An *in situ* biological weighting function for UV inhibition of phytoplankton carbon fixation in the Southern Ocean

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**ABSTRACT:** A daily integrated *in situ* biological weighting function (BWF) for inhibition of primary production by ultraviolet radiation (UVR, 280 to 400 nm) was determined for a natural community of Antarctic diatoms maintained under daylight conditions. The derived daily averaged BWF had a radiation amplification factor of 0.91 for the environmental radiation conditions under which it was determined, and displayed greater sensitivity to UV-B than BWFs determined for laboratory cultures of temperate latitude phytoplankton (Cullen et al. 1992. *Science* 258:646–650). In addition, the function was shown to accurately predict the UVR-dependent *in situ* rates of primary production when the same community was under different stratospheric ozone (O³) conditions. An error estimate for the BWF is also provided and the predictive limitations of the function are discussed briefly. In the early austral spring of 1993 near Palmer Station, Antarctica, surface samples were maintained in 6 spectrally distinct outdoor incubators over the course of a single day and the spectral sensitivity of photosynthetic carbon fixation rates and phytoplankton pigmentation was quantified. The changes in spectral sensitivity to O³-dependent UV-B (280 to 320 nm radiation) and O³-independent UV-A (320 to 400 nm radiation) was resolved on time scale of 2 h intervals over the course of the 10 h incubation. Besides determining the daily peak of cell sensitivity to UVR damage, the derived short-term kinetics for the 6 different spectral light treatments provided the database for resolving a robust action spectrum for the UVR inhibition of *in situ* rates of primary production. For the diatom community being studied, daily exposure to ambient levels of UVR resulted in a 34% reduction in averaged carbon fixation without any significant effect on the cellular pigment content. The UV-B portion of the solar spectrum photoinhibited daily rates of primary production by 15%, while UV-A was responsible for a 19% reduction in daily averaged rates of carbon fixation. It appears that springtime diatom-dominated communities are equally or more sensitive to UV-B photoinhibition of daily primary production than pynmesiophyte-dominated communities, analyzed during the 1990 ‘Icecolor’ expedition (Smith et al. 1992. *Science* 258:952–959).

**KEY WORDS:** UV Primary production · Phytoplankton · Antarctica · Action spectra · Photoinhibition · Ozone

**INTRODUCTION**

It is widely acknowledged that the severity (i.e. magnitude, area and duration) of the Antarctic ozone (O³) 'hole' has increased significantly in the last decade. It is now common to observe springtime O³ levels 30 to >50% less than levels measured pre-O³ hole or at lower latitudes ‘outside’ the O³ hole [ca 250 to 400 Dobson Units (DU)] (Smith et al. 1992, Stolarski et al. 1992, Madronich 1993, Booth et al. 1994). The maximum area covered by the O³ hole has expanded nearly to the limit of the south polar vortex and routinely passes over the majority of the Southern Ocean. In addition, O³-poor air masses commonly reach South America and Australia (Roy et al. 1994). The duration of the O³ hole appears to be lengthening while the depletion was first observed only in October, it has lately been evident from late September until early
December. In 1993, the magnitude of the OЗ hole was remarkable in that stratospheric OЗ levels over the South Pole were the lowest on record (<100 DU) and significant OЗ depletion was observed over large regions of the Antarctic continent prior to the beginning of spring (WMO 1995). All of the above changes, as well as the observations that OЗ levels are declining (although less severely) over much of the rest of the world are consistent with reports that the global inventory of OЗ has decreased in recent years (Stolarski et al. 1992).

The direct link between OЗ depletion, increased UV-B irradiance (EUVB, 280 to 320 nm), and associated changes in the spectral balance of UV-B, UV-A and photosynthetically available radiation (PAR) (Table 1), has raised concern and prompted research into the potential impacts of environmental increases in EUVB on the ecological balance of Antarctica (Weiler & Penhaie 1994). Given that the vast majority of Antarctic communities are marine and dependent upon the productivity of phytoplankton, and that the timing of the greatest relative increase in EUVB coincides with the spring growing season, the impact of ultraviolet radiation (UVR) on autotrophic carbon fixation in the Southern Ocean has received special attention in recent years (El-Sayed et al. 1990, Karentz et al. 1991a, b, Helbling et al. 1992, Smith et al. 1992, Holm-Hansen et al. 1993, Prézelin et al. 1994a, Weiler & Penhaie 1994). While laboratory studies have a place in defining mechanisms of EUVB damage, it is increasingly recognized that environmental concerns, and their possible remediation, cannot be addressed adequately until OЗ-related effects are quantified and evaluated under natural conditions (see UNEP 1994). Relevant field experiments, Prézelin et al. (1994a) argued, had to consider that phytoplankton are polychromatic organisms. Modifying the spectral balance of their light fields on time scales of a few hours to days as the OЗ hole oscillates overhead could easily upset the balance of the many interdependent photoprocesses (photosynthesis, photorepair, photoadaptation, nitrogen assimilation, taxis etc.) that all plants have evolved over millennia (Mohr et al. 1984, Senger et al. 1986, Middleton & Taramura 1994).

Whereas the spectral increase in UV-B associated with the reduction in stratospheric OЗ is well documented (Lubin & Frederick 1991, Madronich 1993), the effects of the spectral shifts in UV radiation on primary production remain less well understood. Action spectra linking the amount of damage to the wavelength of UVR have long been measured for several photoprocesses in terrestrial animal and plant physiology (Caldwell et al. 1986), but it is only recently that such functions have been described for temperate phytoplanktonic organisms (Cullen et al. 1992). With the advent of the ozone hole, the shape of such spectra has been used as a biological weighting function (BWF) to compensate for the unbalanced effects of UVR on biological systems and quantify a radiation amplification factor (RAF), i.e. the increment of biological damage associated with a diminution in OЗ concentration (National Academy of Sciences 1979, Madronich & de Grujil 1993, Booth & Madronich 1994).

