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TEMPERATURE DEPENDENCE OF UV RADIATION EFFECTS ON ANTARCTIC CYANOBACTERIA[1]

Jan C. Roos[2] *and Warwick F. Vincent*[3]

Département de Biologie et Centre d'Études Nordiques, Université Laval, Sainte-Foy, Québec, Canada G1K 7P4

ABSTRACT

The mat-forming cyanobacterium Phormidium murrayi *West and West isolated from a meltwater pond on the McMurdo Ice Shelf was grown in unialgal batch cultures to evaluate the temperature dependence of ultraviolet radiation (UVR) effects on pigment composition, growth rate, and photosynthetic characteristics. Chlorophyll* a *concentrations per unit biomass were generally reduced in cells grown under UVR (low UV-A plus UV-B).* In vivo *absorbance spectra showed that the carotenoid/chlorophyll* a *ratio increased as a function of photosynthetically available radiation (PAR) and UVR exposure and varied inversely with temperature. Ultraviolet inhibition of growth (percentage reduction of* μ_{max} *at each temperature) increased linearly with decreasing temperature, consistent with the hypothesis that net inhibition represents the balance between temperature-independent photochemical damage and temperature-dependent biosynthetic repair. There was no significant effect of UVR on photosynthesis over the first hour of exposure, but significant UV inhibition was observed after 5 days. Unlike growth, however, there was no apparent effect of temperature on the magnitude of UV inhibition of photosynthesis. These results imply that assays of UVR effects on photosynthesis are not an accurate guide to growth responses and that low ambient temperatures can have a major influence on the UV sensitivity of polar organisms. In a set of assays at 20° C (preacclimation under 300* μ*mol photons·*m^{-2}*·*s^{-1} *and 20° C), growth was strongly depressed by UVR over the first day of exposure but then gradually increased over the subsequent 4 days, approaching the growth rates in the minus UVR control. This evidence of acquired tolerance indicates that the damaging effects of UVR will be most severe in environments where there is a mismatch between the timescale of change in exposure and the timescale of UV acclimation.*

Key index words: acclimation; blue-green alga; carotenoids; chlorophyll; photosynthesis; UV-B radiation

Recent interest in the role of ultraviolet radiation (UVR) in aquatic ecosystems has focused especially on the polar regions, where large increases in the ground-level flux of UV-B wavelengths (280–320 nm) have resulted from stratospheric ozone depletion (Madronich 1994). UV-B is known to have a broad range of direct and indirect inhibitory effects on marine and freshwater biota, including inhibition of underwater photosynthesis (Vincent and Roy 1993, Williamson 1995). Solar UV-A radiation (320–400 nm) has also been implicated in the inhibition of primary production and plays an additional role in activating UV-damage-repair mechanisms (Quesada et al. 1995).

Cold water temperatures are a characteristic feature of the polar aquatic environment, and although a large literature now exists on the independent effects of temperature (Raven and Geider 1988, Davison 1991) and UVR (Neale et al. 1993) on photosynthesis, little is known about the interaction between these two physical variables on micro-algal physiology and growth. There is considerable evidence from higher plants that photoinhibition by photosynthetically available radiation (PAR) becomes increasingly severe at low temperatures (Krause 1994). In the aquatic vascular plant *Lemna gibba,* low temperatures depressed the turnover rate of the chloroplast D1 protein and thereby increased the severity of photoinhibition effects (Gong and Nilsen 1989). Effects of UVR on the related species *Spirodela oligorrhiza* appear to operate at least in part through D1 degradation (Greenberg et al. 1989). In a study of photosynthesis by the cyanobacterium *Synechococcus,* PAR photoinhibition was found to be independent of temperature, whereas the kinetics of recovery were highly temperature dependent at all PAR irradiances (Wünschmann and Brand 1992). These observations led us to hypothesize that if UV inhibition of photosynthesis and growth represents the balance between photochemical damage and biosynthetic repair (Vincent and Roy 1993), this balance will shift increasingly toward damage with decreasing temperature.

We evaluated the UV–temperature hypothesis by way of culture experiments on a freshwater species of cyanobacterium from Antarctica. Cyanobacteria of the family Oscillatoriaceae are widely distributed throughout the Arctic as well as Antarctica, and in shallow water environments they can be the ecosystem dominants (Vézina and Vincent 1997, Vincent 1997). Our aim was to better understand the ecophysiological responses of cyanobacteria to UVR in the changing polar environment and to define the range of optimal growth conditions for a high-latitude oscillatorian. This latter point is also relevant to the current development of tertiary wastewater treatment systems. Benthic Oscillatoriaceae appear to be a promising group of microorganisms for nutrient and contaminant stripping from wastewaters (Talbot and de la Noüe 1993), and pilot studies are

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[2] Present address: Department of Botany and Genetics, University of the Free State, P.O. Box 339, Bloemfontein 9300, South Africa.

[3] Author for reprint requests; e-mail warwick.vincent@bio.ulaval.ca.

in progress using polar cyanobacteria for outdoor tertiary treatment in cold north-temperate climates (Tang et al. 1997).

