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Effects of artificial UV-A and UV-B radiation on carbon allocation in *Synechococcus elongatus* (cyanobacterium) and *Nitzschia palea* (diatom)

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Introduction

Both UV-B (280-320 nm) and UV-A (320-400 nm) radiation penetrate the near surface waters of lakes (HODOKI & WATANABE 1998, SCULLY & LEAN 1994). UV-B radiation is primarily attenuated by the amount of dissolved organic carbon (DOC) and, secondarily, by the phytoplankton/chlorophyll *a* concentration. Although UV-A penetrates more deeply in the water column, less is known about what controls the attenuation of UV-A radiation (however, see SOMMARUGA & PSENNER 1997).

Much emphasis has been placed recently on measuring the effects of UV-B radiation on aquatic life and, in particular, on phytoplankton (reviewed in HÄDER et al. 1995) primarily because of the specter of enhanced UV-B resulting from stratospheric ozone depletion. UV-A radiation, although not expected to increase as a result of ozone depletion, still exerts strong effects on the physiological and ecological responses of aquatic organisms. There is more energy in the UV-A waveband than in the UV-B region in natural sunlight spectra, but applications of presently available biological weighting functions to natural spectra would appear to suggest that the effects of UV-A radiation will be much less significant than those of UV-B radiation. Furthermore, UV-A radiation in natural systems has been implicated in both photo-damage (KIM & WATANABE 1993, 1994) and photorepair (WEINBAUER et al. 1997), although the direction of the effects (positive or negative) has often been equivocal (QUESADA et al. 1995).

In this study we examined the allocation of carbon to the main macromolecular products of photosynthesis (lipids, polysaccharides and proteins) in *Nitzschia palea* (Kutz.) and *Synechoccocus elongatus* Näg. under different dose regimes of UV-B and UV-A radiation, both in combination and separately. We tested the null hypotheses that carbon allocation patterns would not be affected by exposure to UV radiation and that overall the carbon allocation response would be similar between the diatom and cyanobacterium.

Methods

Two species of algae were used in these experiments: *Nitzschia palea* (Bacillariophyceae) and *Synechococcus elongatus* (Cyanophyceae). The algae were grown in 2-L chemostats (Schmizo AG, Switzerland) in a 14:10 light:dark cycle at a PAR intensity of 100 µmol m^{-2} s^{-1}. *Synechococcus* and *Nitzschia* were maintained in modified Woods Hole WC media (GUILLARD & LORENZEN 1972) and CHU media, respectively. Further details of the culture conditions and harvesting of cells can be found in ARTS & RAI (1997).

Just prior to the beginning of each experiment (1–2 h after the onset of the light cycle) we added an aliquot of ^{14}C-sodium bicarbonate to the algae suspension sufficient to achieve an activity level of ≈80,000 DPM mL^{-1}. Prior to adding the ^{14}C we measured chlorophyll *a* concentrations of the cell suspensions as in ARTS & RAI (1997) in order to standardize the subsequent production measurements.

Experiments were conducted in a phototron (RAI & KRAMBECK 1992). Briefly, this apparatus consists of a solid aluminum temperature-controlled block cored out to hold 24 cylindrical glass containers (75 mL each) closed at the bottom but open at the top to permit sampling. Photosynthetically active radiation (PAR: 400–700 nm) was provided from below by individual halogen bulbs (Philips Spot Lamps Model GBE, 18° arc, 12 V, 20 W). A PAR intensity of 100 µmol m^{-2} s^{-1} was maintained by placing individual neutral density metal screens in a tray (6 × 4 array) centered between the bulbs and the cylinders.