### Table 1. Significant symbols and abbreviations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Carbon (mg m$^{-3}$)</td>
</tr>
<tr>
<td>Chl</td>
<td>Chlorophyll a (mg m$^{-3}$)</td>
</tr>
<tr>
<td>Q(λ)</td>
<td>Quantum irradiance at wavelength λ (µmol quanta m$^{-2}$ s$^{-1}$)</td>
</tr>
<tr>
<td>E(λ)</td>
<td>Irradiance at wavelength λ (W m$^{-2}$)</td>
</tr>
<tr>
<td>PAR</td>
<td>Photosynthetically available radiation (400–700 nm)</td>
</tr>
<tr>
<td>UVR</td>
<td>Ultraviolet radiation (280–400 nm)</td>
</tr>
<tr>
<td>UV-A</td>
<td>Ultraviolet A radiation (320–400 nm)</td>
</tr>
<tr>
<td>UV-B</td>
<td>Ultraviolet B radiation (280–320 nm)</td>
</tr>
<tr>
<td>M(λ)</td>
<td>Biological weighting function (BWF) at wavelength λ</td>
</tr>
<tr>
<td>RAF</td>
<td>Radiation amplification factor</td>
</tr>
<tr>
<td>AI$_{EUVB}$</td>
<td>Absolute inhibition of primary production in spectral incubator Tnm</td>
</tr>
<tr>
<td>FI$_{EUVB}$</td>
<td>Fractional UV inhibition of primary production in spectral incubator Tnm</td>
</tr>
<tr>
<td>α</td>
<td>Photosynthetic efficiency [mg C m$^{-3}$ h$^{-1}$ (µmol quanta m$^{-2}$ s$^{-1}$) ]</td>
</tr>
<tr>
<td>β</td>
<td>Slope of the white light photoinhibited portion of the P-I curve [mg C m$^{-3}$ h$^{-1}$ (µmol quanta m$^{-2}$ s$^{-1}$) ]</td>
</tr>
<tr>
<td>$l_k$</td>
<td>Minimum irradiance for onset of light-saturated rates of photosynthesis (µmol quanta m$^{-2}$ s$^{-1}$) (= $P_{max}$/α)</td>
</tr>
<tr>
<td>$l_l$</td>
<td>Minimum irradiance for onset of white light inhibition of photosynthesis (µmol quanta m$^{-2}$ s$^{-1}$) (= $P_{max}/β$)</td>
</tr>
<tr>
<td>$P_{max}$</td>
<td>Volume-specific rate of light-saturated photosynthesis (mg C m$^{-3}$ h$^{-1}$)</td>
</tr>
<tr>
<td>$P_{max}/Chl$</td>
<td>Chl-specific rate of light-saturated photosynthesis (mg C mg Chl$^{-1}$ h$^{-1}$)</td>
</tr>
<tr>
<td>$P$</td>
<td>Volume-specific rates of in situ photosynthesis (mg C m$^{-3}$ h$^{-1}$)</td>
</tr>
<tr>
<td>$P/Chl$</td>
<td>Chl-specific rates of in situ photosynthesis (mg C mg Chl$^{-1}$ h$^{-1}$)</td>
</tr>
<tr>
<td>$P_p$</td>
<td>Photosynthetic rate modelled from P-I parameters (mg C m$^{-3}$ h$^{-1}$)</td>
</tr>
<tr>
<td>$P_{PAR}$</td>
<td>Photosynthetic rate under PAR only (mg C m$^{-3}$ h$^{-1}$)</td>
</tr>
<tr>
<td>Tnm</td>
<td>Incubator designation based on a filter combination that transmits ≥ 50% of incoming light at wavelengths &gt; 'nm'</td>
</tr>
</tbody>
</table>
to spectral shifts in ambient UVR, i.e. the BWF, and therefore link changes in \( E_{UVB} \) (i.e. due to season, time of the day, cloud and ice cover, stratospheric \( \text{O}_3 \) concentration and location in the water column) to the inhibition of carbon uptake in Antarctic phytoplankton.

The present experiment was conducted to estimate the \textit{in situ} daily integrated BWF for primary production inhibition in Antarctic phytoplankton under natural irradiance conditions. We quantified the inhibitory effects of different bandwidths of UVR on springtime primary production of typical Antarctic coastal waters diatom communities. Here, we present and test the function, demonstrate that the sensitivity of Antarctic phytoplankton to UV-B during the early spring transition to higher irradiances is greater than that of temperate terrestrial plants and marine phytoplankton, and show how the newly derived \textit{in situ} BWF can be used to model UV-dependent rates of primary production for similar diatom-dominated communities. We make no assumption regarding the universality of our BWF, as such conclusions can not be reached until more field data are available.

**MATERIAL AND METHODS**

**Location and time of work.** Measurements were made on September 16, 1993 [Day 259 of the Year] in ice-free coastal waters of the Antarctic Peninsula. Working from a Mark V Zodiac near dawn (ca 08:00 h local time, LT), a large volume (20 l) of surface water was collected in blackened polyethylene bottles at the Palmer Long Term Ecological Research (LTER) Station B (64° 46.27' S, 64° 4.35' W). Bottles were returned to the laboratory within 30 min of collection, and samples prepared for outdoor incubations in less than 1 h.

**Outdoor simulated \textit{in situ} (SIS) incubators.** Optical notations and other significant symbols and abbreviations are defined in Table 1. Outdoor SIS incubators described elsewhere (Prézelin et al. 1994a, b) were employed for determination of the BWF, thereby assuring that samples were exposed to the natural fluctuations in the solar light field over the course of a day. For the different spectral light treatments, the containers and the sample compartment dividers were composed of black Plexiglas to eliminate back and side direct radiation and to minimize reflection. Trays were covered with combinations of long-pass filters selected to remove increasingly larger portions of the UV spectrum.

For each of the treatments, 18 \( ^{14} \text{C} \) radiolabelled 200 ml samples and 3 non-radiolabelled 500 ml samples were sealed in nontoxic UV-transparent polyethylene (PE) bags (Prézelin et al. 1994a, b). Once filled, the sample bags had the broad sides exposed to the incoming light field and were in direct contact with incubator lids. A constant flow of sea water was pumped from the nearby harbor and circulated through the incubators keeping the SIS samples within 0.5°C of \textit{in situ} surface water temperature.

