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Effect of short-term exposure to UVA and UVB on potential phytoplankton production in UK coastal waters

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The influence of vertical mixing on phytoplankton sensitivity to UV light has been assessed over an annual cycle. Photosynthesis rates of natural assemblages were compared in samples that were incubated at fixed position in a light gradient and with duplicate samples that simulated vertical mixing by movement in the same gradient with a periodicity of 4 h. This is the typical time-scale of vertical mixing in coastal waters in the English Channel. There were clear seasonal differences in the short-term response of phytoplankton to enhanced UVA+UVB. For most of the year, there was no detectable effect of UV on photosynthetic carbon fixation. But natural assemblages in late winter/early spring, when high UV light may sporadically occur at this latitude, were sensitive to UVA+UVB. In some samples, primary production was 40% of that measured in the absence of UV light. At the time of maximum sensitivity to UV, the phytoplankton assemblage was dominated by diatoms. Simulated vertical mixing resulted in more inhibition of photosynthesis by UVA+UVB light than when samples were at constant light with the same time-integrated irradiance. Transient increases in UVA+UVB due to ozone depletion, such as have been observed over Northern Europe, could have a serious impact on coastal phytoplankton production in late winter/early spring.

INTRODUCTION

Since the discovery of the destruction of the stratospheric ozone layer by synthetic chlorofluorocarbon compounds (see review by Solomon, 1999), there has been considerable interest in the effect of increased ultraviolet B (UVB) radiation on marine phytoplankton. The ozone hole was discovered in the Antarctic and much of the early work was concerned with natural phytoplankton assemblages from the Southern Ocean (Cullen *et al.*, 1992; Smith *et al.*, 1992), ice algal communities (Schofield *et al.*, 1995) and individual phytoplankton cultures (Montero *et al.*, 2002). Subsequently, there was interest in the effect of UVB on freshwater phytoplankton, with a number of publications on UVB and freshwater phytoplankton (e.g. Marwood *et al.*, 2000; Xenopoulos and Schindler, 2003).

There have been fewer studies of temperate coastal seas, perhaps because the assumption has been made that ozone depletion is only a problem for polar seas—and that the ozone hole is an Antarctic problem. Yet there can be significant decreases in stratospheric ozone concentrations in the northern hemisphere. In most winters, ozone concentrations are significantly lower within the stratospheric polar vortex over the Arctic. Crucially, at times this can be displaced over NW Europe, resulting in significant low-ozone events. For example, in winter 1995–96, the stratosphere was colder than for the previous 2 decades and there was a significant decrease in ozone concentration over NW Europe (Manney *et al.*, 1996). Since 1995, climatic conditions continue to result in short periods of ozone depletion over the region when the stratospheric polar

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vortex is displaced. In January 2006, a record low ozone column of 177 Dobson units was measured in SE England (Keil *et al.*, 2007). The effect on marine phytoplankton is poorly understood of these transient decreases in ozone at mid-latitudes in the northern hemisphere. This study aims to understand how phytoplankton assemblages in coastal waters might be affected by short-term exposure to elevated UVA and UVB.

In the temperate waters of the NW European shelf, there is intense tidal mixing and in winter the water column is well mixed. It is only after the development of the seasonal thermocline in April that stratification develops in some areas, although many regions remain well mixed throughout the year as a result of strong tidal mixing. These physical processes have important implications for the development of phytoplankton blooms (Pingree *et al.*, 1976; Holligan *et al.*, 1984) since they limit the period that phytoplankton can photosynthesis to the time when cells are close to the sea surface. Turbulence and vertical mixing can also affect the way in which marine phytoplankton responds to periods of enhanced UVB radiation. Little is known about the possible effects of vertical mixing on UV exposure to phytoplankton in temperate waters and there have been few attempts to include vertical mixing in experimental studies.

Therefore, in this study, we attempt to answer two questions. How important is vertical mixing in enhancing or decreasing the UVB dose received by phytoplankton in UK shelf seas and how do different phytoplankton assemblages, which alter with season, respond to enhanced UVB? Marra (Marra, 1978) published one of the first attempts to manipulate vertical movement within the water column and he investigated the effect on phytoplankton production of moving incubation bottles within a light gradient. Since most incubations are done at fixed depth, it is relevant to ask how representative are static incubations of the natural environment where phytoplankton cells are continuously moving within a light gradient—and in a deep mixed water column, moving out of the euphotic zone completely. Given this gradient of exposure, it is possible that damage by UV radiation may be lessened as a result of vertical mixing. DNA is the main cellular component that is damaged by UVB and the most common effect is the formation of pyrimidine dimers. It as long been known that photoreactivation can repair such damage (Yamamoto *et al.*, 1983). Other metabolic functions are susceptible to UV—for example, D1 and D2 proteins of photosystem II (Vincent and Neale, 2000). High values of PAR can also exacerbate UV damage (Shelly *et al.*, 2003). So the position of a cell in a light gradient is important not only for the potential damage due to UVB absorption but also for photo-repair mechanisms.

The approach taken in this study has been to sample the coastal waters off Plymouth at frequent intervals throughout a year and to incubate those samples in a light gradient in a laboratory-based experimental system. Samples were incubated in the presence and absence of UVA+UVB and the rate of photosynthetic carbon fixation of the phytoplankton assemblage was measured over a 24 h period. The approach was to compare samples that were static in a light gradient with identical samples that simulated vertical mixing by moving up and down the gradient.

METHOD

The experiments were done with water collected ~8 km offshore from Plymouth Sound at station L4 in the English Channel (50° 15′N, 4° 13′W). This station has a significant time series of chlorophyll concentration and phytoplankton composition (Southward *et al.*, 2005). This region of the English Channel stratifies in the summer months and is well mixed in the winter. Water samples were taken from the surface with a clean bucket at ca. 0900 h. Samples of 25 L volume were placed in a clean container and protected from light while transported to the land-based laboratory, a journey time of ~90 min. There was minimal change in temperature of this large water volume in the time between taking the sample and delivering it to the laboratory.

