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Effects of chronic, low levels of UV radiation on carbon allocation in *Cryptomonas erosa* and competition between *C.erosa* and bacteria in continuous cultures

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Abstract. We conducted a long term (4 week) continuous culture study to measure the chronic effects of UV radiation on the alga, *Cryptomonas erosa*, using three different fluence rates of UV radiation. We measured carbon allocation into carbohydrate, protein and lipid pools, as well as chlorophyll *a* concentrations and algal and bacterial density. After 21 days, algal density in the control and lowest UV treatment (treatment 1 = 3.4 W m^{-2} UVR unweighted) was significantly lower than in the two highest UV treatments (treatment 2 = 14.9 W m^{-2} and treatment 3 = 16.2 W m^{-2} UVR unweighted), and did not recover in the following week of no UV exposure. Chlorophyll *a* and carbohydrate content (ng algal $cell^{-1}$) for the control and treatment 1 were clearly lower than treatments 2 and 3 by day 15, and did not recover by day 28. Percentage total lipid for the control and treatment 1 also decreased compared with treatments 2 and 3 by the end of the exposure period. However, by day 21, protein content for the control and treatment 1 was significantly higher than treatments 2 and 3, and demonstrated a further increase by day 28. The results were largely attributed to competition effects between *C.erosa* and bacteria in these non-axenic cultures. Bacterial density was significantly (×4) higher in the control and lowest UV treatment compared with the two highest UV treatments. Our findings suggest a competitive advantage of phytoplankton over bacteria under these conditions. If UV radiation, in general, affects bacteria to a greater extent than algae, there are likely to be changes in (i) bacterial utilization of dissolved organic matter produced by phytoplankton, (ii) competition between phytoplankton and bacteria for nutrient minerals and (iii) predation rates on bacteria by micro-flagellates.

Introduction

Aquatic organisms in shallow ponds, lakes and rivers may increasingly be affected by UV-B (280–320 nm) radiation due to declines in stratospheric ozone levels. There are many documented direct negative effects of UV radiation (UVR, 280–400 nm) on phytoplankton including, for example, inhibition of photosynthesis (Smith *et al*., 1992; Moeller, 1994), decreased motility (Donkor and Häder, 1991), changes in pigment composition (Zündorf and Häder, 1991), reduced nitrogen metabolism (Döhler, 1985) and DNA damage (Buma *et al*., 1995). Although we have begun to understand some of the molecular and cellular mechanisms of damage and repair in single organisms, there is a paucity of information concerning the biological responses to UVR at the population, community and ecosystem levels (Siebeck *et al*., 1994).

Comparatively little research has focused on the indirect effects of UV exposure on microbial communities. Upon exposure to UVR, it is possible, for example, that carbon allocation to the main cellular pools will decline in some species of phytoplankton [e.g. (Arts and Rai, 1997; Arts *et al*., 2000a)], possibly

lowering the quality of food (lipid or protein content and/or composition) available for herbivores. The possibility that changes in food quality may occur is supported by the work of Wang and Chai (Wang and Chai, 1994) and Goes *et al.* (Goes *et al.*, 1994), who have shown that the concentration of essential fatty acids [specifically, eisosapentaenoic (EPA) and docosahexaenoic (DHA) acids] in some marine algae can decline. These declines occurred at levels of UVR below that required to inhibit photosynthesis.

Cosmopolitan algae such as *Cryptomonas* and *Rhodomonas*, which are sporadically abundant, have been described as nutritionally important phytoplankton for zooplankton (Arts *et al.*, 1992, 1993; Chen and Folt, 1993). This is partly due to the fact that *Cryptomonas* contains appreciable amounts of long-chained, polyunsaturated fatty acids (PUFA), particularly EPA and DHA (Ahlgren *et al.*, 1992). Hence, if increased UVR causes a decline in lipid energy stores or a change in fatty acid composition in freshwater algae such as *Cryptomonas*, there may be cascading effects at higher trophic levels. In addition, where there is competition between micro-organisms, UVR could have significant indirect effects on phytoplankton–bacterial interactions (Herndl *et al.*, 1993; Goes *et al.*, 1994; Ferreyra *et al.*, 1997). In order to predict when UVR impacts may be greatest, Jeffrey *et al.* (Jeffrey *et al.*, 2000) indicate that a better understanding of the environmental conditions influencing UVR response, and identification of sensitive (and insensitive) species, is needed.

Most studies on algae have focused on the effects of acute exposure to UVR, and alga–bacterial interactions are often overlooked. Phytoplankton are commonly exposed for only a few hours and the effects of UVR on photosynthetic activity are measured by oxygen production or ^{14}C uptake (Veen *et al.*, 1997). In addition to interactive effects, there is also a need for research into the chronic effects of UVR on algae, because organisms in nature may be (a) exposed for long periods and/or (b) capable of adapting to UVR stress (Plante and Arts, 1998). Stockner and Antia present evidence that initial algal exposures to environmental stressors for as long as 20–40 days may be required for successful adaptation (Stockner and Antia, 1976). However, only a few studies have investigated the effects on phytoplankton after a prolonged (several days) UV exposure (Jokiel and York, 1984; Döhler, 1984, 1989; Behrenfeld *et al.*, 1992; Bothwell *et al.*, 1993; Veen *et al.*, 1997). Thus, we conducted a UV exposure experiment in 2 l continuous cultures over a 3-week period (with a 1 week recovery phase) in order to examine long-term effects due to UVR on a nutritionally important alga, *Cryptomonas erosa*.