**Spectral attenuation within each light treatment.** Knowledge of the transmission characteristics of the incubators and the fluctuating environmental spectral irradiance was required to accurately estimate the phytoplankton exposure within each bag and for any time interval within the day. The optical properties of the different filter materials and the PE bags were determined on a DW-2 Aminco spectrophotometer (0.67 nm resolution) and are presented in Fig. 1. For clarity, the term 'Tnm' is used here to designate each light treatment by its 50% transmittance wavelength, i.e. T299 denotes the light treatment transmitting 50% of 299 nm light and more than 50% all wavelengths greater than 299 nm.

Spectrally integrated \( \text{PAR} \) (Q\text{PAR}, \( \mu \text{mol quanta m}^{-2} \text{s}^{-1} \)) was monitored continuously using a Biospherical 185B photometer placed next to the outdoor incubator.

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**Fig. 1** Comparison of the UVR transmission properties for samples sealed in polyethylene (PE) incubation bags and placed within different spectral incubators during the 'Icecolors 93' field expedition. The rigid long-pass filters consisted of 6.4 mm Plexiglas (UVT, MC, UF4, UF3), or 3.2 mm UVT Plexiglas when used in combination with an inserted 0.13 mm sheet of Mylar (Mylar), 6.4 mm (Pyrex 2) and 3.2 mm (Pyrex 1) Pyrex. The resulting 50% transmission cutoffs wavelengths and average transmission in the PAR region were: (1) <290 nm and 91.2%, (2) 299 nm and 83.0%, (3) 314 nm and 86.8%, (4) 324 nm and 82.9%, (5) 328 nm and 81.4%, (6) 382 nm and 84.2%, (7) 402 nm and 84.6%, (8) >414 nm and 79.5%. For added details regarding outdoor incubators, see Prézelin et al. (1994a, b).
and 5 min averages recorded on a Li-Cor 1000 datalogger. In addition, the NSF (National Science Foundation) polar network for monitoring UV radiation (Booth et al. 1994) provided spectral irradiance at the surface measured every hour, on the hour $E_\lambda (\lambda, 0^\circ)$, $\lambda = 280$ to 400 nm, 0.1 nm step size). Radiation inside the experimental containers was determined by multiplying the irradiance spectra by the transmission spectra of the incubator. For all calculations, transmittance spectra were numerically degraded to 1 nm resolution.

**Chemical analyses.** Chlorophyll a (chl) and accessory pigments as well as particulate carbon (C) and nitrogen (N) concentrations were measured at the sampling site for 3 d prior to the experiment. CN samples (1.5 l) were filtered onto pre-combusted (1 h at 500°C) Whatman GF/F glass fiber filters, dried at 65°C and transported to the Marine Science Analytical Laboratory at UC Santa Barbara, CA, USA, where chemical analyses were made using standard techniques (see Moline et al. 1997, and references within).

Phytoplankton pigmentation was determined by high performance liquid chromatography (HPLC) on a Hitachi HPLC system and using procedures described elsewhere (Prézelin et al. 1982, Moline et al. 1997). Pigment determinations were done on the freshly collected in situ material, the sample retrieved from each incubator at the end of the incubation and a series of samples retrieved every 2 h from T299, which most closely mimics in situ conditions. Triplicate sea water samples (500 ml each) were collected at each sampling interval, pooled, filtered onto 0.4 μm 47 mm Nalgene nylon filters and frozen at -70°C for at least 2 h. At the time of analysis, pigments were extracted in 90% acetone at 0°C for 18 h and the extracts were centrifuged to remove non-pigmented material.

**H$^4$CO$_3^-$ procedures for indoor photosynthetic measurements.** Photosynthesis-irradiance (P-I) relationships were measured in indoor blue-light photosynthetic entities every 2 h for the samples incubated under the full solar spectrum (T299). For each P-I determination, a 30 ml subsample was removed from the non-radiolabelled T299 sample and inoculated with H$^4$CO$_3^-$ to a final concentration of 9 μCi ml$^{-1}$. Then, 25 discrete samples, 1 ml each, were aliquoted into glass scintillation vials, placed in temperature-controlled photosynthetic incubators and incubated for 2 h. Light was provided by a set of tungsten lamps illuminating samples through an overlaying plastic chamber filled with a 1% CuSO$_4$ aqueous solution. This filter/solution combination decreased the heat load on the sample (infrared radiation), removed any incoming UV radiation and provided a better spectral match with the in situ PAR. As such, the filter/solution combination attempted to correct the artificial unbalanced light fields common to unfiltered tungsten white light fields (Prézelin et al. 1989, Schofield et al. 1991). $Q_{PAR}$ values in the photosynthetrons ranged from 4 to 1100 μmol quanta m$^{-2}$ s$^{-1}$ and were adjusted when necessary by using combinations of neutral density plastic filters.

At the end of the incubation, the samples were acidified (glacial acetic acid/methanol, 1/30 v/v) and heat dried for 24 h at 65°C (Prézelin et al. 1987). One ml of distilled water was added to the samples to dissolve salts. Five ml of Ecoscint scintillation cocktail were added and samples vortexed to complete mixing prior to determination of the disintegration rate (DPM). Volumetric primary production (mg C m$^{-3}$ h$^{-1}$) was calculated from the sample DPM and the total activity prior to the incubation as described by Parsons et al. (1984).

The P-I parameters describing the relationship between photosynthesis and irradiance were derived using the relationships (Neale et al. 1987):

$$P_t = P_{\text{max}} \tanh \left(\frac{Q_{PAR}}{I_k}\right)$$

for $Q_{PAR} \leq I_t$

and

$$R_t = P_{\text{max}} \exp \left(-\beta (Q_{PAR} - I_t)\right)$$

for $Q_{PAR} > I_t$

where $P_t$ is the instantaneous rate of primary production (mg C m$^{-3}$ h$^{-1}$), $P_{\text{max}}$ is the light-saturated photosynthetic potential, $I_k$ is the minimum light requirement for the onset of light-saturation of photosynthesis (and is equal to $P_{\text{max}}/\alpha$, where $\alpha$ is the photosynthetic efficiency), $\beta$ is the photoinhibition rate, and $I_t$ is the minimum light requirement for the onset of $Q_{PAR}$ photoinhibition ($= P_{\text{max}}/\beta$). Error estimates for each parameter were determined as described by Zimmermann et al. (1987).