Chlorophyll concentrations were measured by the fluorometric method of Holm-Hansen *et al.* (Holm-Hansen *et al.*, 1965), and 100–200 mL aliquots of water were filtered through glass fibre (GF/F) filters. Pigments were extracted by adding 90% (v/v) acetone to the filters and samples were stored in the dark at 4°C for ~12 h before analysis. Water samples were frozen and batched for later analysis of nutrient concentrations. Nitrate concentrations were determined by colorimetric auto-analysis using the methods of Brewer and Riley (Brewer and Riley, 1965).

The experimental design attempted to simulate as closely as possible the coastal environment of UK waters, where tidal mixing results in the vertical movement of phytoplankton cells within the water column. Since sunlight enters the water column at the surface, mixing processes result in the continuous movement of phytoplankton cells within a light gradient from close to full sunlight just below the sea surface to darkness, if the depth of the surface mixed layer is greater than the depth to which light penetrates. Experiments were done

in a large temperature-controlled tank of sea water. The tank had an overall length of 4 m, the cross-section was ca. 0.5 × 0.5 m and the experiments were conducted in a constant temperature laboratory that was maintained at ambient sea water temperature. The tank was illuminated from one end, so forming a light gradient. Therefore, distance along the tank from the light source was equivalent to depth in the sea. The end window of the tank was made of UV transparent Perspex which gave excellent transmission of UVA+UVB. We aimed to provide photon flux similar to sunlight and this was achieved using three quartz halogen lamps (Osram Power Star, 150 W). Heating effects were not a problem because the samples were incubated in such a large volume of water in the water tank (1000 L). UVA+UVB was enhanced by two metal halide lamps with iron and cobalt additives (Phillips High Power HPA 400S). These lamps have a maximum output of 800 μW cm^{-2} UVA, 115 μW cm^{-2} UVB, and 25 μW cm^{-2} UVC. Since some light of wavelength shorter than 280 nm (UVC) was present in the lamp output, a sheet of cellulose acetate was placed over the end of the tank to absorb any UVC. The water in the tank was sea water taken from station L4. Light was absorbed as it passed through the sea water and a light gradient was established. The Phillips HPA 400S has several strong emission lines that dominate the spectrum (Fig. 1), so that, although the total number of UVB quanta were equivalent to that expected at the sea surface, there were more quanta at certain wavelengths than in natural irradiance. The light gradient was typical of a sunny day, with maximum values of ca. 1600 μmol quanta m^{-2} s^{-1} and minima of ca. 100 μmol quanta m^{-2} s^{-1}. The maximum UVB irradiance at the equivalent of 0.1 m depth was 1.1 μW cm^{-2} nm^{-1}, at 315 nm.

The light in the tank was measured with two instruments. In each experiment, routine measurements of light were made with a 4π calibrated sensor.

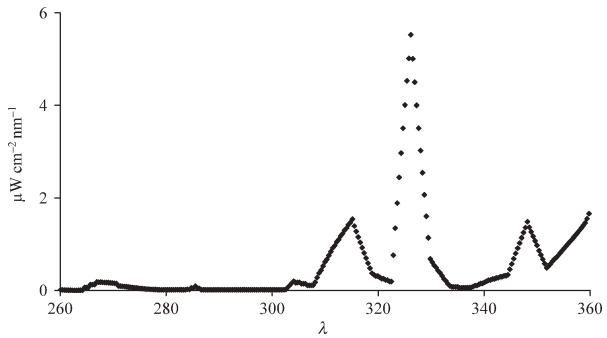

Fig. 1. Spectrum of UV light at a distance of 0.1 m from the light source.

In addition, at bimonthly intervals throughout the study, the spectrum of light received at all fixed positions in the tank was determined with a spectroradiometer. Calibrations were done in-house. UV calibrations were made with a certified deuterium lamp, traceable to the National Physical Laboratory (NPL) and calibrations at visible wavelengths were done with a NIST traceable lamp according to SeaWiFS procotols (Mueller and Austin, 1995).

The experiments compared static incubations (i.e. at constant irradiance) with samples moving in the light gradient, hence simulating the natural processes of vertical mixing by turbulent diffusion. Typical mixing rates in UK coastal water are of the order of 4 h (Uncles and Joint, 1983). That is, an average phytoplankton cell would be at the sea surface every 4 h. This is a simplistic view of vertical mixing which is actually a random process and does not involve cyclic movement (Uncles and Joint, 1983). Nevertheless, the pragmatic laboratory solution to simulating vertical mixing in coastal waters is a cyclical motion. The system constructed for this study consisted of a conveyor belt to which samples were attached and moved in the light gradient. The time to complete one circuit from high light at one end of the tank to low light at the other and back again, was set at 4 h.

In order to compare the effect of UVA+UVB *per se* at both constant light (at a fixed position in the light gradient) and variable light (moving in the gradient), samples were contained in either polyethylene bags, which are UVA+UVB transparent, or in polycarbonate bottles, which are UVA+UVB opaque. Polyethylene bags allowed transmission of light over the complete spectrum, from 240 nm to the infrared. In contrast, polycarbonate bottles absorbed light of wavelength <400 nm but transmission was excellent from 400 nm to the infrared. Polyethylene bags provided a convenient way to enclose water samples and to expose phytoplankton to UVA+UVB in the experimental tank. Polycarbonate bottles provide good controls since no UVB (and little UVA) was transmitted through the bottles to the phytoplankton.

To exclude the possibility that any differences were due to the materials that contained the samples, rather than to the presence of UVB light, experiments with no UV light were done in early March, late April and early September. In the absence of UVA+UVB, estimates of primary production, integrated over the length of the water column, showed a 1:1 relationship between incubations in polyethylene bags and in polycarbonate bottles. There was no significant (*t*-test) difference in either the static or rotating estimates. That is, no effect was found of the container material on production and

any change in the rate of primary production measured when the UVA+UVB light was switched on, were due directly to UV.

