mM $CaCl_2$, 1 mM $MgCl_2$) bubbled with a mixture of 95% O_2 and 5% CO_2. For experiments, slices were bathed in the same solution. Patch-clamp recordings were made [O. P. Hamill *et al.*, *Pfluegers Arch.* **391**, 85 (1981)] from swellings ranging from 5 to 15 μm, which were clearly visible under Nomarski optics at ×600. Pipette-filling solutions for whole cell recordings are given in the figure legends. Additional methodological details can be found in (*8*) and (*9*).
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TECHNICAL COMMENTS

Polyethylene Bags and Solar Ultraviolet Radiation

In their study of the effects of solar ultraviolet (UV) radiation on natural phytoplankton assemblages in Antarctic waters, R. C. Smith *et al.* (*1*) used Whirlpak polyethylene bags as used by Z. Z. El-Sayed *et al.* (*2*) as sample containers during their in situ incubations. On a cruise in the Pacific from Chile to California, in March and April 1992, we placed replicate aliquots of water samples, taken from a depth of 5 to 10 m, in Whirlpak bags (18 ounces) and in round quartz glassware vessels (250 ml). Samples were then placed in deck incubators with the temperature controlled by flowing surface seawater and were exposed to direct solar radiation. Some samples were covered with plastic filters for 6 to 8 hours, centered at local noon. Rates of photosynthesis were determined by standard radiocarbon techniques (*3*); chlorophyll a concentrations were determined by fluorometry after extraction in methanol (*4*).

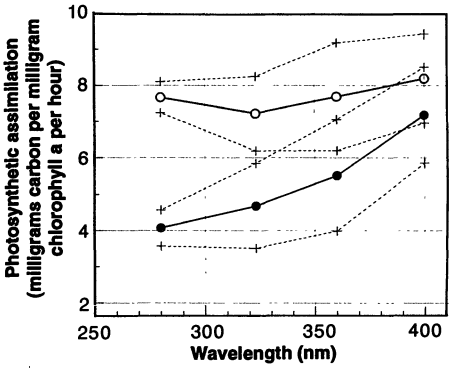

Fig. 1. Rates of phytoplankton photosynthetic assimilation in samples in glassware (open circles) compared with those in polyethylene bags (closed circles), as determined by analysis of variance tests. Some of the samples were covered with mylar (50% transmission at 323 nm), some with Plexiglas (50% transmission at 360 nm), and others with Plexiglas (UF3) (50% transmission at 400 nm). Circles represent the mean value for each treatment (n = 46 paired samples with no prefilter, 15 paired samples with UV radiation cut off at 323 nm, 15 paired samples with UV radiation cut off at 360 nm, and 38 paired samples with UV radiation cut off at 400 nm). Dashed lines indicate one standard deviation around the mean.

We found a significant difference ($P < 0.001$) in photosynthetic assimilation rates for samples in glassware as opposed to bags when the samples were exposed to solar radiation without any filter and when they were covered by mylar, which absorbs ultraviolet B (UVB) radiation (280 to 320 nm) (Fig. 1). This inhibitory effect was not decreased by leaching the bags in 1 N HCl for 12 hours. The results of our transmission tests agree with those in (*2*), which showed only 68% transmission of UVB at 300 nm. It is apparent that polyethylene bags absorb UVB, which results in a toxicity that significantly lowers the rate of CO_2 assimilation.

In spite of artifacts associated with the use of polyethylene bags, the general conclusions reached by Z. Z. El-Sayed *et al.* (*2*) and by Smith *et al.* (*1*) are similar to our results (*3*, *5*), which were obtained with glassware. There does not appear to be a temperature dependence associated with the toxicity produced by polyethylene bags, as similar results were obtained in Antarctic and in tropical waters. We do not know, however, whether different taxonomic groups of organisms would react in the same way as that noted in our experiments. It would therefore seem advisable for those studying the effects of solar UV radiation on microbial populations to first ascertain the validity of measurements made with the use of polyethylene bags.

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Response: Clean techniques and control studies (*1–3*) were performed before, during, and after the Icecolors '90 cruise. We checked and found no evidence for polyethylene bag contamination or toxicity.

Before the cruise, laboratory cultures and field samples of mixed phytoplankton communities were incubated in 250- or 500-ml untreated Whirlpak bags for different periods (up to 8 hours) while being exposed to darkness or to light-saturating fluorescence that had passed through a glass plate [photosynthetically available radiation (PAR) only]. When compared with replicate samples in wide-bottom glass Erlemeyer flasks, we found no decrease in volumetric production rates (mg C/m^3/hour) and no toxic effect.

