after collection.

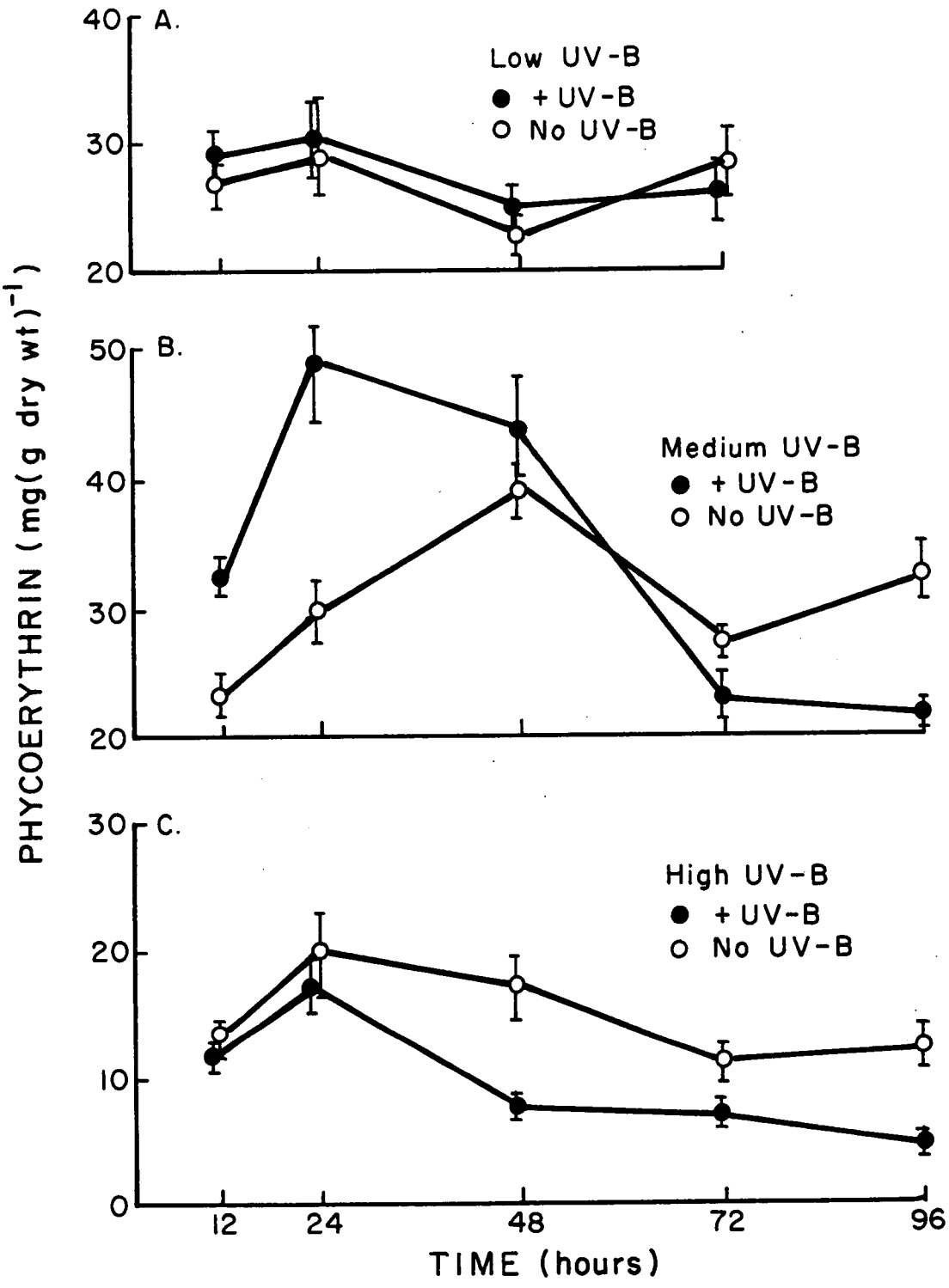
A.
Low UV-B
+ UV-B
No UV-B
B.
Medium UV-B
+ UV-B
No UV-B
C.
High UV-B
+ UV-B
No UV-B
PHYCOERYTHRIN (mg(g dry wt)$^{-1}$)
TIME (hours)
40
30
20
50
40
30
20
30
20
10
0
12
24
48
72
96

Figure 3

TABLE 3. ANOVAs of productivity, chlorophyll concentrations and phycoerythrin concentrations of UV-B- and non-UV-B-irradiated Gracilaria tikvahiae over time for low, medium and high UV-B dose rates.