**H$^4$CO$_3^-$ procedures for in situ BWF measurements.** Measurements of SIS rates of carbon fixation were made in the outdoor incubators for 6 light treatments (T299, T314, T324, T328, T382, T402). The T414 incubator was not used in the BWF determination on September 16, due to technical difficulties on that day. A 131 sample was inoculated with H$^4$CO$_3^-$ to a final concentration of 0.5 μCi ml$^{-1}$. Aliquots, each 200 ml, were quickly dispensed into PE bags and placed in outdoor incubators at 09:35 h LT.

To determine the time course of carbon uptake over a single day, duplicate samples were removed from each incubator every hour from 10:00 to 18:00 h LT, fixed with 0.5% formalin and gently filtered onto a 0.4 μm Nuclepore filter rinsed with filtered sea water. Sample DPMs were measured after placing each filter in Ecoscint scintillation cocktail. All carbon fixation rates were corrected for dark fixation. Volumetric primary production was calculated as described in the previous section.

**Absolute inhibition (AI) and fractional inhibition (FI) calculations.** The light treatments represent overlapping radiation regimes of progressively larger
bandwidths. The inhibition in each incubator was calculated by subtraction: the volumetric carbon uptake rates in the sample exposed to PAR only (\(P_{T402}\), mg C m\(^{-3}\) h\(^{-1}\)) was used as reference to compute the absolute inhibition (\(A_{ITn_m}\), mg C m\(^{-3}\) h\(^{-1}\)) as follows

\[
A_{ITn_m} = P_{T402} - P_{Tn_m}
\]

where \(P_{Tn_m}\) is the primary production measured in incubator \(Tn_m\). The fractional inhibition (\(F_{ITn_m}\), unitless) is then equal to:

\[
F_{ITn_m} = 1 - \frac{P_{Tn_m}}{P_{T402}}
\]

### Analytical approach to BWF determination.

We assumed that the fractional inhibition \(F_{ITn_m}\) over a time interval can be expressed as a function of the average irradiance \(E(\lambda)_{Tn_m, i}\) which impinges on the phytoplankton community inside the incubator and the BWF \(\epsilon(\lambda)\) (W m\(^{-2}\) \(\mu\)m\(^{-1}\)) representing the spectral sensitivity of the organism to such radiation, such that:

\[
F_{ITn_m} = \sum_{\lambda \text{ UVR}} E(\lambda)_{Tn_m, i} \epsilon(\lambda) d\lambda
\]

A similar equation can be written for the absolute inhibition \(A_{ITn_m}\) (mg C m\(^{-3}\) h\(^{-1}\)) as:

\[
A_{ITn_m} = 3600 \sum_{\lambda \text{ UVR}} E(\lambda)_{Tn_m, i} \epsilon(\lambda) d\lambda
\]

where \(\epsilon(\lambda)\) has units of mg C m\(^{-3}\) (J m\(^{-2}\) \(\mu\)m\(^{-1}\)) and is related to \(\epsilon(\lambda)\) by the constant factor \(P_{T402}\).

Since \(F_{ITn_m}\) determinations are broad band measurements, the sensitivity function \(\epsilon(\lambda)\) can not be mathematically derived from Eq. (4) alone. However, the method outlined by Rundel (1983) can be used to extract the spectral dependency of the function from knowledge of the several broad band \(F_{ITn_m}\). Assuming a mathematical form of the BWF \(\epsilon(\lambda)\), one can calculate the expected fractional inhibitions \(F_{\text{exp, }Tn_m}\) for each time interval and in each incubator \(Tn_m\) from the average irradiance \(E(\lambda)_{Tn_m}\) (Eq. 4). The best-fit \(\epsilon(\lambda)\) is obtained when the mean squared error \(\chi^2 = \sum_{i} \left[ F_{\text{exp, }i} - F_{\text{ITn_m}, i} \right]^2 / F_{\text{exp, }i} \) is minimized. We used iterative non-linear fitting techniques to minimize \(\chi^2 \) by varying the parameters describing \(\epsilon(\lambda)\). With 5 data points available in our experiment (6 filters giving 5 \(F_{ITn_m}\) values), the number of adjustable parameters describing \(\epsilon(\lambda)\) is limited to 4. As a result, only simple equations can be used and fine-scale resolution will be lost. Since the sensitivity of various organisms to UVR has been shown to decrease exponentially with wavelength (Rundel 1983), the best-fit BWF was chosen from results obtained using increasingly complex exponential decay functions.

The accuracy of this deconvolution technique increases with the number of filters used and depends on the distribution along the UV region of the spectrum of the transmittance cut-offs of the filters. To compensate for the limited number of suitable long-pass filters, we concentrated the highest resolution in the UV-B region of the spectra, where the slope of the BWF has been shown to vary the most for other plant photoprocesses (Caldwell 1986) and where findings would have greatest significance to assess the effects of changing O\(_3\)-dependent UV climatology on marine primary production.

### RAF determination.

The sensitivity of a given photoprocess to a decrease in O\(_3\) concentration can be described by a single parameter, the radiation amplification factor (RAF). RAF is a measure of the relative increase in biologically effective UVR for a given level of O\(_3\) reduction. It has recently been mathematically described for large variations in O\(_3\) concentration by Madronich (1993) using the power formulation:

\[
\frac{A_{I_N}}{A_{I_{360}}} = \left( \frac{N_{360}}{N_N} \right)^{RAF}
\]

where \(A_{I_N}\) and \(A_{I_{360}}\) are the inhibition corresponding to ozone concentration of \(N\) DU (N\(_N\)) and 360 DU (N\(_{360}\)). The best-fit value for RAF was calculated for \(N\) varying between 100 and 360 DU in 10 DU increments. The corresponding inhibition values were determined using Eq. (5), with input data for daily average solar irradiances obtained from the clear-sky model of Madronich (1993) applied to September 16 at a latitude of 64°S.