Ultraviolet radiation was supplied by up to six Bachofer GmbH bulbs (Model W-IL-200-M, peak intensity at 312 nm and/or Model W-IL-200-L, peak intensity at 365 nm, both types were 8 W, 200

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mm long) arranged in a scaffolding above the phototron. Sample spectra for 6 Bachofer UV-A or UV-B bulbs suspended 33 cm above the phototron, as well as two natural solar spectra, are shown in Fig. 1. The Bachofer UV-B lamps emit more energy than solar radiation below 320 nm, less energy than solar radiation above 320 nm and very little visible light (ARTS & RAI 1997). The UV-B bulbs were not intended to simulate the UV-B component of natural sunlight, but rather provide an illustration of the effects of the most deleterious wavelengths responsible for the predominant pathological effects of solar UV-B radiation (KARENTZ et al. 1991). These bulbs are hereafter referred to as UV-B and UV-A bulbs, respectively. All UV bulbs were "burned in" for a minimum of 100 h. Different dose rates were achieved by raising or lowering the bulbs, changing the number of bulbs, and/or through the use of metal screens placed on top of the incubation cylinders. We measured the resulting UV spectra using an Optronics OL-754 spectroradiometer fitted with an integrating sphere cosine-sensor (Model OL 754-O-PMT). The spectroradiometer was calibrated using a dual calibration and gain check module (Optronic Laboratories, Model OL 752-150) employing a fluorescent 4 W mercury emission lamp and a 5-W stable tungsten filament lamp to check wavelength and gain, respectively. We positioned the spherical sensor at a position representative of the surface of the media in the incubation bottles at the various dose regimes. Figure 2 provides an example of the different UV-A (unweighted) dose rates that were obtained for one to six Bachofer UV-A bulbs using no filter, a coarse, or a fine metal filter.

All incubations proceeded for a total of 4 h during which time the temperature was maintained at 10 °C. At various times during the incubations we removed two, replicate, 10-mL aliquots of cell suspension, which were then filtered onto pre-combusted 25 mm Whatman GF/F filters. The filters were frozen and, at a later date, subjected to an extraction procedure originally developed by LI et al. (1980). The extraction procedure yielded four macromolecular classes: low molecular weight metabolites (LMW), lipids, polysaccharides and proteins. PAR-only controls (4-h incubations) were run in triplicate and standard errors around the mean production values (for the different macromolecular classes) were never more than 15%, and typically <10%, of the mean.

In total, three different experiments were conducted. Experiment 1 consisted of exposing *Nitzschia* or *Synechococcus* to two doses (0.026 and 0.11 mW cm^{-2}) of UV-B radiation. Cells were exposed to UV-B for 10, 20 60, 120, 180 or 240 min at which time duplicate samples were collected for macromolecular extraction. The total incubation was always 4 h even when the bottles were sampled at UV-B exposures of <240 min. However, in those incubation bottles we continued the exposure and a further sample was also collected at 4 h to check for replicability. Hence, for each dose at 4 h we collected a total of eight samples (three from the dedicated 4-h sample and one from each of the 10, 20 60, 120, 180 or 240 min bottles). Experiment 2 consisted of exposing *Nitzschia* to two doses of UV-A (1.2 and 0.6 mW cm^{-2}) for the same time periods as in Experiment 1. Additional samples were also collected at 4 h. This experiment was not performed with *Synechococcus*. In Experiment 3, *Nitzschia* or *Synechococcus* was exposed to three combinations of UV-B/UV-A (0.222 mW cm^{-2} UV-B and 0.060 mW cm^{-2} UV-A; 0.096 mW cm^{-2} UV-B and 0.030 mW cm^{-2} UV-A; 0.060 mW cm^{-2} and 0.015 mW cm^{-2} UV-A); and three doses of UV-B alone (0.222 mW cm^{-2}, 0.096 mW cm^{-2}, and 0.060 mW cm^{-2}). When in combination, the UV-B bulbs were shut off at the time of sampling but the UV-A lamps were left on for the duration of the 4-h experiment. The UV-B alone portion of the experiment was conducted similarly to Experiment 1 but without the replicate samples at 4 h.