Experiments were done at approximately 2 weekly intervals over an annual cycle to determine seasonal variations in the response of natural phytoplankton assemblages to enhanced UVA+UVB. Photosynthetic carbon fixation was measured in incubations that started at midday on the day of collection and continued for 24 h. In each experiment, following screening to remove large zooplankton, a single 5 L water sample was inoculated with 9.25 MBq (250 μCi) $NaH^{14}CO_3$ and 75 mL aliquots were dispensed into 30 polycarbonate bottles and 30 polyethylene bags. Duplicate bottles and bags were attached at 10 positions along the light gradient at 0.1, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 m from the light source, with equivalent percentage of the maximum light of 100%, 54%, 49%, 37%, 24%, 17%, 12%, 9%, 7% and 5% (measured values). In addition, 10 bottles and 10 bags were equally spaced along the gradient on a conveyor belt system that rotated along the 4 m length and back every 4 h. The samples were exposed to continuous light for 24 h and there was no dark period. At the end of 24 h, the samples were removed from the tank after measuring the photosynthetically available radiation immediately behind each bottle or bag. Samples were filtered through 0.2 μm pore-size polycarbonate filters, fumed in HCl to remove unfixed ^{14}C and dried before counting in a liquid scintillation counter. Counting efficiency was determined by the external standard, channels ratio method. Primary production was determined by the methods of Joint and Pomroy (Joint and Pomroy, 1983, 1993). Production of labelled dissolved organic carbon was not measured.

Photosynthesis/irradiance (P/E) parameters were derived from the ^{14}C fixation data (Joint and Pomroy, 1986) using the procedure of Platt *et al.* (Platt *et al.*, 1990). The parameters were P_m^B (the maximum rate of chlorophyll-specific photosynthesis), α^B (the initial slope of the curve), β^B (the slope of the photoinhibited part of the curve) and E_k (the derived parameter that is the value of light at which extrapolation of the initial slope meets the value of P_m^B). E_k can be considered as the optimum light for photosynthesis.

RESULTS

Environmental context

We investigated the seasonal effect of UVA+UVB on natural phytoplankton assemblages over a 12-month period. The assemblage changed in composition and response and there were seasonal variations in water temperature, insolation, nutrient concentration, grazing pressure and phytoplankton species abundance. Figure 2 shows the range of temperature measured when the water samples were taken and the concentration of chlorophyll. Sea surface temperature varied from 9.5°C in the winter to 16.4°C in the summer. Salinity was relatively constant and was generally >34.5. The minimum value was 33.80 in early March and the maximum was 34.98 in May 1999. The spring bloom occurred in late April and the maximum measured chlorophyll concentration was 8 μg L^{-1} (Fig. 2). During this study, nutrient concentrations were only measured from March to November 1999. The data are plotted with chlorophyll concentration in Fig. 3. At the end of winter, nitrate concentration was ~10 μmol N L^{-1} and this declined to undetectable (<0.2 μmol N L^{-1}) as the phytoplankton spring bloom developed. Concentrations began to increase again in September and reached a value of 4 μmol N L^{-1}.

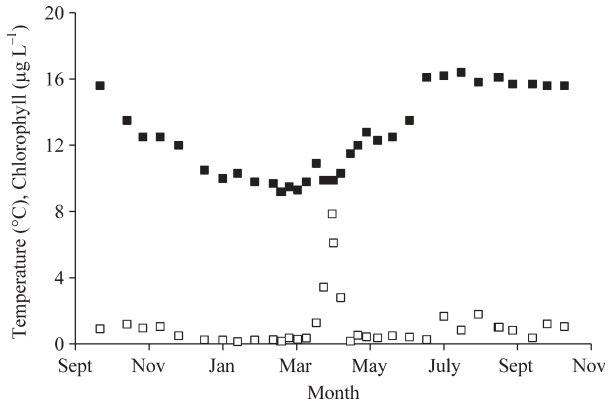

Fig. 2. Temperature (■) and chlorophyll concentration (□) measured throughout the study, from September 1998 to October 1999.

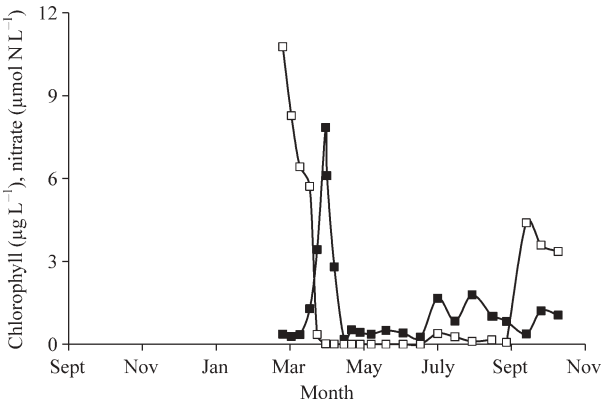

Fig. 3. Nitrate (□) and chlorophyll (■) concentrations; nitrate was only measured from March to October 1999.

Phosphate concentrations in March were 0.38 μmol P L^{-1} and were undetectable (<0.02 μmol P L^{-1}) throughout the summer. In March, silicate concentrations were 4.8 μmol Si L^{-1} and declined to ~0.5 μmol Si L^{-1} after the spring bloom and remained constant until the autumn.

Response of phytoplankton assemblages to UVA+UVB in the winter

There were seasonal differences in the response of phytoplankton to UVA+UVB. Results from 30 November 1998 are shown in Fig. 4a, in an experiment that is typical of the winter period. The data are for samples that were incubated at fixed positions in the light gradient. These stationary samples showed a typical P/E response, with decreased carbon fixation at high irradiance, i.e. closest to the light source, as a result of photoinhibition. The samples exposed to UVA+UVB showed an even greater decrease in photosynthesis at high light than those in UV-opaque polycarbonate but there was little difference along the rest of the light gradient.