### RESULTS

#### Physical environment

In 1993, the ozone hole over Antarctica developed very early. At the beginning of August, the stratospheric O\(_3\) above Palmer Station had already reached column concentrations below 200 DU (Fig. 2), an arbitrary value previously used to define the outer boundary of the O\(_3\) hole (Smith et al. 1992). On September 16, the fourth passage of the hole over Palmer Station since the start of our field program was observed. The spring growing season was therefore initiated with an enhanced level of UV-B. The potential UV-B exposure was increased further at our study site as the area was virtually ice-free throughout the months of August and September 1993.

On September 16 (Day 259 of the Year), the O\(_3\) concentration was 260 DU, in the middle of a 3 d transition period from 180 to 320 DU as the O\(_3\) hole moved away from the site (Fig. 2). While the amount of UV-B radiation reaching the earth surface was slightly enhanced due to the O\(_3\) diminution, it was still low due to the large solar zenith angle. However, daylength was increasing rapidly (ca 20 min \(d^{-1}\)) and on September
Fig. 2. Changes in (A) daylength and (B) stratospheric ozone column concentration (DU) above Palmer Station, Antarctica (Meteor 3 TOMS data) and midday (16:00 h GMT) UV-B (280 to 320 nm) irradiance (µW cm⁻²) at Palmer Station during the first half of the 'Icecolors '93' expedition. Arrows indicate date when a daily averaged biological weighting function (BWF) for UV inhibition of in situ primary production was determined (September 16, 1993, Day of the Year 259) for surface water phytoplankton, and date (September 9, 1993, Day of the Year 252) when data was collected to test the BWF ability to predict UV inhibition of in situ primary production under different levels of stratospheric O₃ concentrations before solar noon (12:00 h LT) on September 16. Early morning hours were characterized by completely overcast skies, clearing intermittently in late morning when the maximum Q_PAR(0°) was recorded and approached 700 µmol quanta m⁻² s⁻¹ (Fig. 3A). Sudden white-out conditions occurred from a passing blizzard between noon and ca 14:00 h LT. Skies were clear for the last 2 h of daylight.

Monitoring of the UV spectral irradiances was limited to once an hour, on the hour. Sampling frequency was adequate to measure the major diurnal variations in total UV-A and total UV-B radiation. Fig. 3B shows the sharp decrease at 12:00 h LT, the shoulders at 14:00 h LT, when the blizzard stopped and at 16:00 h LT after the complete clearing. Under clear-sky conditions, the E_UVB:E_UVA ratio increase with solar zenith angle to a midday maximum. The E_UVB:E_UVA ratio over the day shows that the relative proportion of UV-B radiation was greatest and relatively stable between 11:00 and 14:00 h LT (Fig. 3B).

While the incubated samples were kept at a constant temperature due to high volume circulation of surface sea water, the outside air temperature ranged from −11.5 to −6.5°C with an average of −8.8°C. Constant attention was given to the incubators and light sensor to assure proper functioning of the equipment at these low temperatures and during snowfall. The easterly winds (230°) peaked at 31 knots (15.5 m s⁻¹) in the afternoon blizzard and averaged 9 knots (4.5 m s⁻¹) over the day.

Weather perturbations, typical of episodic patterns routinely experienced in Antarctica on the time scale of a single day, caused significant fluctuations in the natural light field (Fig. 3). While solar radiation generally increased with decreasing solar zenith angle, maximum quantum flux occurred

16, the photoperiod was 11 h 25 min long, with sunrise occurring at 06:20 h LT and sunset at 17:45 h LT

Weather perturbations, typical of episodic patterns routinely experienced in Antarctica on the time scale of a single day, caused significant fluctuations in the natural light field (Fig. 3). While solar radiation generally increased with decreasing solar zenith angle, maximum quantum flux occurred

Fig. 3. Solar light conditions on September 16, 1993, when the BWF or UV inhibition of in situ primary production was determined. (A) Comparison of daytime variation in PAR quantum irradiance at the surface [Q_PAR(0°)] and zenith angle with the arrow indicating the time of field collection and the shaded area under the Q_PAR curve indicating the outdoor incubation period. (B) Comparison of daytime variations in UV-A (E_UVA, 320 to 400 nm), UV-B radiation (E_UVB, 280 to 320 nm) and the E_UVB to E_UVA ratio (note the change of scale)
Photosynthetic parameters

Hydrographic, optical and pigment profiles of the in situ characteristics of the water column made at the time of sampling, as well as several days before and after sampling, confirmed that the experimental sample, while dilute (0.195 mg chl m⁻³), was representative of the late winter/early spring phytoplankton communities (Prézelin unpubl.). The presence of fucoxanthin as the main photosynthetic pigment (fucoxanthin/chl = 0.49 w/w) confirmed the microscope observation that the surface phytoplankton community was dominated by diatoms, commonly found at this time of the year within the near shore region of Arthur Harbor (Krebbs 1983).

Knowledge of the diurnal variation of photosynthetic characteristics of the population provides necessary information to assess the success of simulated in situ experiments, to document the physiological state of the natural community during the incubation and to provide intercomparable rates of primary production. The diurnal variations in light-saturated and light-limited rates of chl-specific photosynthesis were monitored every 2 h by measuring P-I curves in indoor PAR-only incubators (photosynthe-trons), from samples exposed to full solar radiation (T299). Chl-specific rates of light-saturated photosynthesis showed Pₘₐₓ/chl to vary 4-fold over the day with a peak value of 3.94 ± 0.57 mg C mg chl⁻¹ h⁻¹ measured close to solar noon (Fig. 4A). The minimum PAR required to saturate photosynthesis (Iₖ) varied 2-fold over the day (data not shown). Midday values averaged 109 ± 11 µE m⁻² s⁻¹ and decreased to 57 ± 9 µE m⁻² s⁻¹ before sunset. The ratio of incident irradiance Qₚₐᵣ to Iₖ varied between 1 and 4, clearly indicating that surface phytoplankton photosynthesis was light-saturated during most of the day and rarely inhibited by bright white light (Qₚₐᵣ/Iₖ > 4; Lewis et al. 1984). Light limitation (Qₚₐᵣ/Iₖ < 1) occurred only in the first and last hour of sunlight (Fig. 4A). Chl-specific photosynthetic efficiency (µ/chl) was nearly constant during the first half of the day [0.03 mg C mg chl⁻¹ h⁻¹ (µE m⁻² s⁻¹)⁻¹, n = 3] increasing at the end of the day when the cells became light-limited (data not shown).