ANOVA	Treatment	DF	MS	F
Productivity	Low; no UV-B	6	7.48	32.51**
	Error	11	0.23	
	Low; + UV-B	6	7.55	24.35**
	Error	11	0.31	
	Medium; no UV-B	7	0.08	0.48
	Error	14	0.17	
	Medium; + UV-B	7	4.31	22.66**
	Error	14	0.19	
	High; no UV-B	8	2.03	12.69**
	Error	16	0.16	
	High; + UV-B	8	5.18	21.39**
	Error	16	0.24	
Chlorophyll	Low; no UV-B	2	0.24	0.65
	Error	4	0.37	
	Low; + UV-B	2	0.21	0.49
	Error	3	0.43	
	Medium; no UV-B	3	0.30	4.29
	Error	6	0.07	
	Medium; + UV-B	3	5.63	13.98*
	Error	6	0.26	
	High; no UV-B	3	0.06	0.55
	Error	6	0.11	
	High; + UV-B	3	0.84	10.50*
	Error	6	0.08	
Phycoerythrin	Low; no UV-B	2	32.38	0.67
	Error	4	48.05	
	Low; + UV-B	2	26.87	0.85
	Error	3	31.54	
	Medium; no UV-B	3	75.98	3.59
	Error	6	21.18	
	Medium; + UV-B	3	622.30	11.24*
	Error	6	55.37	
	High; no UV-B	3	37.35	2.72
	Error	5	13.71	
	High; + UV-B	3	97.00	10.58*
	Error	6	9.17	

*Significant at p = 0.01.
**Significant at p = 0.001.

control in the low and medium UV-B dose rate treatments was 29.78 ± 5.47 (n = 7, algae used within two weeks of collection), whereas mean phycoerythrin concentration of the control in the high UV-B dose rate treatment was 15.79 ± 3.33 (n = 5, algae used 7 weeks after collection). The difference in mean phycoerythrin concentrations may be due to length of time of maintenance in the aquaria. Previously reported phycoerythrin concentrations of Gracilaria tikvahiae were from 15 to 20 mg (g dry wt)$^{-1}$ for .13I_o (13% of incident light intensity; Ramus and van der Meer, 1983).

Beneficial Effects of Low Doses of UV-B Radiation

In the low and high UV-B dose rate treatments, no beneficial effects of UV-B radiation were observed. However, in the medium UV-B dose rate treatment, productivity was stimulated and pigment concentrations were increased in UV-B-irradiated algae compared to the controls for the first 12 and 24 h, respectively (Figures 1B, 2B and 3B; Tables 4, 5 and 6). During the first 12 h of UV-B exposure in the medium UV-B dose rate treatment, mean productivity of the UV-B-irradiated algae was 3.63 ± 0.36 mg C (g dry wt)$^{-1}$ h^{-1} (n = 4), 66% higher than the mean control productivity of 2.19 ± 0.28 mg C (g dry wt)$^{-1}$ h^{-1} (n = 4). After 24 h of UV-B exposure, productivity of the UV-B-irradiated algae was significantly less than that of the control ($p < 0.05$). The occurrence of decreased productivity at 24 h while the pigment concentration was still increased indicated that decreased productivity was not a secondary effect of decreased pigment concentrations.

TABLE 4. Effects of UV-B radiation on productivity of Gracilaria tikvahiae over 96 h for three dose rate treatments.[a]