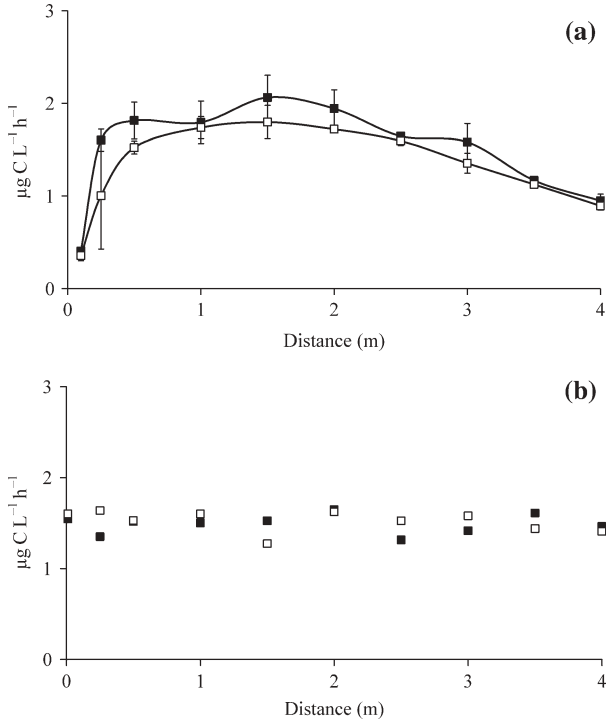

Fig. 4. Primary production in a light gradient in the presence and absence of UVA+UVB on 30 November 1998. (**a**) Static samples incubated with (□) and without (■) UVA+UVB at fixed positions from the light source (0 m) for 24 h. Error bars indicate 95% confidence intervals. (**b**) Primary production measured with (□) and without (■) UVA+UVB in samples to simulate vertical mixing that rotated from high to low light in the light gradient with a periodicity of 4 h.

Results from experiments that simulated turbulent mixing in the water column, when phytoplankton cells were continually moving within the vertical gradient of light, are shown in Fig. 4b. Although the data are plotted as distance from the light source, this is merely the position of the sample at the end of the 24 h incubation period. Each sample had experienced an identical light dose because each sample had completed six circuits within the light gradient. The results are plotted against distance for convenience and as a comparison with Fig. 4a. It is clear that there was a little difference in the rate of photosynthesis measured in the nine bottles or bags which were continuously moving in the gradient.

A convenient method of comparing production from different sites and seasons is to integrate the data for the total length of the illuminated water column. In this case, the water column was 4 m long and the depth-integrated production of the fixed samples was estimated by linear interpolation between the estimated production at each distance from the light source. For the circulating samples, depth-integrated production was estimated from the mean rate of carbon fixation determined in the rotating bottles and bags. This rate of μg C L^{-1} h^{-1} (i.e. mg C m^{-3} h^{-1}) was multiplied by the length of the tank (4 m) to give depth-integrated primary production (mg C m^{-2} h^{-1}). The estimates obtained are compared in Fig. 5. It is clear that for the experiments done on 30 November, there were no significant differences in the estimated primary production in the four treatments. All samples that were continuously moving in the light gradient, both in the presence

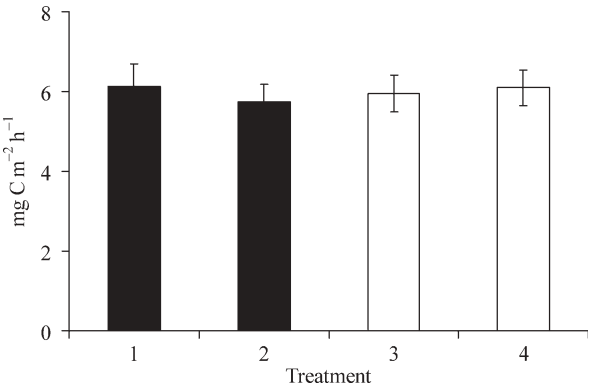

Fig. 5. Primary production (mg C m^{-2} h^{-1}), integrated along the length of the light gradient for 30 November 1998. Estimates are for fixed and rotating samples, with and without UVA+UVB. (1) Samples incubated at constant light at fixed distances from the light in the absence of UVB, (2) samples continuously moving in the light gradient in the absence of UVB, (3) samples incubated at constant light at fixed distances from the light in the presence of UVB, (4) samples continuously moving in the light gradient in the presence of UVB. The 95% confidence intervals of the estimates are shown.

and absence of UVA+UVB, gave the same estimate of depth-integrated water column primary production as the fixed light incubations. That is, on 30 November 1998, there was no measurable decrease in the rate of primary production in samples that were incubated in the presence of UVA+UVB. Similar results were obtained throughout the winter months of the study.

Response of phytoplankton assemblages to UVA+UVB in spring

The response of natural assemblages sampled in the spring was very different to the autumn and winter, with phytoplankton appearing to be more sensitive to enhanced UVA+UVB. Figure 6a shows the results of 4 May 1999, of incubating samples in the presence and absence of UVA+UVB at fixed positions in the light gradient. It is clear that photosynthesis was decreased in all samples in the presence of UVA+UVB up to a distance of 3 m from the light source. These phytoplankton cells were exposed to continuous light and the results suggested that continuous exposure to UVA+UVB might have damaged the photosynthetic apparatus. However, those samples that were continuously moving in the light gradient also showed decreased rates of photosynthesis in the presence of UVA+UVB (Fig. 6b). This is in contrast to the situation earlier in the season, with no difference between fixed and rotating samples. That is, even relatively short-term exposure to the highest levels of UVA+UVB at the surface, and to the decreasing UV flux along the gradient, was sufficient to decrease the rates of primary production. The integrated primary production estimates demonstrate the degree of UVA+UVB inhibition (Fig. 7), with a 25% decrease in the estimate of primary production at fixed light and 37% decrease in the samples that were incubated at varying light.