As modeled using Eq. (1), the chl-specific rates of photosynthesis (Pᵢ/chl, Fig. 4B) and volumetric production rates (Pᵢ, Fig. 4C) followed the diurnal pattern in irradiance with midday maximum of 4.5 mg C mg chl⁻¹ h⁻¹ and 0.91 mg C m⁻³ h⁻¹, respectively. Volumetric and chl-specific rates of production covaried over the day due to the relative constancy of chl abundance over the time course of the experiment.

Simulated in situ carbon uptake under 6 spectral treatments

The time course of cumulative carbon fixation (mg C m⁻³) by the phytoplankton assemblages exposed to the 6 spectral light treatments is shown in Fig. 5. Error bars represent the range of values for the duplicate measurements. For all treatments over most of the day, cumulative volumetric production increased with the rate of increase being greatest near midday when Qₚₐᵣ was greatest. For all UV treatments (1 to 5), the cumulative radiolabelled carbon declined during the last hour of daylight.

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Fig. 4. Diurnal variation in the photophysiology of Antarctic surface phytoplankton maintained in the full spectral incubator (T299) under simulated in situ conditions on September 16, 1983. (A) Comparison of derived estimates of the light saturation index (ratio of incident photosynthetically available radiation Qₚₐᵣ to the minimum light requirement to saturate photosynthesis Iₖ) and the chl-specific light-saturated rate of photosynthesis (Pₘₐₓ/chl, mg C mg chl⁻¹ h⁻¹). (B) Diurnal variation in derived rates of photosynthetic performance (Pᵢ/chl, mg C mg chl⁻¹ h⁻¹). (C) Comparison of daytime changes in volumetric production rate (Pᵢ, mg C m⁻³ h⁻¹) and the chlorophyll concentration (chl, mg m⁻³). Error bars represent the estimate of ± 1 SD around the derived parameters.
Under the experimental conditions, divergence in cumulative fixed carbon between different spectral treatments was significant after 2.5 h of incubation. Accumulation of radiolabelled carbon was enhanced by the removal of shorter bandwidths of UVR (Fig. 5). Samples exposed to unfiltered radiation (T299) showed the lowest uptake rates whereas primary production was highest in the QpAR-only treatment (T402) or QPAR with the addition of the longest wavelengths of UV-A (T383).

Average volumetric primary production, assimilation rates and doubling times in each UV treatment over the incubation time are summarized in Table 2. The doubling times estimates obtained from carbon fixation measurement on samples incubated under QPAR (T402, 3.9 d) and from P-I modeled estimates (3.8 d) were similar. The progressive addition of shorter UVR increased the community generation time to 6.3 d for the cells exposed to full incident UVR.

Estimates of the daily average UV inhibition of carbon fixation rates in each treatment were obtained by regressing primary production measured at each time interval in each incubator with the primary production measured under QPAR only (Fig. 6A). Such a normalization procedure eliminated considerations of the diurnal QPAR-dependent variation of primary production present in all incubators. Any decrease in the slope of the regression from unity is a measure of the fractional inhibition of primary production by selected UVR bandwidth. Primary production measurements under all UV treatments were well correlated to the primary production measurements under QPAR only (average \( r^2 = 0.97 \pm 0.01 \)). The average daily inhibition by UVR was 34.3 ± 2.6% (n = 10) and decreased with the progressive removal of the shorter wavelengths of UVR (Table 2). The value for the inhibition due to the radiation removed by T314, which can be considered to approximate UV-B radiation, was 14.6%, or 43% of the total UVR inhibition. By difference, UV-A radiation was responsible for 19.7% inhibition of photosynthetic potential, though radiation between 383 and 402 nm appeared to slightly enhance primary production (Table 2).

To estimate the goodness of the linear fit used to estimate \( \Delta P_{\text{PAR}} \), we analyzed the residuals (measured minus predicted values) normalized to the predicted value (Fig. 6B). A positive residual implied that the

<table>
<thead>
<tr>
<th>Outdoor incubator</th>
<th>Volumetric primary production (mg C m(^{-2}) h(^{-1}))</th>
<th>Assimilation rate (mg C mg chl(^{-1}) h(^{-1}))</th>
<th>Doubling time (d)**</th>
<th>Measured daily averaged inhibition relative to ( P_{\text{PAR}} ) (%)</th>
<th>Modeled daily averaged inhibition relative to ( P_{\text{PAR}} ) (%)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>T299</td>
<td>0.33 ± 0.02</td>
<td>1.82 ± 0.03</td>
<td>6.3 ± 0.8</td>
<td>34.3 ± 2.6</td>
<td>31.4 ± 3.0</td>
</tr>
<tr>
<td>T314</td>
<td>0.40 ± 0.02</td>
<td>2.18 ± 0.03</td>
<td>5.3 ± 0.7</td>
<td>22.4 ± 2.4</td>
<td>26.4 ± 2.5</td>
</tr>
<tr>
<td>T324</td>
<td>0.40 ± 0.02</td>
<td>2.18 ± 0.04</td>
<td>5.3 ± 0.7</td>
<td>19.7 ± 2.7</td>
<td>19.7 ± 1.9</td>
</tr>
<tr>
<td>T328</td>
<td>0.48 ± 0.04</td>
<td>2.61 ± 0.10</td>
<td>4.4 ± 0.6</td>
<td>12.9 ± 2.3</td>
<td>12.2 ± 1.1</td>
</tr>
<tr>
<td>T383</td>
<td>0.53 ± 0.02</td>
<td>2.89 ± 0.07</td>
<td>4.0 ± 0.5</td>
<td>-0.02 ± 0.12</td>
<td>-0.01 ± 0.0</td>
</tr>
<tr>
<td>T402</td>
<td>0.54 ± 0.09</td>
<td>2.94 ± 0.26</td>
<td>3.9 ± 0.6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Indoor photosynthetron</td>
<td>0.55 ± 0.13</td>
<td>3.03 ± 0.41</td>
<td>3.8 ± 0.7</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Carbon-based estimates over the incubation time given a measured C/chl ratio of 398 ± 50 w/w. The particulate carbon content represents the average concentration at the surface for the 3 d prior to the experiment