MATERIALS AND METHODS

Isolation and culture. The mat-forming cyanobacterium *Phormidium murrayi* West and West was isolated from an ice-shelf pond in Antarctica (Quesada and Vincent 1993) and grown in unialgal culture (nonaxenic but low bacterized) on sterilized BG-11 medium (Rippka et al. 1979). The stock culture was homogeneously dispersed with a Teflon pestle before inoculation.

Growth experiments. Incubations were for 5 days in a double illuminated system, with PAR from below and UVR from above, at 5°, 10°, 15°, 20°, and 25° C. The stock culture of dispersed, exponentially growing cells was diluted to an absorbance at 750 nm of 0.035 ± 0.005; 20-mL aliquots were then transferred to each 25-mL petri dish. The experiments were conducted in temperature-controlled culture chambers at 5°, 10°, 15°, 20°, and 25° ± 1.0° C. The specific irradiances (10, 30, 60, 100, 350, and 500 ± 10% $\mu mol \cdot m^{-2} \cdot s^{-1}$) were obtained by positioning the petri plates on a glass light table overlain with neutral density filters and illuminated from below with a 400-W Philips metal-halide circular lamp (MH 400/C/U). The lamp was enclosed in a cupola reflector to provide a concentric gradient of PAR on the light table. The UV lamps were placed over the petri dishes, and their height was adjusted to obtain a UV-A flux of 125 ± 15 $\mu W \cdot cm^{-2}$ and a UV-B flux of 25 ± 5 $\mu W \cdot cm^{-2}$. This gave a UV-B/UV-A ratio of about 0.2, which causes moderate growth inhibition in *P. murrayi* (Quesada et al. 1995). The UV-A source (320–400 nm) was a Spectroline X40 lamp combined with a Spectroline ENF 260 C lamp (both Spectronics Corp., Westburg, New York). Shortwave ultraviolet radiation (UV-B) was provided by a UVM-57 lamp (UVP Inc.) and by a Spectroline XX15B lamp (Spectronics). The output spectra for these UVR sources are given in figure 1 of Quesada et al. (1995). Ultraviolet radiation flux was measured beneath the petri dish lid with a DRC-100X digital radiometer (Spectroline), with calibrated sensors DIX-365 for UV-A and DIX-300 for UV-B radiation. Measurements of PAR were made with a 4π light meter (Biospherical Instruments Inc., San Diego, California). We used continuous illumination to avoid the biochemical and physiological changes introduced by light/dark cycles and in keeping with the continuous light regime during the polar summer. However, unlike the natural regime, the cells were exposed to constant UV-B/UV-A and UVR/PAR within each treatment. Although the UVR flux and UV-B/UV-A ratio were held constant across all UV treatments, the UVR/PAR ratio varied between treatments in inverse proportion to the PAR flux; that is, the lowest PAR treatment was exposed to the highest ratio of UVR/PAR.

The initial biomass, chlorophyll *a* concentration (chl *a*), and photosynthetic parameters were measured at the beginning (T_0) of the 5-day growth period. The algal biomass accrual, pigment composition, and photosynthetic characteristics were also monitored after 1 day (T_1), 3 days (T_3), and 5 days (T_5) by randomly selecting two petri dishes from each PAR irradiance. Biomass was estimated by optical density (OD) at 750 nm using a Milton Roy Spectronic 1001 plus spectrophotometer, as in Quesada and Vincent (1993). Previous work with this strain has shown that OD (750 nm) provides an accurate index of cell concentration (Quesada et al. 1995). Specific growth rates (μ) were then calculated as $\ln (B_f/B_i)/5$, where B_i is the initial biomass and B_f is the biomass at the end of the 5 days of incubation. Growth was represented as for photosynthesis by the Webb et al. (1974) equation (see below).

Pigment analyses. In vivo absorbance spectra were determined with a diode array spectrophotometer (Hewlett-Packard model HP-8452A) fitted with an integrating sphere (Labsphere Inc., North Sutton, New Hampshire, model RSA-HP-84). The measurements were from 400 to 800 nm every 2 nm with a total scanning time of 1 s, thereby eliminating the traditional problem associated with cell sedimentation during a spectral scan. A relative measure of cellular pigment concentration was then obtained from the *in vivo* absorbance values corresponding to the pigment peaks (chl *a* at 680 nm, phycocyanin-C [PC] at 612 nm, and carotenoids [Carot] at 490 nm). These values were normalized to the absorbance at 750 nm as a measure of relative concentration per cell.

Absolute concentrations of chl *a* concentration were determined on duplicate samples of 5 mL collected by filtration onto Whatman GF/C filters. The filters were homogenized and extracted in 10 mL of 90% acetone at 4° C in the dark for at least 1 h (in a refrigerator), centrifuged for 10 min at 10,000 rpm, and then assayed by spectrophotometer (Strickland and Parsons 1972). The duplicate analyses typically agreed to within 5%.