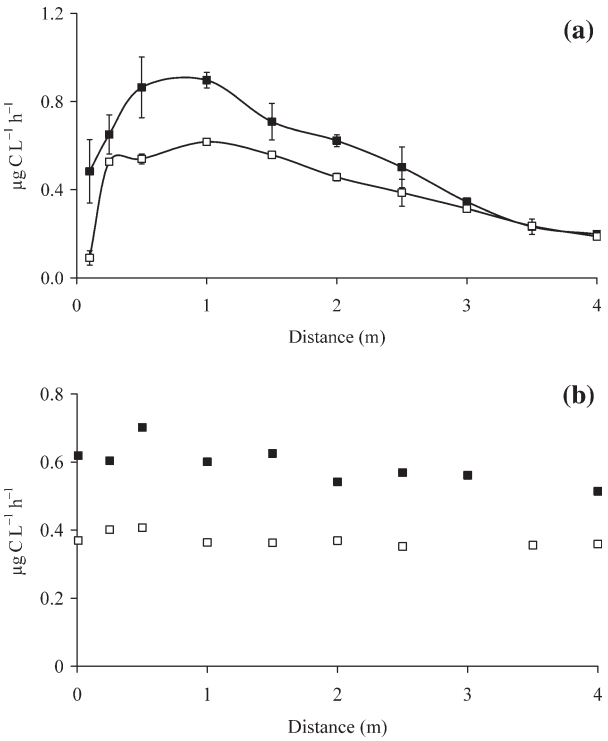

Fig. 6. Primary production in a light gradient in the presence and absence of UVA+UVB on 4 May 1999. (**a**) Static samples incubated with (□) and without (■) UVA+UVB at fixed positions from the light source (0 m) for 24 h. Error bars indicate 95% confidence intervals. (**b**) Primary production measured with (□) and without (■) UVA+UVB in samples rotated from high to low light in the light gradient with a periodicity of 4 h.

The seasonal response of phytoplankton assemblages to UVA+UVB

The values of depth-integrated carbon fixation measured during the study are summarized in Table I as mgC m^{-2} h^{-1}. Table I compares the carbon fixed by samples contained in polycarbonate or polyethylene and which were either incubated at constant light or moving through the light gradient with a periodicity of 4 h. The changes in carbon fixation reflect variations in phytoplankton biomass in the samples, with clear maxima in production at the time of the spring bloom in April and again in July and August when there were transient increases in chlorophyll concentration (Fig. 2). Generally, the estimate of the fixed position incubation was very similar to that obtained by averaging the rotating samples. However, there were seasonal differences in

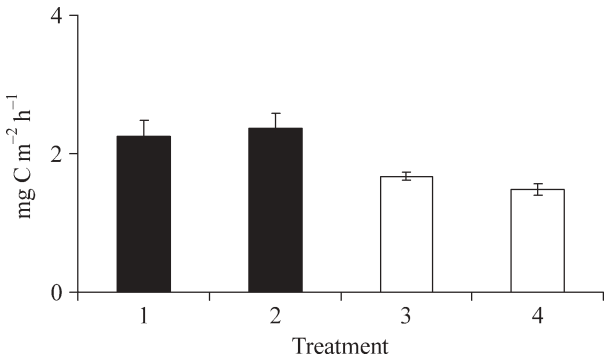

Fig. 7. Estimated primary production for 4 May 1999; values are for the total production integrated along the length of the light gradient. (1) Samples incubated at constant light at fixed distances from the light in the absence of UVB, (2) samples continuously moving in the light gradient in the absence of UVB, (3) samples incubated at constant light at fixed distances from the light in the presence of UVB, (4) Samples continuously moving in the light gradient in the presence of UVB. The 95% confidence intervals on the estimates are shown.

Table I: Comparison of seasonal changes in depth-integrated carbon fixation of samples exposed or not exposed to UVA+UVB

	Fixed		Rotating	
Date	No UV (mg C m^{-2} h^{-1})	+ UVA+UVB (mg C m^{-2} h^{-1})	No UV (mg C m^{-2} h^{-1})	+ UVA+UVB (mg C m^{-2} h^{-1})
12 Oct	5.61 (0.56)	6.92 (0.47)	7.63 (1.55)	7.73 (1.44)
3 Nov	4.17 (0.43)	4.79 (0.36)	4.77 (0.96)	5.00 (0.29)
16 Nov	5.44 (0.93)	6.32 (0.30)	5.64 (0.71)	6.32 (0.27)
30 Nov	6.03 (0.72)	5.75 (0.42)	5.87 (0.61)	6.09 (0.46)
15 Dec	2.39 (0.42)	2.22 (0.18)	2.65 (0.20)	2.36 (0.17)
5 Jan	1.72 (0.11)	1.51 (0.06)	1.62 (0.32)	1.75 (0.14)
20 Jan	1.07 (0.10)	0.79 (0.07)	1.18 (0.12)	0.87 (0.07)
1 Feb	1.80 (0.21)	1.83 (0.13)	1.79 (0.20)	1.74 (0.16)
15 Feb	2.19 (0.33)	1.39 (0.20)	1.96 (0.68)	1.19 (0.12)
2 Mar	2.28 (0.24)	1.83 (0.34)	2.27 (0.35)	1.47 (0.24)
8 Mar	2.53 (0.36)	2.16 (0.17)	2.56 (0.67)	1.65 (0.22)
15 Mar	4.18 (0.93)	3.82 (0.32)	4.27 (0.74)	3.14 (0.30)
22 Mar	3.37 (0.42)	2.97 (0.25)	3.58 (0.48)	2.68 (0.18)
29 Mar	2.80 (0.44)	2.20 (0.24)	3.70 (0.56)	1.61 (0.35)
6 Apr	16.36 (1.95)	10.54 (2.28)	18.54 (4.35)	7.66 (1.39)
12 Apr	38.83 (3.66)	40.35 (3.60)	34.08 (5.78)	22.38 (5.46)
19 Apr	41.92 (11.75)	41.31 (3.21)	41.45 (11.11)	36.42 (12.35)
26 Apr	9.83 (1.32)	6.62 (0.56)	10.68 (1.95)	3.96 (1.16)
4 May	2.25 (0.23)	1.67 (0.06)	2.42 (0.28)	1.53 (0.14)
10 May	8.91 (0.90)	8.86 (0.75)	8.40 (1.95)	7.02 (0.63)
17 May	5.74 (0.58)	4.45 (0.86)	5.73 (1.25)	4.18 (0.44)
26 May	3.81 (0.31)	2.65 (0.10)	4.56 (0.54)	2.39 (0.09)
7 Jun	8.55 (0.83)	5.29 (0.34)	8.50 (1.41)	5.13 (0.16)
21 Jun	6.45 (1.15)	5.92 (0.20)	7.13 (1.55)	5.31 (0.36)
5 Jul	4.22 (0.23)	3.82 (0.28)	4.39 (0.35)	3.60 (0.19)
19 Jul	23.71 (4.90)	22.54 (2.47)	18.15 (2.64)	17.17 (1.97)
2 Aug	5.01 (1.01)	4.07 (0.56)	4.48 (0.78)	4.32 (0.51)
16 Aug	11.13 (1.42)	11.61 (0.45)	12.48 (1.79)	11.62 (0.87)
01 Sep	7.10 (1.40)	8.53 (0.68)	8.44 (1.16)	9.16 (0.58)
13 Sep	12.14 (1.12)	11.95 (0.94)	11.71 (1.31)	13.19 (2.60)
29 Sep	5.72 (0.66)	4.51 (0.19)	6.00 (0.67)	4.95 (0.28)
11 Oct	13.42 (1.67)	11.43 (0.49)	15.28 (1.17)	11.75 (0.59)
25 Oct	4.12 (0.79)	2.96 (0.33)	4.10 (0.41)	2.87 (0.27)