**Modelled estimates were determined using Eqs. (3) & (4)
phytoplankton were less sensitive to the different UVR exposures than what would be predicted from a linear fit. Results showed that the percent residuals decreased with time, following the increase in the cumulative signal to noise ratio. Although the absolute residuals were small for incubation greater than 2 h, they represented a significant fraction of the signal (~10%) and did display a systematic diurnal deviation.

**BWF and RAF for Antarctic phytoplankton**

The daily average *in situ* BWF for the present study of Antarctic phytoplankton was deconvoluted from the knowledge of the 5 broadband daily inhibition values ($E_{s(i)}$) and the corresponding average spectral irradiances responsible for the damage (Fig. 7A). The phytoplankton sensitivity to shortest wavelengths of UVR decreased approximately exponentially as wavelength increased. Best fit to the data was obtained with a double exponential decay function, negatively offset to allow for enhancement of primary production by the longest wavelengths of UVR, expressed as:

$$e(\lambda) = \exp(a + b\lambda + c\lambda^2) + m$$  \hspace{1cm} (7)

The parameters describing the daily integrated BWF $e(\lambda)$ ($[\text{W} \text{m}^{-2} \text{nm}^{-1}]$) are: $a = 119.65$, $b = -2.223 \times 10^{-1}$, $c = 7.670 \times 10^{-4} \text{nm}^{-2}$, $m = -4.03 \times 10^{-3}$; and those describing the chl-specific function $e'(\lambda)$ ($[\text{mg C mg chl}^{-1}]$) ($[\text{J} \text{m}^{-2} \text{nm}^{-1}]$) are: $a = 112.5$, $b = -6.223 \times 10^{-3}$, $c = 7.670 \times 10^{-4}$, $m = -3.17 \times 10^{-6}$ with $\chi^2 = 0.212$ ($p = 0.0052$). The associated RAF (Eq. 5) for the BWF on this date was $0.91 \pm 0.3$ units.

Quantifying how sensitive the determination of the BWF was to a change in the inhibition input parameters...
ters $F_{\text{ran}}$ required a realistic estimate of the error around $F_{\text{ran}}$ as well as a way to quantify the associated change in the BWF. We chose the RAF as an estimator of the general shape of the BWF. As described above, the residuals from the linear fit used to determine $F_{\text{ran}}$ represented $\pm 10\%$ of the signal. To estimate the sensitivity of the BWF algorithm to the input parameters, we determined the impact of $\pm 10\%$ variation in each $F_{\text{ran}}$ on the RAF. These 10 manipulated BWFs had associated RAFs ranging from 0.87 to 0.96, representing an average 2.4% change from the original RAF.

**DISCUSSION**

The negative impact of UV radiation on phytoplankton short-term production is widely accepted (El-Sayed et al. 1990, Karentz 1991, Karentz et al. 1991a, Helbling et al. 1992, Smith et al. 1992, Bothwell et al. 1993, Holm-Hansen et al. 1993, Bothwell et al. 1994, Prézelin et al. 1994b) and the degree of inhibition appears relatively consistent between workers (Prézelin et al. 1994a). The present study quantifies the degree to which several UV broadbands decrease short-term estimates of primary production under simulated in situ conditions and provides an estimate of the spectral variation of the inhibition of Antarctic phytoplankton under conditions of enhanced levels of UV-B radiation. We discuss the shape of newly deconvoluted biological weighting in comparison with action spectra for other processes, explain how such a function can be used to estimate the UV effect of the Antarctic ozone hole on primary production and test its application to correct PAR-dependent measurements of Antarctic primary production with the sole knowledge of the spectral UV field.

A comparison of the principal published BWFs normalized to 295 nm is presented in Fig. 7B. The slope of the functions at the lowest wavelengths depicts the sensitivity of each process studied to the unbalanced increase in UVR associated with the depletion of ozone. The RAF quantifies this sensitivity by directly relating the increase in damage due to the decrease in ozone. At the end of the austral winter, Antarctic phytoplankton appeared more susceptible to an increase in the $Q_{\text{UVB}}/Q_{\text{DNA}}$ balance than temperate terrestrial plants (Jones & Kok 1966, Caldwell 1971) and temperate marine phytoplankton (Cullen et al. 1992). The RAF for Antarctic phytoplankton production (0.91 ± 0.3 units) lies in the upper region of the range in RAFs measured for various plant damages (as tabulated by Madronich 1993). It was 40% higher than the RAFs measured for the inhibition of photosynthesis in 2 temperate phytoplankton species in culture (Cullen et al. 1992). Whether this enhanced sensitivity is a result of a change in species composition, the exposure history or the experiment conditions remains to be established and is under ongoing investigation.

On the contrary, the RAFs associated with the 2 DNA action spectra (Setlow 1974, Hunter 1979) as well as all RAFs determined for other DNA-related effects (Madronich 1993) were much greater than our estimate. RAFs are a function of the absorption spectra of the target molecule and the negative impact of the energy absorbed onto the process studied (e.g. quantum yield of inhibition). As a consequence, BWFs describing the UV damage of different target molecules are not constant, since the absorption spectra of the molecules are different. With whole organisms, the issue is further complicated by photoprotective absorption and photoprocesses counteracting the inhibition effects. For instance, in the case of plant carbon production, the sensitivity of the cells will decrease with intracellular photoprotectant concentration or with changes in the rates of counterprocesses such as photosynthesis (Tevini & Teramura 1989, Prézelin et al. 1993) or photorepair (Karentz et al. 1991a, b, Raven 1991). It is therefore not surprising to find intact plant photosynthetic machinery to be less sensitive to UV radiation than naked DNA.