Photosynthesis. Photosynthetic rates were first measured in cultures that had been preincubated for 5 days at low light (60 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR; no UVR) and high temperature (20° C) and then transferred to the light table in the incubation chamber set to 5°, 10°, 15°, or 20° C. These measurements were made immediately after transfer (after allowing 1 h for temperature equilibration prior to the ^{14}C addition) and are referred to as T_0. The cultures were then allowed to grow for another 5 days with and without UVR at each PAR irradiance and temperature, and the photosynthetic measurements were repeated. Note that in these photosynthesis–irradiance (P-E) measurements at T_5, each photosynthetic value represents a ^{14}C incubation under the same PAR, UVR, and temperature conditions as those experienced during growth (unlike the T_0 measurements).

For each measurement, 10-mL aliquots of algal culture from the light table were transferred to 25-mL petri dishes and then injected with ^{14}C-labeled sodium bicarbonate at a final specific activity of 0.125 $\mu Ci \cdot mL^{-1}$. The petri dishes were placed on the light table and incubated for 1 h. Two control samples were also incubated with DCMU for dark (background) values. After incubation, 2-mL samples of duplicate cultures were transferred from the petri dishes to scintillation vials, acidified with 0.5 mL of 6N HCl, and mechanically shaken on a table for 30 min. The liquid was then neutralized with 0.5 mL of 6N NaOH, 10 mL of EcoLite (+) LSC-cocktail was added, and the vials were counted with an LKB 1219 Rackbeta liquid scintillation counter. Dissolved inorganic carbon was measured by pH determinations and potentiometric titration.

Carbon fixation rates were normalized to chl *a* (P^B), plotted as a function of irradiance (the P-E curve), and fitted using nonlinear regression (Frenette et al. 1993) to the model of Webb et al. (1974) to calculate the photosynthetic parameters:

$$P^B = P^B_{max} \cdot [1 - \exp(-\alpha^B E / P^B_{max})]$$

In this equation, P^B_{max} (mg C·(mg chl *a*)$^{-1}$·h^{-1}) is the light-saturated photosynthetic rate per unit chl *a*, α^B [mg C·(mg chl *a*)$^{-1}$·mol^{-1}·m^2] is the initial slope of the light-limited portion of the P-E curve, and E is irradiance. Two of the P-E curves showed evidence of photoinhibition, and for these the Platt et al. (1980) model was used:

$$P^B = P^B_S \cdot [1 - \exp(-\alpha^B E / P^B_S)] \cdot [\exp(-\beta^B E / P^B_S)]$$

where P^B_S is the theoretical maximum photosynthetic rate in the absence of any photoinhibition and β^B is the photoinhibition parameter. All the curve fits were by nonlinear regression using the Marquardt-Levenberg algorithm (SigmaStat, version 2.0). The light adaptation index, E_k, was calculated as P^B_{max}/α. The shape of the growth–irradiance (μ-E) curves was similar to that of the saturating exponential P-E curves, so we used the Webb et al. (1974) model with nonlinear regression estimates of maximum growth rates (μ_{max}) and the initial slope of the growth curve (α_μ). The light adaptation index for growth ($E_{k\mu}$) was calculated as μ_{max}/α_μ.

RESULTS

Pigment composition. Pigmentation of *P. murrayi* was strongly influenced by PAR as well as the UVR regime during the 5-day growth period. Figure 1 gives the data for a culture series incubated at 20° C. Chlorophyll *a* per unit biomass initially increased

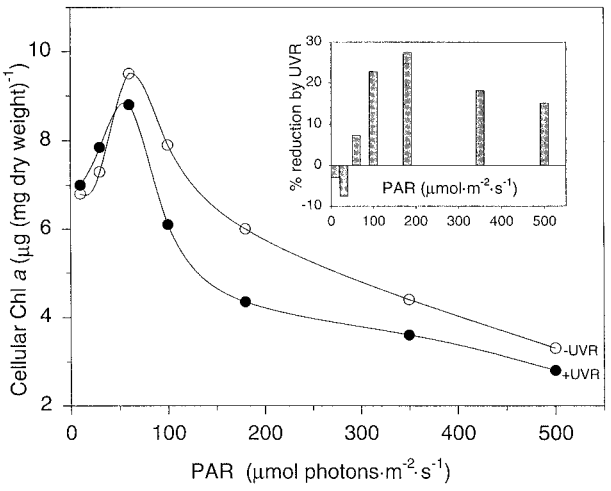

FIG. 1. The chl *a* content of *P. murrayi* as a function of PAR in the presence and absence of UVR. Each value is the mean of duplicate subsamples. Insert: the percentage reduction of chl *a* induced by UVR.