Results

Experiment 1

Exposures of *Nitzschia* to UV-B radiation of >60 min produced similar effects at both dose rates (Fig. 3). Synthesis of LMW metabolites was consistently higher than the controls but there was no apparent trend with exposure duration. Lipid synthesis was enhanced by short-term exposures to UV-B especially at the 0.026 mW cm^{-2} dose rate; however after 1 h of exposure there was no difference between the UV-B exposed and the control *Nitzschia* (Fig. 3). Polysaccharides, although initially stimulated by UV-B radiation, appeared to decline steadily as a function of exposure duration. Protein synthesis was initially stimulated by short exposures (≤1 h) to UV-B. However after 1 h protein synthesis slowly declined relative to controls. The net effect of these two UV-B dose rates on the various macromolecular classes was that, for short exposures (≤20 min), net production was stimulated relative to the PAR-only controls.

With the exception of LMW metabolites, the

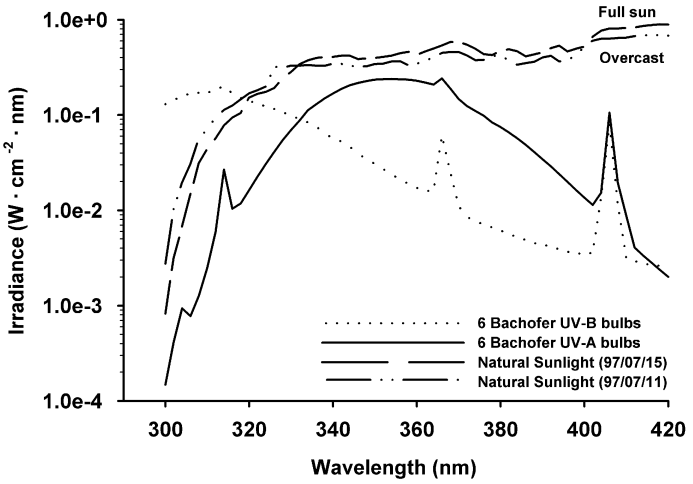

Fig. 1. Irradiance spectra achieved by placing six Bachofer UV-A (peak 365 nm) and UV-B (peak 312 nm) bulbs at 33 cm height above the phototron. Also shown, for comparison, are natural sunlight spectra for a sunny day (97/07/15) and an overcast day measured in Saskatoon, Canada on 97/07/15 and 97/07/11, respectively. Note log scale.

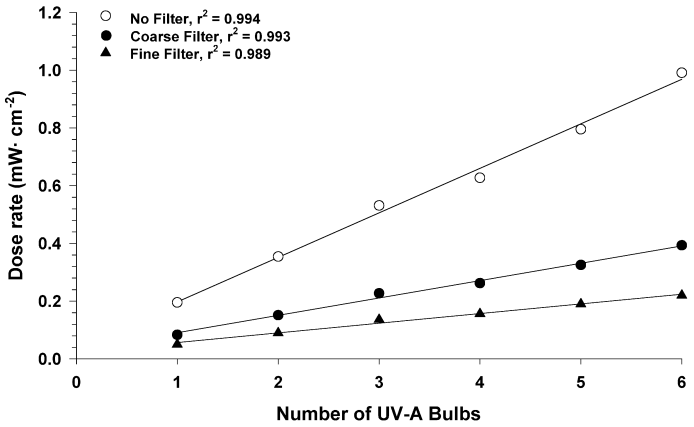

Fig. 2. Sample of different UV-A (320 to 400 nm) dose rates (unweighted) for different numbers of Bachofer bulbs with either no mesh filter, a coarse metal-mesh filter or a fine metal filter. In this example the scaffolding holding the UV-A bulbs was suspended at 33 cm above the phototron.

same two dose rates produced nearly identical patterns as a function of exposure duration (Fig. 4) in *Synechococcus*. The higher dose rate (0.026 mW cm^{-2}) stimulated LMW production compared with the 0.011 mW cm^{-2} dose rate and the control. Exposures of ≤1 h appeared to stimulate the synthesis of all the macromolecular classes, especially lipid and protein (Fig. 4). After 1 h of exposure to UV-B radiation the production of LMW metabolites, lipids and proteins began to decline but polysaccharide production remained relatively constant and higher than the controls. The net effect of these two dose rates on *Synechococcus* was stimulatory for exposure durations of ≤1 h but not different from the PAR controls after 1 h (Fig. 4).