"Fixed" refers to samples which were held at constant light during the 24 h experimental period and "Rotating" to samples which moved through the light gradient with a periodicity of 4 h. Values in brackets are (± SD).

response to UVA+UVB which are summarized in Fig. 8. Here we attempt to illustrate the effect of UV light by plotting the ratio of carbon fixation estimated from samples incubated in UVA+UVB to the carbon fixation in the same samples incubated without UVA or UVB. If there is no effect of UV light, the ratio should be 1.

At the beginning of the study, from October 1998 to January 1999, there was little effect of UV light and the ratio was close to 1. In some experiments, there was an indication that carbon fixation was slightly enhanced in the presence of UV light, especially in the samples incubated at fixed positions in the light gradient. There appeared to be less effect on the samples that were moving in the light gradient. However, from February there was a change and most of the incubations resulted in ratios that were significantly less than 1. That is, there was clearly less carbon fixation in the samples that were incubated in UV light. Generally, the ratio was lower in the samples that were revolving in the light gradient than in the samples incubated at constant light.

There was some variability in this ratio from experiment to experiment and, interestingly, the highest measured rates of production, on 19 April, showed only a small effect of UV on carbon fixation. But Fig. 8 demonstrates significant inhibition by UVA+UVB for a considerable part of the year. Maximum sensitivity to UVA+UVB appeared to be from February to June—the time of the maximum phytoplankton biomass and production in the English Channel (Jordan and Joint, 1984).

Photosynthetic parameters

The deleterious effect of UVB is likely to be on DNA, but in this study, we used carbon fixation to assess the effects of UV light. It is therefore relevant to ask if the lower rates of carbon fixation are merely a proxy for

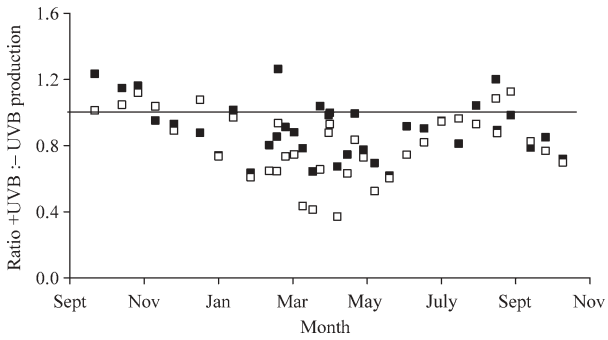

Fig. 8. Effect of UVB on carbon fixation throughout the year is shown as the ratio of production in samples contained in polyethylene (i.e. +UVB) to that of samples in polycarbonate (i.e. without UVB). Fixed samples (■) were at constant light and rotating samples (□) were moving in the light gradient. The horizontal line indicates the 1:1 ratio, which is the value that would result if there was no effect of UV light.

DNA damage, and concomitant reduction in the physiological fitness, or if UV light has a direct damaging effect on photosynthesis. An indication of a direct effect on photosynthesis can be obtained by comparing photosynthesis/irradiance (P/E) curves. The data from the fixed incubations were used to determine P/E parameters. The derived parameter, E_k, offers a convenient way to compare the response of phytoplankton assemblages to changing seasonal conditions, since this is the optimum light for photosynthesis and can indicate if phytoplankton assemblages are adapted to high light conditions. It may also provide an indication of sensitivity to the effects of UVA+UVB.

E_k changed throughout the study period for samples incubated in the presence and absence of UVA+UVB (Fig. 9). The E_k value in the presence of UV light was always lower than when the phytoplankton was not exposed to UV. Values of both α^B and P_m^B were often, but not always, lower in the presence of UVB, suggesting that both the light harvesting and carbon fixation processes were affected by UVB. However, for the most part, the lower values of E_k were due to increases in the slope of inhibition (β^B) which has the effect of decreasing P_m^B. Lowest E_k values were measured during the winter months from December to February. That is, the phytoplankton assemblage in the winter was not adapted to high light. This is not surprising given the low irradiance during winter months and the high degree of vertical mixing that phytoplankton cells experience in the winter. So these communities are analogous to shade plants in showing a high degree of inhibition at high light. However, at this time of year, the phytoplankton assemblage was not very sensitive to UVA+UVB, as judged by the ratio of carbon fixation in the presence and absence of UV light (Fig. 8). Values of E_k increased from February to May; that is the phytoplankton changed from being a shade adapted population to one that was adapted to high light situations with less susceptibility to photoinhibition at high light. However, this was also the period of greatest sensitivity to UVA+UVB (Fig. 8). Values of E_k remained high throughout the summer (Fig. 9) even after the phytoplankton ceased to show the same degree of sensitivity to UVA+UVB (Fig. 8). Therefore, it appears that UVA+UVB sensitivity is not closely related to the seasonal photosynthetic adaptation that occurs in natural phytoplankton assemblages.