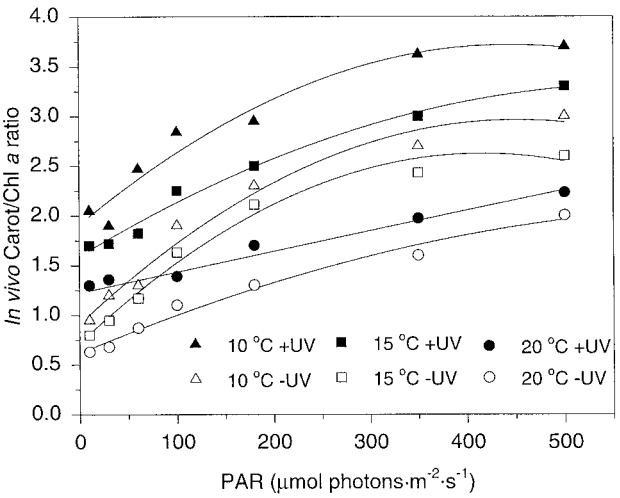

FIG. 2. The *in vivo* carotenoid to chl *a* ratio of *P. murrayi* as a function of PAR, UVR, and temperature. The lines have been fitted to the data by polynomial regressions.

with increasing PAR and reached a maximum concentration at 60 µmol·m^{-2}·s^{-1}. A further increase in PAR resulted in reduced concentrations of cellular chl *a*, with values at 500 µmol·m^{-2}·s^{-1} that were 82% below the maximum. A similar pattern was observed in the presence of UVR, with rising values over the range 10–60 µmol·m^{-2}·s^{-1} and decreasing values at higher PAR. For PAR irradiances <60 µmol·m^{-2}·s^{-1}, the cellular chl *a* was approximately the same with or without UVR; however, at higher PAR values, the UVR exposure resulted in an 8%–28% reduction.

In vivo absorbance spectra showed that the changes in chl *a* were accompanied by shifts in the relative concentration of carotenoids. Three types of effect were observed in the Carot/chl *a* ratios (Fig. 2). First, values increased with increasing PAR by a factor of about three over the range 10–500 µmol PAR·m^{-2}·s^{-1}. Second, there was an increase in this ratio with decreasing temperature; for example, at 500 µmol PAR·m^{-2}·s^{-1}, there was a 50% higher Carot/chl *a* ratio at 10° C relative to 20° C. This ratio was not determined for the 5° C treatment because the cell concentrations were too low to give reliable absorption values. Third, there was an additional increase induced by the exposure to UVR at all PAR irradiances, with the least effect at the highest temperatures; for example, at 500 µmol PAR·m^{-2}·s^{-1}, the presence of UVR caused Carot/chl *a* ratios to increase by 23% (10° C), 24% (15° C), and 12% (20° C).

The combined effect of all three variables on the pigment ratio Carot/chl *a* was examined by stepwise multiple linear regression using UVR as a dummy variable (set to 0 or 1). The data set (n = 41) met the criteria of normality and homoskedasticity and was closely fit (r^2 = 0.952) by the model

$$\text{Carot/chl } a = 1.92 + 0.0032\ \text{PAR} + 0.91\ \text{UVR} - 0.068\ \text{Temperature}$$

All coefficients in this model were highly significant at $P < 0.0001$. Further analysis by forward stepwise multiple regression showed that PAR explained 48.0% of the variance in Carot/chl *a*; UVR explained an additional 34.9% (r^2 = 0.83 for PAR + UVR) and temperature an additional 12.3%.

Photosynthesis. Photosynthesis versus light at T_0 followed a saturating exponential relationship with little evidence of photoinhibition up to 500 µmol·m^{-2}·s^{-1}; an exception to this pattern was at 20° C in the presence of UVR (Fig. 3a). There was no significant effect of temperature on α^B, but P^B_{max} increased with increasing temperature by a factor of 2.7 between 5° and 20° C (Table 1). The UVR exposure during incubation caused, on average, a 5.6% reduction in P^B_{max}, but a paired *t*-test for the four temperatures showed that this effect was not significant (t = 2.62, df = 3, P = 0.079). There was no discernible effect of either temperature or UVR on α^B values.

The P-E curves at T_5 similarly followed a saturating exponential relationship in which photoinhibition was not apparent, with the exception of 10° C + UVR (Fig. 3b). The photosynthetic rates exhibited a wider spread, reflecting the acclimation of cultures to the range of temperatures and light regimes and the longer-term influence of UVR exposure. There was no effect of UVR or of temperature on α^B values; however, P^B_{max} was significantly reduced by the presence of UVR (paired *t*-test: t = 3.94, df = 3, P = 0.029) to an average of 30% below the minus UVR treatments at T_5 (Table 1). There was no discernible trend in this effect with temperature.