Time (h)	Productivity[b] (mg C (g dry wt)$^{-1}$ h^{-1})					
	Low UV-B dose rate		Medium UV-B dose rate		High UV-B dose rate	
	No UV-B	+ UV-B	No UV-B	+ UV-B	No UV-B	+ UV-B
3	5.07 ± 0.18b	3.45± 1.10b	1.99 ± 0.73	3.78 ± 0.50a*	3.47 ± 0.18ab	3.75 ± 0.51a
6	4.85 ± 0.87b	5.84 ± 0.19a	2.61 ± 0.33	4.05 ± 0.73a	4.00 ± 0.10a	3.32 ± 0.50ab
9	5.73 ± 0.69ab	5.23 ± 0.72a	2.10 ± 0.33	3.26 ± 0.55a*	2.33 ± 0.47cde	3.08 ± 0.38ab
12	6.50 ± 0.79a	4.66 ± 0.68a*	2.07 ± 0.28	3.42 ± 0.84a*	2.04 ± 0.30de	2.44 ± 0.26b
24	4.28 ± 0.37c	3.63 ± 0.40ab	2.18 ± 0.03	1.59 ± 0.15b*	2.43 ± 0.27de	1.58 ± 0.36c*
48	3.14 ± 0.08c	2.14 ± 0.28bc*	2.13 ± 0.34	1.42 ± 0.22b*	2.69 ± 0.40bc	1.02 ± 0.49c*
72	1.95 ± 0.18d	1.53 ± 0.21c*	2.07 ± 0.18	1.41 ± 0.35b*	2.84 ± 0.16bcd	0.95 ± 0.88cd*
96	---[c]	---	2.22 ± 0.35	1.32 ± 0.22b*	1.96 ± 0.30c	0.43 ± 0.15d**

[a]Productivity values with different letters in the same vertical column are significantly different over time at the 0.05 level according to the Student-Newman-Keuls test.

[b]Values are given as mean ± one standard deviation based on three replicates.

[c]No data available.

*Significantly different from the "no UV-B" control at same time and dose rate according to Student's t-test, p = 0.05.

**Significantly different from the "no UV-B" control at same time and dose rate according to Student's t-test, p = 0.01.

TABLE 5. Effects of UV-B radiation on chlorophyll concentrations of Gracilaria tikvahiae over 96 h for three dose rate treatments.[a]

Time (h)	Chlorophyll[b] (mg (g dry wt)$^{-1}$)					
	Low UV-B dose rate		Medium UV-B dose rate		High UV-B dose rate	
	No UV-B	+ UV-B	No UV-B	+ UV-B	No UV-B	+ UV-B
12	2.73 ± 0.14	2.19 ± 0.24*	1.46 ± 0.10	2.32 ± 0.28**	1.61 ± 0.14	1.54 ± 0.25
24	2.02 ± 0.63	2.50 ± 0.46	2.46 ± 0.27	3.09 ± 0.20a*	1.46 ± 0.22	1.87 ± 0.34a
48	1.79 ± 0.47	1.93 ± 0.28	2.16 ± 0.55	2.50 ± 0.84ab	1.59 ± 0.27	1.43 ± 0.40ab
72	2.32 ± 0.58	2.14 ± 0.73	2.11 ± 0.11	1.62 ± 0.04b**	1.77 ± 0.38	0.91 ± 0.37bc*
96	----[c]	---	1.69 ± 0.36	0.56 ± 0.43c*	1.46 ± 0.35	0.64 ± 0.26c*

[a]Chlorophyll concentration values with different letters in the same vertical column are significantly different over time at the 0.05 level according to the Student-Newman-Keuls test. 12-h values are not included in Student-Newman-Keuls test because of diel rhythm variance.

[b]Values given as mean ± one standard deviation based on three replicates.

[c]No data available.

*Significantly different from the "no UV-B" control at the same time and dose rate according to Student's t-test, p = 0.05.

**Significantly different from the "no UV-B" control at the same time and dose rate according to Student's t-test, p = 0.01.

TABLE 6. Effects of UV-B radiation on phycoerythrin concentrations of Gracilaria tikvahiae over 96 h for three dose rate treatments.[a]

Time (h)	Phycoerythrin[b] (mg (g dry wt)$^{-1}$)					
	Low UV-B dose rate		Medium UV-B dose rate		High UV-B dose rate	
	No UV-B	+ UV-B	No UV-B	+ UV-B	No UV-B	+ UV-B
12	26.93 ± 5.78	29.26 ± 3.64	23.99 ± 4.95	33.12 ± 3.12*	13.58 ± 1.26	11.96 ± 2.16
24	29.76 ± 6.90	30.78 ± 5.79	30.50 ± 7.05	48.68 ± 9.36a*	19.94 ± 6.31	17.52 ± 4.91a
48	23.59 ± 3.12	24.41 ± 4.12	39.18 ± 1.42	45.16 ± 9.03a	17.34 ± 6.02	7.96 ± 1.15b*
72	28.54 ± 6.65	26.60 ± 4.16	27.54 ± 1.66	22.72 ± 3.45b	13.37 ± 1.88	7.75 ± 1.69b*
96	---[c]	----	34.32 ± 5.61	21.51 ± 4.46b*	11.56 ± 3.13	4.34 ± 1.61b*