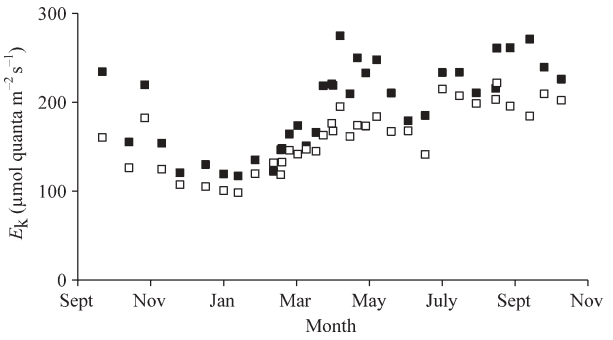

Fig. 9. Changes in the optimum light (E_k) for photosynthesis measured with (□) and without (■) UVA+UVB.

DISCUSSION

This study has shown that coastal phytoplankton assemblages, typical of UK coastal waters, are sensitive to elevated UVA+UVB and that there are seasonal variations in the degree of this effect. Phytoplankton in the spring and early summer appear to be particularly susceptible. This is the period of the year when a significant proportion of annual primary production occurs, so increased UVA+UVB could have a strong impact on coastal marine ecosystems through decreased phytoplankton activity.

We have used photosynthetic carbon fixation to assess the effect of UV light. In doing this, we acknowledge that DNA is likely to be the cellular constituent that will be most the susceptible to UV damage. However, we are primarily interested in the potential ecological effects of UV on the planktonic assemblage and carbon fixation is a sensitive and meaningful measure that indicates the physiological health of the phytoplankton cells. It is also appropriate to test if photosynthesis, which is a light utilizing process, is also sensitive to wavelengths other than those involved in the light reaction of photosynthesis.

Limitations of the experimental design

It is technically very difficult to achieve high flux rates of UVB light in the laboratory. We aimed to have photosynthetically active radiation PAR that was close to that experienced at the sea surface on a sunny day, and to expose samples to UVA and UVB at realistic levels. We have achieved these photon fluxes using quartz halogen and metal halide lamps with iron and cobalt additives. Although these give appropriate quantities of light, the UV energy in particular was limited to specific wavelengths. The spectral distribution (Fig. 1) was not the same as sunlight, although total UVA+UVB photon flux was environmentally realistic. The light gradient used in every experiment was typical of a sunny day, and maximum UVB was equivalent to an increase of about 30% of the maximum UVB currently measured in the UK. This is an appropriate value to test the effect of UVB, since it is within the range that has been measured recently in the UK (Keil *et al.*, 2007).

It was also important to deal with variations in day length through the period of the study. Since the aim was to compare phytoplankton assemblages from different times of the year, it was decided to incubate the samples in continuous light for 24 h. The disadvantage is that all assemblages were exposed to more light than they had previously experienced in the sea—and in winter, this will be three times the day length. The advantage is that it is possible to directly compare the response of different assemblages at different times of the year because the experimental conditions are identical. On balance, it was decided that this advantage outweighed to disadvantages of exposing the experimental samples to longer light periods. A 24 h exposure period was adopted because, assuming that DNA was the site of UV damage, it was necessary to have sufficiently long period of exposure to ensure that there would be a measurable effect on DNA expression, as judged by carbon fixation. Longer exposure to light would also maximize any photo-activated DNA repair, so minimizing potential effects of UV damage and provide a realistic simulation of the processes that occur in nature.

Identical production estimates in rotating and fixed incubations

An innovation in this study was the simulation of vertical movement in the water column by moving incubation bottles and bags along a light gradient. The mixing time was equivalent to that which occurs in UK coastal waters (Uncles and Joint, 1983), although constant movement along a gradient is unlikely to occur in a tidally mixed water column where there is turbulent mixing. Nevertheless, it is a realistic, if pragmatic, attempt to simulate how phytoplankton cells will be exposed to UV light in the coastal ocean. Since all incubations included bottles that were held at fixed light as well as bottles that rotated in the light gradient, it was possible to test the consequences of moving within a light gradient. That is, were the production estimates the same in fixed and moving incubations? These experiments resulted in very similar estimates of depth-integrated primary production (Table I). There was a strong correlation ($r^2 = 0.98$) between the two estimates, although overall, the estimated production in the samples that moved in the light gradient was slightly less (92%) than that obtained from samples incubated at constant light. This finding has relevance to the validity of ship-board primary production experiments (Pemberton *et al.*, 2006), which are often done either by incubating at constant depth in the ocean or in on-deck incubators to simulate light at different depths (Joint *et al.*, 2001). This type of incubation does not attempt to account for vertical mixing processes. The similarity of results obtained in this experiment gives confidence that this type of incubation is appropriate and that the lack of mixing does not compromise the estimation of depth-integrated production at sea.

The effect of UVA+UVB on phytoplankton production

There were clear seasonal differences in the response to UV, both in incubations at fixed light and moving in the light gradient. At the beginning of the study in autumn 1998, there appeared to be little or no effect of UV (Figs 5 and 8). However, in late winter, the phytoplankton assemblages began to show greater sensitivity and by March, there were large differences in the carbon fixation rate in the presence and absence of UVA+UVB (Fig. 8). The greatest difference appeared in the samples that were moving in the light gradient. One other report (Hernando and Ferreya, 2005) has considered the effect of mixing but they found no significant effect on the sensitivity of Antarctic phytoplankton to UVB. On only one occasion, when ozone was low, was there an effect in that study. However, in the present study, phytoplankton appeared to be much more sensitive, particularly throughout the spring, and vertical mixing was not able to compensate for the detrimental effects of UVB.

Why did the samples exposed to constant light appear to show less sensitivity to UVA+UVB than those moving in the light gradient? From Fig. 6 it can be seen that there was little difference in ^{14}C fixation

rate at the low-light end of the gradient between samples in the presence and absence of UV. The spectroradiometer used did not detect any UV at the low-light end of the gradient and it is probable that UVB quanta at this end of the tank were too few to affect the phytoplankton, either by damage to DNA or to photosystem integrity. So part of the water column would have shown no difference in the estimated production in the presence or absence of UVA+UV (Fig. 6). At high light, there was very significant inhibition by UV within 0.25 m of the light source. However, when depth-integrated production was calculated, this part of the water column made only a very small contribution to the estimated production value.