Growth. Figure 4 illustrates the average 5-day growth rates (µ) versus irradiance (E) for *P. murrayi* in a single experiment in which the cultures were incubated at four different temperatures in the presence and absence of UVR. As for photosynthesis,

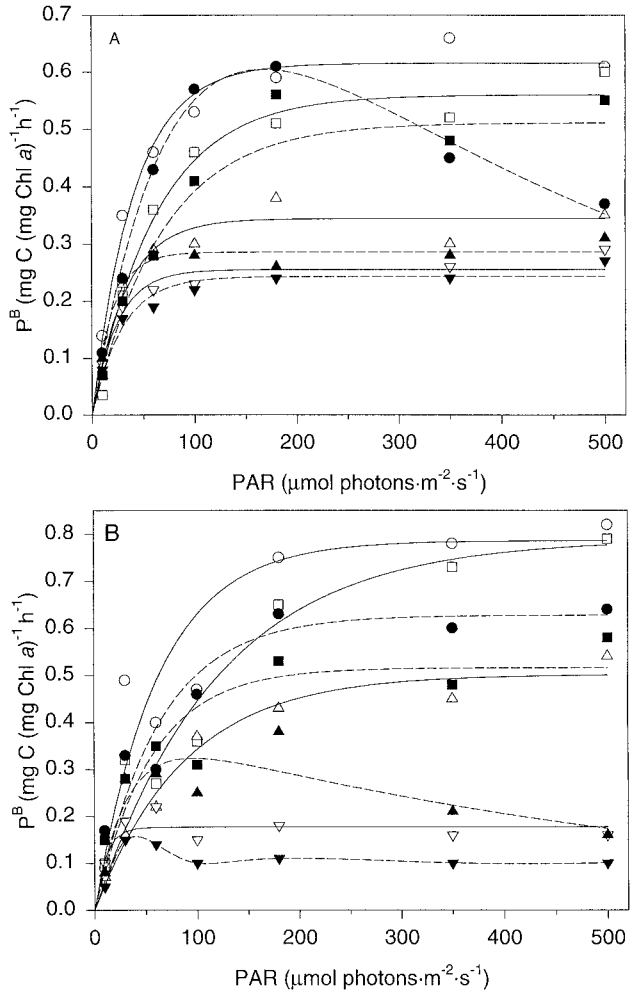

FIG. 3. The P-E curves for the *P. murrayi* cultures at different temperatures in the presence (closed symbols, dashed lines) and absence (open symbols, solid lines) of UVR. The lines have been fitted by the saturating exponential or double exponential model; the regression parameters are given in Table 1. Circles: 20° C; squares: 15° C; triangles: 10° C; inverted triangles: 5° C. (a) Cultures at T_0. (b) Cultures at T_5.

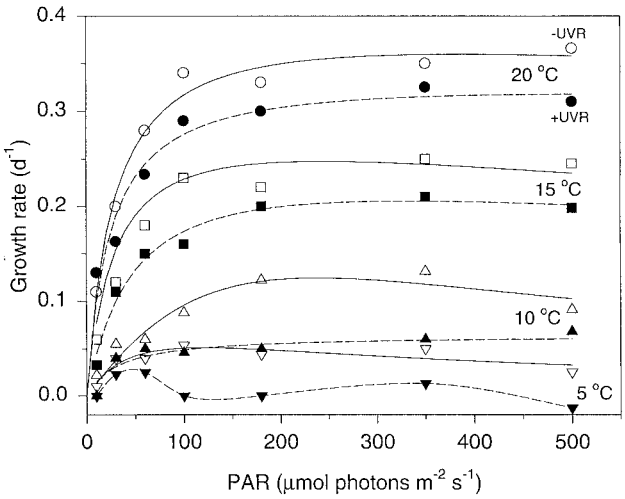

FIG. 4. Growth versus irradiance at four temperatures in the presence (closed symbols, dashed lines) and absence (open symbols, solid lines) of UVR. The lines have been fitted by the saturating exponential model; the regression parameters are given in Table 2.

these curves followed a saturating exponential relationship and were generally well described by the Webb et al. (1974) equation. The poorest fit was for the cultures at 5° C, in which growth rates were extremely slow and close to the limits of estimation (Table 2). There was a sevenfold drop in μ_{max} over the temperature range 20°–5° C and a fivefold drop in α_μ; the net result was a much smaller change in the saturation irradiance for growth $E_{k\mu}$, which showed no consistent change with temperature.

Growth rates were reduced by the presence of UVR at all PAR irradiances. The greatest effect was on μ_{max}; a paired *t*-test comparing plus and minus UVR over this temperature range gives $t = 9.92$, df = 3, $P = 0.0022$. A lesser but still significant effect was seen on α_μ ($t = 5.55$, df = 3, $P = 0.0115$). There was no significant effect of UVR on $E_{k\mu}$ ($t = 0.922$, df = 3, $P = 0.4246$).