[a]Phycoerythrin concentrations with different letters in the same vertical column are significantly different over time at the 0.05 level according to the Student-Newman-Keuls test. 12-h values are not included in Student-Newman-Keuls test because of diel rhythm variance.

[b]Values are given as mean ± one standard deviation based on three replicates.

[c]No data available.

*Significantly different from the "no UV-B" control at the same time and dose rate according to Student's t-test, p = 0.05.

During the first 24 h of UV-B exposure in the medium UV-B dose rate treatment, mean chlorophyll concentration was 2.71 ± 0.54 mg (g dry wt)$^{-1}$ (n = 2), 38% higher than the control chloropohyll concentration of 1.96 ± 0.71 mg (g dry wt)$^{-1}$ (n = 2) and mean phycoerythrin concentration was 40.92 ± 10.97 mg (g dry wt)$^{-1}$ (n = 2), 50% higher than the control concentration of 27.25 ± 4.60 mg (g dry wt)$^{-1}$ (n = 2). Because the first pigment concentration measurements were not taken until after 12 h of UV-B exposure, it is not known how rapidly this response began. A gradual decrease over 48 h from increased levels in pigment concentrations to significantly lower levels than the control values contrasts with the relatively rapid decrease (12 h) in productivity.

Decreased Productivity Resulting from UV-B Radiation

Productivity was depressed by UV-B radiation in all treatments after 12 h (Figure 1, Table 4). In the low UV-B dose rate treatment (Figure 1A) after 9 h of no effect, productivity was significantly depressed from the control throughout the experiment ($p < 0.05$) although the control productivity was simultaneously decreasing. In the medium UV-B dose rate treatment (Figure 1B, in which the UV-B transmittance by the cellulose acetate decreased with time), mean productivity was 1.44 ± 0.11 mg C (g dry wt)$^{-1}$ h^{-1} (n = 4) until the end of the experiment; this was a 33% decrease from the control and was significant at the p = 0.05 level. In the high UV-B dose rate treatment (Figure 1C, in which the high UV-B transmittance was maintained by changing the cellulose acetate every 24 h), productivity was significantly decreased from the control ($p < 0.05$) from 24 to 72 h and also at 96 h ($p < 0.01$).

Decreased Pigment Concentrations Resulting from UV-B Radiation

Light-harvesting pigment concentrations were reduced by UV-B radiation, but not until after at least 48 h (Tables 5 and 6). Increasing the UV-B dose rate accelerated the time of occurrence of decreased pigment concentrations. Chlorophyll and phycoerythrin concentrations remained unchanged in the UV-B-irradiated algae in the low UV-B dose rate treatment (Figures 2A and 3A). In the medium UV-B dose rate treatment (Figures 2B and 3B), pigment concentrations in UV-B-irradiated algae were decreased by 72 h. The phycoerythrin concentration decrease was maintained at 96 h while the chlorophyll concentration had decreased further at 96 h. In the high UV-B dose rate treatment, the phycoerythrin concentration of the UV-B-irradiated algae was decreased earlier at 48 h (Figure 3C) while the chlorophyll concentration in the UV-B-irradiated algae was again decreased at 72 h (Figure 2C). In this treatment, pigment concentration decreases were maintained at about 50% lower than control concentrations until the end of the experiment. Statistically significant decreases in the phycoerythrin concentrations in the UV-B-irradiated algae in both treatments coincided with visual color change in the algal segments from purple to yellow-green. There was no significant change in the pigment concentrations or color of the algal segments in the controls.