In contrast, all samples that moved within the light gradient were exposed to UVA+UVB, albeit of varying intensity. It appears that in spring, the phytoplankton assemblage was particularly sensitive to UV, that short-term exposure to high UVA+UVB flux (with a cycling time of 4 h, samples were at the highest UVA+UVB photon flux for about 10 minutes) was sufficient to damage the phytoplankton and to result in decreased primary production rates. In the winter and summer months, phytoplankton appeared to be less sensitive to UVA+UVB and there was less evidence of UV inhibition in either fixed or rotating samples. It is important to stress that the same light gradient was used for all experiments, so seasonal variations in response are due to different responses of the changed phytoplankton assemblages.

Abundant phytoplankton taxa at times of UV sensitivity

In winter, the phytoplankton assemblage was characterized by small flagellates that made up the majority of the biomass (Fig. 10). As the water column began to stabilize in March, flagellates increased in abundance but by April, diatoms were growing strongly and replaced flagellates to form most of the elevated biomass that comprised the spring bloom. Diatoms continued to be a dominant component of the phytoplankton through the spring and into early summer, with a second diatom bloom in May. As is often the case at this station in the English Channel, there was a late summer bloom of dinoflagellates. However, during the period of greatest sensitivity to UVA+UVB—March to June (Fig. 8)—diatoms were the dominant phytoplankton group.

Other studies have found that diatoms are sensitive to UV light. An investigation of laboratory cultures of *Phaeodactylum tricornutum* and *Chaetoceros mülleri* (Liang *et al.*, 2006) found that both species were inhibited by

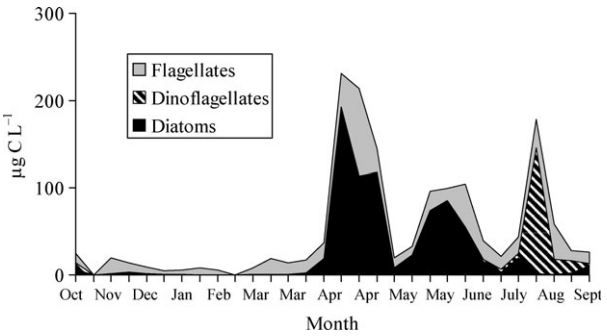

Fig. 10. Changes in abundance of major phytoplankton groups during study period; carbon content is calculated from cell volume.

exposure to UVA+UVB and there was an increase in the value of E_k. In contrast to the 24 h continuous light used in the present study, they used a 16 h light 8 h dark period. In the present study, there was no correlation between E_k and sensitivity to UV. There were seasonal changes in E_k, which suggested that winter assemblages were adapted to low light conditions without showing great sensitivity to UV. However, phytoplankton cells from the spring assemblage that was dominated by diatoms and sensitive to UV, did not appear able to be adapted to high light. E_k values increased during the period from February to May and phytoplankton assemblages in the late summer, autumn and winter had higher values of E_k. These were not dominated by diatoms and did not appear to be particularly sensitive to UVA+UVB.

Other studies have shown that representatives of the phytoplankton taxa that are present at station L4 can be affected by UV. For example, Mostajir *et al.* (Mostajir *et al.*, 1999) found that UVA+UVB affected the cell size of *Prymnesiophytes*, an important phytoplankton group in most oceanic provinces, including the English Channel. Cells became larger in the presence of UVA+UVB, and they suggested that this was due to effects on cell division; they also found that photosynthetic rates declined. Buma *et al.* (Buma *et al.*, 2000) found that *Emiliana huxleyi* was very sensitive to UVB. At station L4 in the English Channel, *E. huxleyi* frequently forms blooms in July or August. In 1999, the maximum biomass occurred on 19 July (2727 cells mL^{-1}). However, this assemblage did not have lower carbon fixation rates in the presence of UV (Fig. 8) suggesting that this *E. huxleyi*-dominated population was not sensitive to UV.

Phytoplankton species vary in their resistance to UV damage partly because of the presence of photoprotective pigment production and some species appear to be able to induce the production of UV-absorbing compounds (Buma *et al.*, 2006). One particularly effective group of UV absorbing compounds are mycosporine-like amino

acids (MAAs). In a study at station L4, Llewellyn and Harbour (Llewellyn and Harbour, 2003) demonstrated that UV absorbing MAAs were produced throughout the year. They found increases in specific MAA concentrations that were associated with particular phytoplankton. For example, a bloom of *Phaeocystis pouchetii* in spring had high concentrations and there was another maximum in late summer associated the diatom *Guinardia striata*. The experiments in the present study demonstrate that, even if MAAs or photoprotective pigments were produced, they did not prevent inhibition by UV of carbon fixation in the spring.

CONCLUSION

These experiments indicate how seasonal changes in phytoplankton species composition and environmental factors can combine to increase UVA+UVB sensitivity in phytoplankton assemblages in the temperate coastal ocean. This study did not attempt to consider acclimation to UV. Experiments were done over 24 h periods, and this is not enough time for adaptation to occur. But this reflects the situation in Northern Europe where ozone depletion is sporadic and likely to last only a few days until a displaced stratospheric polar vortex returns north. The results from this study need to be interpreted in the context of ozone depletion over NW Europe. This only occurs in late winter/early spring. Interestingly, this is the time when the phytoplankton assemblages appear to be most sensitive to UVB. So the greatest danger is at a time of year when assemblage is most vulnerable. This study suggests that vertical mixing in the water column could not provide a refuge from UV, since samples moving in a light gradient were just as sensitive as those incubated at fixed photon flux.

The rapid onset of ozone depletion means these phytoplankton assemblages cannot adapt to mitigate the impact of higher UVA+UVB. Sensitive taxa, such as diatoms, cannot acclimate their photosynthetic apparatus or photorepair mechanisms because UV flux increases within a few hours as the ozone depleted stratosphere moves across the region. Within a few days, there could be significant decreases in primary production, sensitive phytoplankton species might be replaced by UV-tolerant species, and this could lead to changes in the dominant phytoplankton species. With the removal of anthropogenic ozone depleting chemicals from the environment, stratospheric ozone depletion should disappear within the next century. Until that time, it is possible that short lived ozone depletion events over temperate regions may decrease primary production, change species composition and have a measurable impact on the whole of the pelagic food web in coastal waters.

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