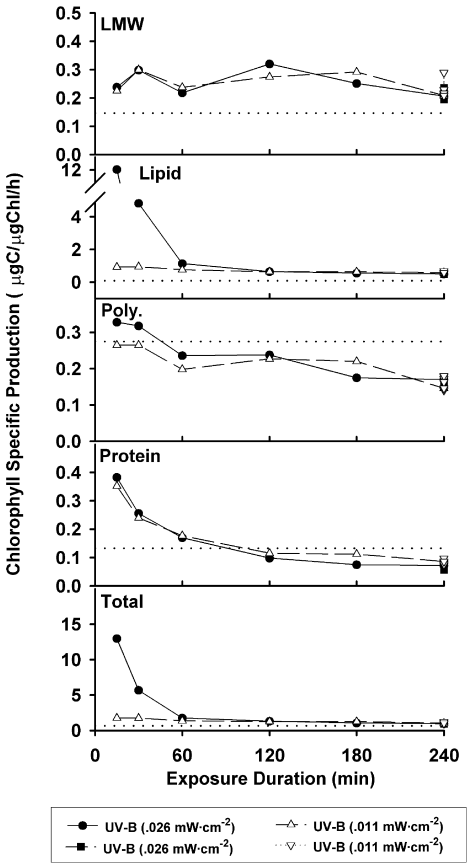

Fig. 3. Chlorophyll-specific production of photosynthates in *Nitzschia palea* exposed to two different doses from the Bachofer UV-B bulbs for various lengths of time. The dotted lines represent the light controls (no UVR) after 4 h. Solid squares and downward-pointing open triangles signify additional samples collected from the <240 min UV-B exposures but at the end of the experiment (240 min).

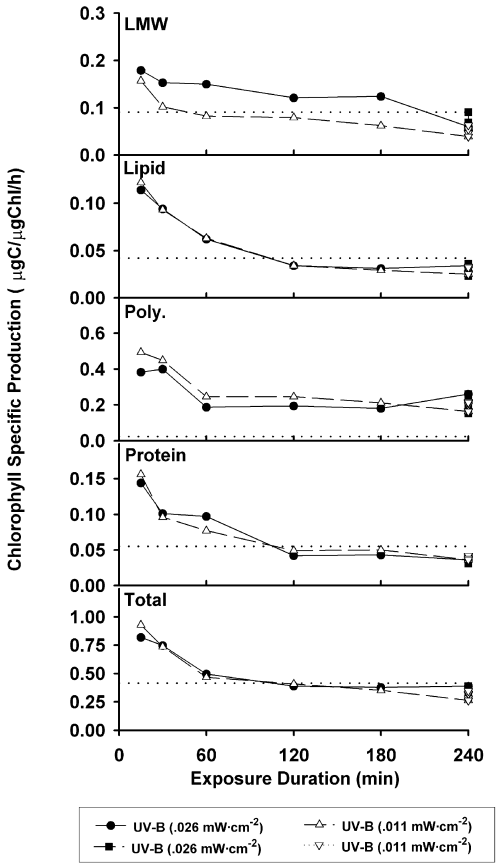

Fig. 4. Chlorophyll-specific production of photosynthates in *Synechococcus elongatus* exposed to two different doses from the Bachofer UV-B bulbs for various lengths of time. Symbols as in Fig. 3.