A plot of μ_{max} versus temperature using data pooled from all our experiments with this isolate (n

TABLE 1. *Photosynthetic parameters for* P. murrayi *at four temperatures in the presence and absence of UVR. The estimates (±SE) are derived from nonlinear regression fits of the data in Figure 3 to the Webb et al. (1974) model or for two curves showing evidence of photoinhibition by the Platt et al. (1980) model. * = P^B_{max} for the two photoinhibited curves calculated from P^B_S.*

Temperature (° C)	P^B_{max} (10^{-1} mg C·(mg chl *a*)$^{-1}$·h^{-1})		α^B (10^{-2} mg C·(mg chl *a*)$^{-1}$·h^{-1}) (µmol·m^{-2}·s^{-1})$^{-1}$)	
	Minus UVR	Plus UVR	Minus UVR	Plus UVR
Day 0 (T_0)				
5	2.55 (0.10)	2.43 (0.09)	1.05 (0.18)	0.83 (0.13)
10	3.38 (0.17)	2.86 (0.09)	1.09 (0.20)	1.50 (0.22)
15	5.60 (0.27)	5.37 (0.27)	0.89 (0.09)	0.78 (0.10)
20	6.16 (0.17)	6.04*	1.51 (0.14)	1.11 (0.08)
Day 5 (T_5)				
5	1.77 (0.12)	1.15 (0.11)	1.75 (0.70)	1.09 (0.63)
10	5.02 (0.24)	3.24*	0.57 (0.06)	1.24 (0.49)
15	7.89 (0.73)	5.17 (0.48)	0.65 (0.12)	0.94 (0.25)
20	7.87 (0.79)	6.22 (0.46)	1.12 (0.29)	1.00 (0.21)

TABLE 2. *Growth versus irradiance parameters for* P. murrayi *grown in the presence and absence of UVR. The saturating exponential model of Webb et al. (1974) was fit to the data in Figure 4 by nonlinear regression; ±SE for the regression estimate of each parameter is given in parentheses.*

Treatment	μ_{max} (d^{-1})	α_μ (10^{-3}·d^{-1}·(μmol photons·m^{-2}·s^{-1})$^{-1}$)	$E_{k\mu}$ (μmol photons·m^{-2}·s^{-1})
Minus UVR			
5° C	0.043 (0.006)	1.8 (1.0)	25
10° C	0.11 (0.011)	1.9 (0.5)	55
15° C	0.24 (0.006)	5.8 (0.5)	42
20° C	0.35 (0.009)	10.3 (1.0)	35
Plus UVR			
5° C	0.007 (0.005)	0.9 (0.7)	19
10° C	0.06 (0.004)	1.8 (0.4)	42
15° C	0.20 (0.007)	4.6 (0.5)	46
20° C	0.31 (0.015)	8.9 (1.5)	36

= 2–4 at each temperature) shows that the light-saturated growth rate of *P. murrayi* was maximal around 20° C and that UVR had a consistent negative effect on growth throughout this temperature range (Fig. 5). The plus and minus UVR curves lie roughly parallel to each other, indicating a similar absolute impairment of μ_{max}, regardless of temperature. However, the proportional effect of UVR on μ_{max} strongly increased with decreasing temperature (inset, Fig. 5) and could be approximated by the negative linear relationship

$$\text{Percentage reduction in } \mu_{max} = 115 - 4.3 \text{ temperature } (r^2 = 0.875)$$

Acclimation to UVR. Changes in growth rate over the course of a 5-day incubation were examined in cultures that had been preacclimated (preceding 5 days) to high temperature (20° C) and high irradiance (300 μmol photons·m^{-2}·s^{-1}). The growth parameters during the subsequent 5-day incubation period without UVR were reasonably constant at the different irradiances from day 1 (T_1) to day 5 (T_5) (Fig. 6A). Paired *t*-tests show no significant difference between growth rates calculated for days 0–1 (labeled T_1 in Fig. 6A) and 1–3 (labeled T_3): t = 1.43, df = 6, P = 0.20. Similarly, there was no significant change between the T_3 growth estimates and those for the period day 3 to day 5: t = 2.02, df = 6, P = 0.09. However, in the presence of UVR (Fig. 6B), the growth rates at all PAR irradiances increased significantly from T_1 to T_3 (t = 8.74, df = 6, P = 0.00012) and again from T_3 to T_5 (t = 6.508, df = 6, P = 0.00063). Between the periods T_1 and T_5, μ_{max} increased by 28%. However, the final growth rates in the presence of UVR were still significantly below those in the control (minus UVR) at T_5 (t = 2.974, df = 6, P = 0.025). These increased growth rates with time imply physiological acclimation of *P. murrayi* to UVR over the course of the experiments.

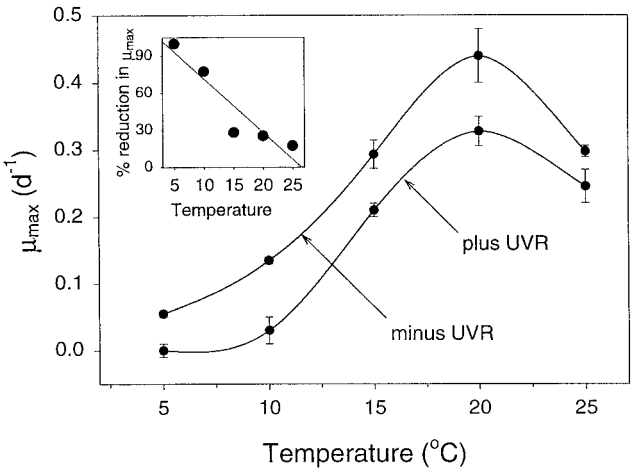

FIG. 5. Maximum growth rates (μ_{max}) of *P. murrayi* as a function of temperature in the presence and absence of UVR. For this plot, data from all our experiments have been combined, and the error bars represent ± SE for the mean of two to four estimates of μ_{max} at each temperature. Insert: UV inhibition (as a percentage reduction in μ_{max}) as a function of temperature for this pooled data set.