With the knowledge of the BWF, the in situ spectral irradiance and the primary production under PAR $P_{\text{PAR}}$, one can estimate UV-dependent rates of primary production (Eqs. 3 & 4). The description of the earth surface UV climatology has greatly improved in recent years due to the development of radiative transfer models and monitoring networks (Smith & Wan 1992, Madronich 1993, Booth et al. 1994). Primary production of the oceans remains difficult to characterize due to its large temporal and spatial variability. In recent years, parameterizations of the variation of carbon uptake with increasing irradiance ($P-I$ curves) have become a common way to describe ocean productivity in the field. This technique has been preferred over in situ incubations because it allows for high frequency sampling strategies and is very suited to studying the effects of environmental factors on phytoplankton production (Cote & Platt 1983, 1984, Prézelin et al. 1987, Smith et al. 1987, Platt & Gallegos 1988, Prézelin et al. 1992). There is evidence that short incubations often overestimate daily in situ primary production. A commonly evoked mechanism is that $P-I$ curves are a measure of gross production, whereas some fixed radio-labelled carbon can be catalyzed through respiration during long-term incubations, progressively lowering determination to net production values. Another inherent problem of laboratory incubations is the spectral bias introduced by the use of an artificial light source (Kiefer & Strickland 1970, Loh-
renz et al. 1992). One of the effects commonly investigated is the red-light enrichment associated with use of a tungsten light source replacing the 'bluer' in situ light field. Another bias is the removal of the damaging ultraviolet radiation by the laboratory incubators, the samples being incubated under PAR only. Results from our experiment show that, after a 2 h incubation under PAR, no significant difference in P-I parameters were detected between samples originating from different UV treatments. Furthermore, the time course of primary production as modeled using the diurnal variation of the P-I parameters \( P_I \) (Eq. 1) closely approximated primary production measured in the PAR-only incubator (T402, Fig. 8). This demonstrates that most of the production enhancement in the short-term incubations was due to the removal of UV light and that a 2 h incubation under PAR only was sufficient to release the cells from the UV stress to a degree which made any decrease in photosynthetic potential not detectable with the precision of our method. This is consistent with the theory that the 32 kDa photosystem II reaction center protein, which is rapidly turned over (half-life < 25 min) in the presence of visible light (Rogers et al. 1986, Greenberg et al. 1989), is the main photosynthetic machinery target degraded under UV radiation in higher plants (Greenberg et al. 1989) and in phytoplankton (Schofield et al. 1995). Therefore, P-I measurements over long incubations (2 h) provide a way to model in situ primary production under PAR light only \( (P_{PAR}) \). One caveat is that only P-I curves determined over short incubation (<30 min) will reflect UV stress if incubated without UV. Another is that natural phytoplankton populations are likely to recover from some of the reversible damages at dawn and dusk, when the UVR/PAR ratio is low. The degree to which the cost of the repair decreases long-term growth remains to be investigated.

With estimates of irradiance field, PAR-dependent rates of photosynthesis and the BWF, we can now model UV-dependent rates of photosynthesis using Eqs. (3) & (4). To examine the model's applicability in reproducing diurnal patterns of UV-dependent primary production and its adaptability to changes in the UV-B to UV-A balance in the external light field, 2 tests were applied. In a first internal test, we compared the modeled UV-dependent rates of primary production with the measured values in the 6 incubators (Fig. 8). Modeled primary production was calculated using the daily average BWF and PAR-dependent rates of photosynthesis determined either from the primary production of samples exposed to PAR only \( (P_{T402}) \) or primary production modeled from P-I estimates \( (P_I) \). Good agreement (within 3% on average) was found between the 2 models and the diurnal patterns were similar. Differences in incubation lengths between the 2 h P-I and the cumulative daily incubations could introduce differences on the sole basis of gross versus net photosynthesis. The similarity of the diurnal pattern of both measurements (Fig. 8) suggests, however, that such differences were not observed. Within the experiment

![Figure 8](image-url)
conditions, \( P-I \) values were, therefore, good estimators of primary production released from UV stress.

Time courses of modeled primary production in all UV treatments compared well with the measured values although some deviations were apparent, especially for the lowest wavelength of UVR (T299). Such deviations, also observed in the residuals plot (Fig. 6), are a reflection of the diurnal variation of the quantum requirement for inhibition, particularly enhanced in our experiment by the extreme fluctuation in weather pattern (Fig. 3). Even though UV inhibition of production does vary diurnally, using a constant BWF over the course of 1 d will, in a first approximation, provide accurate estimates of \textit{in situ} inhibition of primary production, as shown from the correlation \( r^2 = 0.97, n = 54 \) between modeled and measured estimates.

In a second external test, we used the BWF to compare modeled estimates of \textit{in situ} primary production to hourly measurements made in 2 incubators (T299 and T328) on a different day (September 9, 1994). On that day, the phytoplankton assemblages and the ice coverage were similar to September 16. Stratospheric ozone concentration, however, was reduced to 205 DU, resulting in an increase in the UV-B to UV-A ratio and thus providing a good test of the spectral robustness of the model. The total measured inhibition was 26.7\%, 15.8\% of which was due to UV-A radiation. The model very closely estimated daily results from \({ }^{14} \text{C}\) measurements, with a modeled total inhibition of 26.6\% and a slightly enhanced UV-B to total inhibition ratio (50.4 vs 40.8\%). Similarly, the diurnal variation of modeled UV-dependent rates of primary production (Fig 9) compared well with the measured values \( r^2 = 0.97, n = 10 \) for T299; \( r^2 = 0.99, n = 10 \) for T328.

Predictions of UV-dependent rates of primary production in natural Antarctic phytoplankton communities are possible, given that \textit{in situ} production in the absence of UV radiation and UV fluence rates on a time and space scale matching production estimates are available. The \textit{in situ} BWF provides a valuable tool to emulate primary production under fluctuating levels of the UV-B to UV-A ratio. Further information, however, must be gathered about the behavior of the BWF and its predictive limits, particularly with varying community structure and light history.

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