Experiment 2

Exposure of *Nitzschia* to UV-A at two dose rates (Fig. 5) produced a different pattern of macromolecular synthesis compared with Experiment 1. Exposures of ≤20 min were generally stimulatory for all macromolecular classes; protein for example was c. 2.5 times higher in the two experimental treatments after 10 min exposure to UV-A than in the PAR-only controls. At exposure durations of ≥1 h, the effect of UV-A radiation on component and total (net) macromolecular synthesis was inhibitory with the higher dose rate exhibiting the most negative effect (Fig. 5).

Experiment 3

Although total net photosynthetic carbon fixation in *Nitzschia* was not markedly affected by either UV-B alone or UV-B + UV-A in combination, there were pronounced differences in the response amongst the various macromolecular classes when comparing UV-B alone to UV-B + UV-A (Fig. 6). LMW metabolites were generally stimulated by UV-B + UV-A; how-

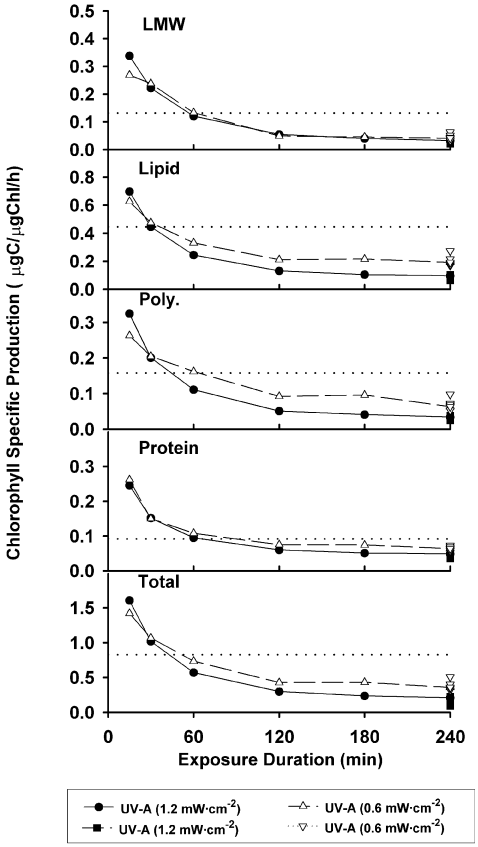

Fig. 5. Chlorophyll-specific production of photosynthates in *Nitzschia palea* exposed to two different doses from the Bachofer UV-A bulbs for various lengths of time. Symbols as in Fig. 3.

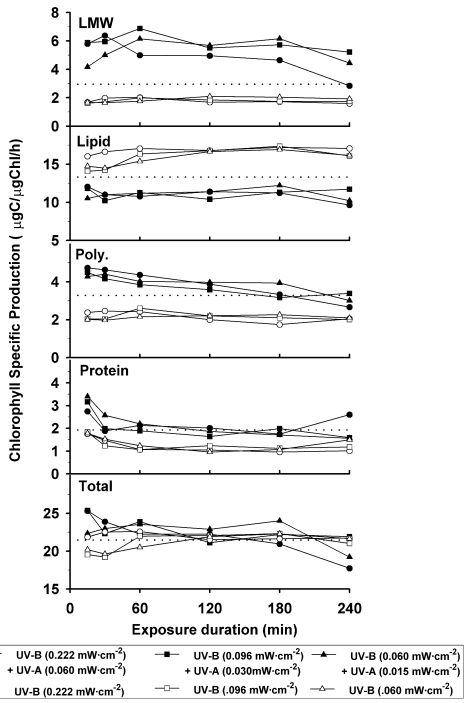

Fig. 6. Chlorophyll-specific production of photosynthates in *Nitzschia palea* exposed to 3 different doses of UV-B and UV-A or 3 different doses of UV-B alone for various lengths of time. Symbols as in Fig. 3.

ever, this effect diminished with the highest dose combination at the 4-h mark. The same dose of UV-B caused LMW metabolite synthesis to be depressed compared to the PAR-only controls (Fig. 6). Lipid production was stimulated by UV-B alone and inhibited by UV-B + UV-A whereas the reverse was true for polysaccharide synthesis. Polysaccharide synthesis in the UV-B + UV-A dose regimes declined steadily as a function of dose rate (Fig. 6). Protein production was depressed in the UV-B alone condition and similar to the PAR controls for incubations of ≥20 min. There was some suggestion that protein synthesis was stimulated in the UV-B + UV-A treatment for short (10 min) exposure periods.