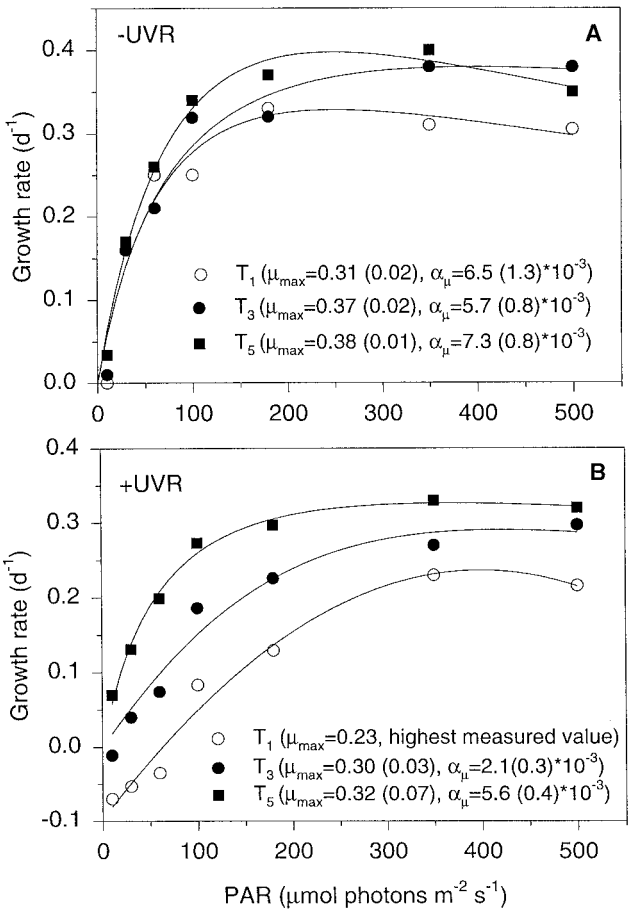

FIG. 6. Growth-irradiance curves for *P. murrayi* calculated for the periods days 0–1 (labeled T_1), days 1–3 (T_3), and days 3–5 (T_5) at 20° C in the absence (A) and presence (B) of UVR.

DISCUSSION

Strong UVR radiation is known to photo-oxidize and bleach all types of photosynthetic pigment (Häder and Worrest 1991), and it can also inhibit the biosynthesis of pigments, including chl *a* (Strid et al. 1990). At the moderate dosage rates employed in our experiments, UVR radiation affected the pigmentation of *P. murrayi* but to an extent that depended on temperature and PAR. Ultraviolet-induced reductions in chl *a* per unit biomass were observed only at higher PAR. Below 60 μmol photons·m^{-2}·s^{-1}, there was no significant UVR effect, and the chl *a* content decreased with decreasing irradiance. This latter response may reflect the tradeoff between light-capturing ability and the energetic costs of building light-harvesting complexes (Markager and Sand-Jensen 1994).

Ultraviolet radiation and bright PAR had similar but independent effects on the carotenoid/chl *a* ratio, consistent with the protective role of carotenoids against reactive oxygen species (ROS) and excess excitation energy (Vincent and Roy 1993). Low temperatures caused a significant increase in the carotenoid/chl *a* ratio and in this way mimicked the effects of bright PAR. Similar effects have been observed in cultures of *Chlorella* by Maxwell et al. (1994, 1995). These authors concluded that photosynthetic acclimation is a response to ''excitation pressure,'' that is, the imbalance between the supply of excitation energy and its dissipation by means of photochemical quenching; thus, low-temperature, low-light-acclimated cells have photosynthetic (including pigment) characteristics that are almost identical to high-temperature, high-light-acclimated cells. Such observations also suggest that low-temperature cells are under light stress at even moderate PAR irradiances and that the combined effect of low temperature and high PAR may render the cells especially sensitive to the additional stress of UVR exposure. The changes in pigment ratio observed here are likely to be the combined result of increased carotenoid pigmentation and decreased chl *a* concentrations in the cells. For example, at 20° C, we observed a measurable reduction in chl *a* under UVR at high but not low PAR irradiances (Fig. 1), whereas at the same temperature Carot/chl *a* ratios increased under UVR at all PAR irradiances, implying changes in the cellular concentration of both pigments.

The photosynthetic assays at T_0 indicated that the UVR dosage rates used here were too low to cause any statistically significant short-term effects on photosynthesis. However, at the longer timescale of the 5-day growth experiments, the UV-treated cells had lower maximum photosynthetic rates at all temperatures. The time dependence of this effect implies a dose response to UVR exposure. Although studies to date on algal responses to UVR have focused on dosage rate (i.e. UVR flux rate) rather than dose (Cullen and Lesser 1991), there is evidence from photosynthetic studies on natural communities of marine phytoplankton of dose type effects, albeit over short (hours) timescales (Behrenfeld et al. 1993). However, neither dose nor dosage-rate dependency is an adequate description of long-term effects that depend on the relative timescales of damage versus recovery and on the timescales of acclimation to changes in the ambient UVR plus PAR flux.