Recovery during Exposure to Visible Light

Decreased productivity which had occurred after 48 h of UV-B exposure at the high UV-B dose rate showed no recovery after subsequent exposure to 48 h of visible light from two Sylvania cool white fluorescent

lamps (approximate irradiance = 6.2 W m^{-2}, Table 7). The decreased pigment concentrations which had occurred after 72 h of UV-B exposure at the high UV-B dose rate also showed no recovery after subsequent exposure to 48 h of visible light (Table 7). Because the phycoerythrin concentration was decreased by 48 h, the lack of recovery of productivity could have been because of lack of repair of the phycoerythrin-membrane system in the chloroplasts, although permanent damage to other components of the photosynthetic or nuclear systems may also have been involved.

TABLE 7. Recovery of decreased productivity and pigment concentrations in UV-B-irradiated Gracilaria tikvahiae after exposure to visible light.

Analysis	No UV-B	+ UV-B[a]	+ UV-B + 48 h visible light
Productivity (mg C (g dry wt)$^{-1}$ h^{-1})	1.98 ± .13	1.29 ± 0.38	0.91 ± 0.36
Chlorophyll (mg (g dry wt)$^{-1}$)	1.77 ± .37	0.91 ± 0.37	0.42 ± 0.23
Phycoerythrin (mg (g dry wt)$^{-1}$)	13.37 ± 1.88	7.55 ± 1.69	6.26 ± 1.48

[a]Productivity measurements were made on UV-B- and non-UV-B-irradiated algal segments after 48 h of exposure to high dose rate UV-B (0.43 - 0.79 W m^{-2}). Pigment concentration measurements were made on UV-B- and non-UV-B-irradiated algal segments after 72 h exposure at the high UV-B dose rate. In all cases, UV-B-irradiated values were significantly lower ($p < 0.05$) than control values.

IV. DISCUSSION AND CONCLUSIONS

Although low doses of UV-B (dose rate x exposure time) in the medium UV-B dose rate treatment stimulated productivity and increased light-harvesting pigment concentrations, no beneficial effects of low doses of UV-B were observed in the low or high UV-B dose rate treatments. This may indicate that the low UV-B dose rate was less than the dose rate required to produce beneficial effects and that the high UV-B dose rate was above a UV-B dose rate threshold for beneficial effects.

The increased productivity of the first 12 h of UV-B radiation in the medium UV-B dose rate treatment was accompanied by increased pigment concentrations for the first 24 h of UV-B exposure. This suggested that increased pigment concentrations were a contributing factor to increased productivity. The more rapid decrease in productivity than in pigment concentrations suggested that other components of the photosynthetic system were also stimulated by UV-B radiation.

Productivity was decreased in all dose rate treatments after 12 h of UV-B irradiation. By increasing the dose rate, the occurrence of decreased pigment concentrations was accelerated. However, at least 48 h were required before the gradual decrease in pigment concentrations became significant. The earlier decrease in productivity than in chlorophyll and phycoerythrin concentrations indicated that UV-B radiation damaged components of the photosynthetic system other than the pigment-membrane light-harvesting apparatus.

The period of 12 h of no discernable effect before UV-B radiation depressed productivity in G. tikvahiae at the high UV-B dose rate

appeared to contrast with the decreased ^{14}C-uptake in phytoplankton apparent after 4 to 5 h incubations at high UV-B dose rates (Worrest, 1981a). Therefore, G. tikvahiae appeared to be more tolerant to UV-B radiation than phytoplankton. This could result from the higher growth rate of phytoplankton when compared to macroalgae, implying that phytoplankton have a more rapid metabolic turnover and thus exhibit UV-induced productivity damage more rapidly. It is also possible that Gracilaria tikvahiae has evolved UV-B defense mechanisms to cope with exposure to high UV and visible levels in its natural environment, and that the apparent difference between G. tikvahiae and phytoplankton reflects a real difference between the two groups' responses to UV-B stress.

The decreased productivity and chlorophyll and phycoerythrin concentrations described in this study are potential effects of accumulated high UV-B doses and do not indicate the effects of increased solar UV-B under natural conditions. However, as a result of this study, studies of differential sensitivity of macroalgal species to predicted increases of solar UV-B can be related to a potential decrease in productivity and light-harvesting pigment concentrations. Studies of contributing factors to depressed productivity other than decreased pigment concentrations are indicated. The effect of UV-B radiation on macroalgal spores and on productivity and growth rate during rapid-growth stages might also prove to be important.

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