In *Synechoccocus*, neither the UV-B + UV-A nor the UV-B alone treatments produced macromolecular synthesis patterns that differed markedly from the PAR control (Fig. 7). There was some suggestion that lipid production was enhanced during short exposure durations (≤20 min) in all but the highest dose of UV-B alone and that UV-B + UV-A produced slightly higher lipid synthesis rates especially after 3–4 h. Protein production was generally depressed relative to the light control but perhaps slightly less so for the UV-B + UV-A treatment (Fig. 7). There were no clear patterns in total net production.

Discussion

Our results clearly demonstrate that exposing

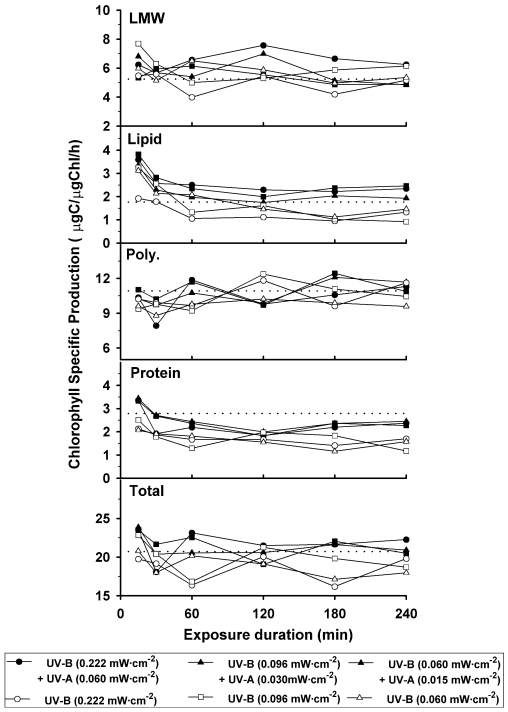

Fig. 7. Chlorophyll-specific production of photosynthates in *Synechococcus elongatus* exposed to 3 different doses of UV-B and UV-A or 3 different doses of UV-B alone for various lengths of time. Symbols as in Fig. 3.

algae to low levels or UV-B or UV-A radiation (Experiments 1 and 2) had marked effects on the apportioning of carbon to the main macromolecular end products of photosynthesis (lipids, proteins and polysaccharides). Exposure to low levels of UV-B or UV-A radiation for short durations (<1 h) generally stimulated carbon fixation to all photosynthate classes. However, increased exposure (>1 h) shifted the balance from stimulation to inhibition of production for most macromolecular classes. Because production of some photosynthates were stimulated while others were inhibited under low levels of UV-B radiation, the net effect on total chlorophyll-specific production was often indistinguishable from the light controls (Figs. 3 and 4). Despite this apparent lack of a net effect on photosynthesis for longer exposure periods (>1 h), the production of specific macromolecular classes was nonetheless affected (e.g. polysaccharides).

We observed similar effects with slightly higher doses of UV-B in that, although there were no strong trends with regard to total chlorophyll-specific production, the carbon allocation to the four classes of photosynthates responded differently (Figs. 6 and 7). For example, in the case of *Synechococcus*, lipids appeared to be stimulated with very short (10 min) exposures (Fig. 7). The superimposition of UV-A radiation together with these UV-B doses markedly changed the direction and magnitude of carbon fixation to the various macromolecular classes, most strikingly in the case of *Nitzschia* (Figs. 6 and 7). The addition of UV-A was either stimulatory (e.g. LMW metabolites in *Nitzschia* and lipids in *Nitzschia* and *Synechococcus*) or inhibitory (e.g. polysaccharides in *Nitzschia*).