An alternative explanation of the delayed UV effect on P^B_{max} is that the cells were initially responding to the transfer from the preacclimation regime of moderately low irradiance (60 μmol·m^{-2}·s^{-1} PAR; no UVR) and high temperature (20° C). For example, the transfer to 200 μmol·m^{-2}·s^{-1} PAR at 5° C would result in a high excitation pressure that could dominate the photosynthetic response and thereby obscure any short-term UV effect. By this argument, the plus and minus UVR differences at T_5 were the result of retarded acclimation to PAR and temperature (i.e. to excitation pressure) in the presence of UVR over the 5-day period of the experiment. In support of this explanation, the average P^B_{max} for the minus UVR treatments was 27% higher at T_5 than at T_0. However, this difference was not significant (paired *t*-test: $P = 0.172$), and even over the low irradiance range of the P-E curves (e.g. at the preacclimation irradiance of 60 μmol·m^{-2}·s^{-1}), there was little effect of UVR at T_0.

Contrary to its nonsignificant or relatively small effect on photosynthesis, UVR exposure strongly inhibited growth rates over the 5-day incubation period. This apparent disparity may be the result of several factors. First, the cumulative, debilitating effects of UVR on photosynthesis and thus growth may have become important only at larger, cumulative doses (i.e. later in the experiment). On the other hand, such effects would be offset by the gradual acclimation of cells toward growth under UVR. Second, the photosynthetic rates were normalized to chl *a* for the standard analysis of P-E parameters. However, the chl *a* content per unit biomass decreased with UVR exposure, so the photosynthetic rate per cell must also have decreased. Finally, growth represents the net balance between photosynthesis, respiration, and excretion; the UVR treatment could have affected the relative magnitude of each of these processes and disrupted the cellular division cycle. Even at 5° C, the effect of UVR on photosynthesis (5% decrease in P^B_{max} at T_0, 35% at T_5) was much smaller than on growth (84% decrease in μ_{max} in this experiment). Regardless of the exact cause of this disparity, these results show that the immediate, short-term effects of UVR on photosynthesis can be a misleading guide to the longer-term effects on growth.

Consistent with our hypothesis, the percentage reduction in growth rates induced by UVR increased with decreasing temperature. It is of interest that the absolute magnitude of μ_{max} depression was similar

between temperatures in both the initial experiment (Table 2) and pooled data set (Fig. 5). This suggests a constancy of absolute effect set by the uniform UVR regime (but gradually reduced by UVR acclimation), with the result that relative impairment was most severe for cells growing at the slowest rates (i.e. at the lowest temperatures). This temperature effect was not apparent in the P-E assays at either T_0 or T_5, again implying that the results from standard photosynthetic measurements should be interpreted with caution. The strong influence of low temperatures is in accord with observations elsewhere (e.g. Lesser et al. 1994) that UV inhibition represents damage minus repair and that this net effect can be worsened by either increasing the rate of damage (e.g. increased UVR dosage rate or decreased ROS scavenging) or decreasing the rate of repair (e.g. general inhibition of biosynthesis by temperature, toxins, or nutrient limitation).

Our results imply that phototrophic organisms living in cold environments may be especially prone to the damaging effects of ultraviolet radiation. These findings are especially relevant to the perennially cold waters found in the north and south polar zones, where stratospheric ozone depletion and the associated increases in ambient UV-B radiation are proceeding most rapidly. However, offset against this evidence of toxic UVR effects was the observation that *P. murrayi* gradually increased its growth rate under UVR, indicating an ability to build up tolerance against this environmental stress. This acclimation could proceed by a variety of mechanisms, including increased efficiency of damage repair (e.g. increased cell concentrations of repair enzymes) or of ROS quenching (e.g. increased carotenoids, peroxidases, catalases, and/or superoxide dismutases). The speed of these acclimation processes will depend on biosynthetic rates and thus, like the direct effects of temperature on the damage-repair balance, will also be affected by temperature.

The acclimation process observed in *P. murrayi* has repercussions for obtaining and applying dose-response curves and biological weighting functions to predict the effects of UVR on aquatic communities. The results imply that individual species of micro-algae and cyanobacteria could yield a broad family of curves with major differences between experiments determined by the timescale of measurement, the speed of repair processes, and the changing acclimation state of the organism during and immediately prior to the assay. This ability to develop an increased tolerance also implies that the negative effects of UV-B radiation in the polar environment will be most severe where there is insufficient time for organisms to acclimate to a change in their incident UVR field. Defining critical times or locations of this mismatch between the timescales of UVR exposure and acclimation may be a useful way of identifying when and where biological communities are most likely to respond to stratospheric ozone depletion.

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