At sea, we observed that extended (up to 14 hours) exposure to low amounts of UVB or UVA radiation, or both, had no effect on the in situ primary production

Table 1. The effect of UV radiation on polyethylene bags used for the measurement of phytoplankton photosynthetic rates. In studies A and B, 200 ml of sterile seawater medium was placed in untreated bags (400-ml capacity). In study A, pretreatment consisted of incubating medium-containing bags for either 1 hour in darkness or under a germicidal lamp (GE G30T8). In study B, pretreatment consisted of incubating bags that contained medium outdoors for 24 hours in temperature-controlled incubators in the presence and absence of natural UV. Then in both studies, rates of radioactively labeled carbon fixation were measured by adding a 50-ml mixed sample of the diatom *Thalassiosira weisflogii* and the dinoflagellate *Hetercapsa pygmae*, which had been inoculated with ^{14}C-$NaHCO_3$ (final 250-ml sample concentration was 0.05 mCi/ml). Replicate pretreated samples were incubated in light and dark. At the end of the incubations, samples were fixed with formalin and 30-ml samples were passed through 0.4-μm Nuclepore filters (Nucleopore, Pleasanton, California). Additional methodology for controls, counting, and calculations have been described in (*6*). Dark, D; light, L.

Pre-treatment (hours)	Incubation conditions (hours)	Productivity ± SE (mg C/m³/hour)	*n*
	Study A		
1 D	2.5 D	2.82 ± 0.09	3
1 UV	2.5 D	2.77 ± 0.46	3
1 D	2.5 L	45.84 ± 0.94	11
1 UV	2.5 L	46.23 ± 0.86	6
	Study B		
24 D			
PAR only	3.0 D	3.68 ± 0.91	3
PAR + UV	3.0 D	3.98 ± 0.53	3
12 L:12 D			
PAR only	3.0 L	53.35 ± 0.87	12
PAR + UV	3.0 L	51.69 ± 0.99	9

measured in polyethylene bags (*1*, *2*, *6*). For phytoplankton communities present and incubated below about 25 m, this was always the case for both volume- and chlorophyll-specific rates, as no differential rates of photobleaching of pigmentation were evident in these low-light experiments [figure 5 in (*1*)].

Replicate surface samples were incubated in natural UV for 4 to 6 hours during the day (*2*). Our data about the effects of UV on diurnal rates of chlorophyll-specific, light-saturated maximum production (P_{max}), and light-limited (α) carbon fixation can be directly compared with the data of Holm-Hansen and Helbling. Even with full exposure to enhanced UV under the ozone hole, no toxic effect from the incubation bags was detected.

However, we found (*2*) that for near-surface irradiances, rates of photobleaching of chlorophyll pigmentation can differ under different light treatments. These data show that it would be incorrect to calculate percent UV inhibition of photosynthesis-irradiance parameters from chlorophyll-specific data if they are derived from different light treatments (*2*). In short, inhibition by UV of photosynthetic rates can be masked in chlorophyll-specific data and resolved in volume-specific data. Comparison of the latter revealed significant UVB inhibition of primary production in Antarctic surface waters (*2*, *6*).

If there were a UVB-induced toxicity in polyethylene bags, there would have been as much inhibition in surface samples as in subsurface samples, and longer exposure to this toxicity should lead to higher rates of UVB inhibition, but this has been shown not to be the case (*1*, *2*, *4*, *5*, *6*).

During the Icecolors '90 program, studies were made of UV effects on growth rates for several Antarctic phytoplankton species incubated for days in polyethylene bags. Results indicated no UVB- or UVA-induced toxic effects from the bags (*7*) [figure 4 in (*1*)].

Recently we performed additional tests (Table 1), in response to the comment by Holm-Hansen and Helbling. Carbon fixation rates were essentially the same for samples in UV- and non-UV–treated bags, and neither short exposures to anomalously high amounts of UV radiation nor long exposures to small amounts of UV showed any toxic effect.

We also have several remarks about the comment by Holm-Hansen and Hebling. The glassware and polyethylene bags they used have different optical geometries. Because the effects we are discussing are a function of exposure, this lack of controls makes intercomparison problematic.

They present grouped comparison of chlorophyll-specific data where only the initial chlorophyll concentrations were determined (*8*), and do not account for differential chlorophyll photobleaching on the basis of flux (which is important because of the lack of optical geometry controls) or for diurnal variability. Finally, figure 1 of their comment could be misleading: the "points" at 280 nm refer to full broadband incident irradiance; those at 400 nm refer to all ambient radiation above this wavelength (*8*).

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