Within the limited scope of these experiments we cannot identify a specific mechanism to explain these results. Rather we speculate that the differences we observed between the two species may be a reflection of the way the different chromophores (chlorophyll and accessory pigments) are affected by the UV radiation, the ability of each species to affect repair and, perhaps most importantly, the species-specific effects of UV-B and/or UV-A on the production rates and ratios of NADPH and ATP.

Taken together, our data indicate that both UV-B and UV-A alone, or in combination, can affect the allocation of carbon to the main macromolecular end products of photosynthesis, even at UV-B and/or UV-A levels that do not markedly affect the overall rate of photosynthesis relative to PAR-only controls. Similar results, using Bachofer UV-B bulbs at higher dose rates, were reported earlier for *Cryptomonas* sp., *N. palea* and *S. elongatus* (Arts & Rai 1997). These findings have important implications for aquatic communities for several reasons. First, short-term exposures (<1 h) to low levels of ultraviolet radiation, such as may occur at depth in natural waters, may actually stimulate carbon fixation perhaps resulting in short-lived increases in food energy available to con-

sumers. Second, the prevailing ratios of UV-B:UV-A:PAR to which the algae are exposed may fundamentally affect the nutritional value of algae by altering both the magnitude and direction of carbon allocation to photosynthates. The natural chromophoric DOC concentration, in particular, will make a significant contribution to the final value of these ratios at depth and will thus have a large influence on the outcome of carbon allocation in phytoplankton. Finally, recent evidence suggests that there may be more subtle compositional changes occurring within each macromolecular class that will further alter both the physiology of the algae and their food value to consumers. For example, GOES et al. (1994) and WANG & CHAI (1994) recently demonstrated that exposing marine phytoplankton to natural solar UV-B, at levels below that required to inhibit photosynthesis, changed their fatty acid composition. Similarly, UV-B radiation, again at levels below that still permitted the uptake of carbon into cells, has also been shown to alter amino acid (GOES et al. 1995) and carbohydrate (GOES et al. 1996) composition in marine phytoplankton.

Examples of strong effects due to exposure to UV-A are less common than published reports of negative impacts of UV-B on phytoplankton. KIM & WATANABE (1994) demonstrated, in laboratory studies, that growth rates in *Melosira* spp. and *Chlorella ellipsoidea* were depressed when the algae were exposed to UV-A radiation but that both species acclimatized to prolonged UV-A exposure by reactivation of the photosystem and enhanced chlorophyll *a* synthesis. However, in field studies conducted in four lakes, KIM & WATANABE (1993) showed that rates of photosynthesis in natural phytoplankton communities were reduced by roughly half by the presence of UV-A radiation. Similarly, in a Swiss lake (BÜHLMANN et al. 1987), a Canadian lake (MILOT-ROY & VINCENT 1994) and four lakes in Pennsylvania U.S.A. (MOELLER 1994), exposure to UV-A radiation was shown to significantly inhibit ^{14}C assimilation in situ. Finally, in detailed biochemical studies, DÖHLER (1995) and DÖHLER & BUCHMANN (1995) have demonstrated that exposure to UV-A radiation changes the rate of ^{15}N-ammonium uptake in marine phytoplankton. The direction of change was seen to be either positive or negative depending on whether or not UV-B was present. Furthermore, DÖHLER & BUCHMANN (1995) showed that UV-A may alter the composition of accessory pigments in *Pavlova lutheri*.

Clearly, UV-A radiation is a potent force regulating biological productivity in aquatic systems. The degree to which this occurs will depend in part on the species composition of the algal community because sensitivity to UV-B and UV-A is species-specific. In addition, models attempting to assess the environmental significance of current levels of UV-A and UV-B and anticipated increased levels of UV-B radiation on wetlands will have to account for the effects of DOC concentration and composition as well as the effects of wind-induced vertical mixing, since these elements will be important in controlling dose rate and exposure duration, respectively.

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