Irradiance as a function of depth, measured in 16 ponds with high levels of dissolved organic carbon (DOC) at the St Denis National Wildlife Refuge, Saskatchewan, were used as the basis for determining our UV treatments (Arts *et al.*, 2000b). Average UV irradiances found at the 10 cm and subsurface (0–3 cm) depths, as well as a UV irradiance that was 14% higher than the subsurface level, comprised our exposure treatments. We investigated the null hypothesis that there is no long-term effect of UVR on *C.erosa* with respect to chlorophyll *a* content, or carbon allocation to carbohydrate, protein or lipid, for exposed versus unexposed (control) algae.

Method

Field investigation/light climate in wetlands at St Denis

An Optronics scanning spectroradiometer (Model OL-754, Optronics, Orlando, FA, USA) fitted with a submersible right-angle teflon cosine sensor (Model OL-86-T-WP) was used to measure natural solar spectral irradiance, as a function of depth, in 16 ponds at the St Denis National Wildlife Refuge, Saskatchewan (Arts *et al*., 2000b). This meter uses a double monochromator design and a temperature-controlled photomultiplier detector. Before any measurements were taken, the instrument was calibrated (250–800 nm) against a NIST-traceable 200 W tungsten–halogen standard lamp (OL752–10). Wavelength (±0.1 nm) and gain accuracy were assessed using an OL752–159 Dual Calibration and Gain Check Source Module. The average irradiances obtained from these measurements were used to determine the treatments in the experiments described here [and see Table 1 (Plante and Arts, 1998)].

Here, near-surface levels of UVR measured on sunny summer days in high DOC wetlands at the St Denis sites (52°13′N, 106°06′W) were simulated in laboratory experiments. Locations closer to the equator, or at higher elevations, receive higher UVR than latitudes further from the equator or at lower altitudes (WHO, 1994). Thus, more pronounced changes in carbon allocation or other effects on *C.erosa* might be observed at high elevation, nearer the equator and/or in low DOC waters. Alternatively, cryptomonads may adapt to higher UVR environments by developing enhanced photorepair mechanisms or by increasing protective UV-absorbing pigments. Thus, the irradiances and observed effects in our experiments are likely to be conservative (due to the high DOC values in the wetlands used to model our fluence rates) compared with the experience of cryptomonads elsewhere. On the other hand, cryptomonads in nature have the option of swimming down into the water column to avoid high UVR levels, whereas in our continuous cultures there is no such refuge.

The UV treatments (in W m^{-2}) applied in this experiment were comparable with some laboratory and field studies (Worrest *et al*., 1981; Behrenfeld *et al*., 1992; Ferreyra *et al*., 1997; Arts and Rai, 1997; Kasai and Arts, 1998), but were much lower than many other experiments (Döhler, 1984; Häder and Lui, 1990; Herndl *et al*., 1993; Döhler and Lohmann, 1995; Jeffrey *et al*., 1996; Lindell and Edling, 1996; Veen *et al*., 1997). Differences between these studies and ours reflected such things as the type of UV bulb used, the method of UV shielding and/or the distance of the UV source from the algal culture. Furthermore, laboratory studies using fluorescent bulbs cannot truly duplicate natural solar spectra due to the differences in spectral distribution. Thus, where experiments were conducted under natural solar irradiance, depending on the location of the study site, UVR could be either quite comparable with those applied in our study, or significantly higher. Generally, in experiments conducted in lakes or rivers, the irradiance tended to be more similar to ours, while irradiances from marine studies were typically higher than those used here. These observations illustrate the need for careful characterization (and justification) of the UV irradiance applied in laboratory experiments to ensure that they will be at least comparable with natural irradiances.

Table I. Unweighted and biologically-weighted irradiances of UV radiation measured at the centre of the algal culture inside the continuous cultures

Treatment	UV-B (W m^{-2}) (280–320 nm)			UV-A (W m^{-2}) (320–400 nm)			UV-B + UV-A (W m^{-2}) (280–400 nm)		
	1	2	3	1	2	3	1	2	3
Unweighted dose rate	4.2E-2	2.8E-1	3.2E-1	3.3E0	1.5E1	1.6E1	3.4E0	1.5E1	1.6E1
Biologically-weighted dose rate:									
Jones and Kok chloroplast (1966; normalized to 300 nm)	3.0E-2	2.0E-1	2.3E-1	9.8E-1	4.3E0	4.7E0	1.0E0	4.5E0	4.9E0
Caldwell Plant (1971; 300 nm)	2.0E-3	1.4E-2	1.5E-2	0.0E0	0.0E0	0.0E0	2.0E-3	1.4E-2	1.5E-2
Cullen *et al. Phaeodactylum* sp. (1992; 300 nm)	9.0E-3	5.7E-2	6.6E-2	1.3E-1	5.9E-1	6.3E-1	1.4E-1	6.4E-1	7.0E-1
Cullen *et al. Prorcentrum micans* (1992; 300 nm)	7.0E-3	4.8E-2	5.5E-2	7.0E-2	3.1E-1	3.4E-1	7.7E-2	3.6E-1	4.0E-1
Setlow DNA (1974; 300 nm)	2.0E-3	1.1E-2	1.3E-2	0.0E0	0.0E0	0.0E0	2.0E-3	1.1E-2	1.3E-2

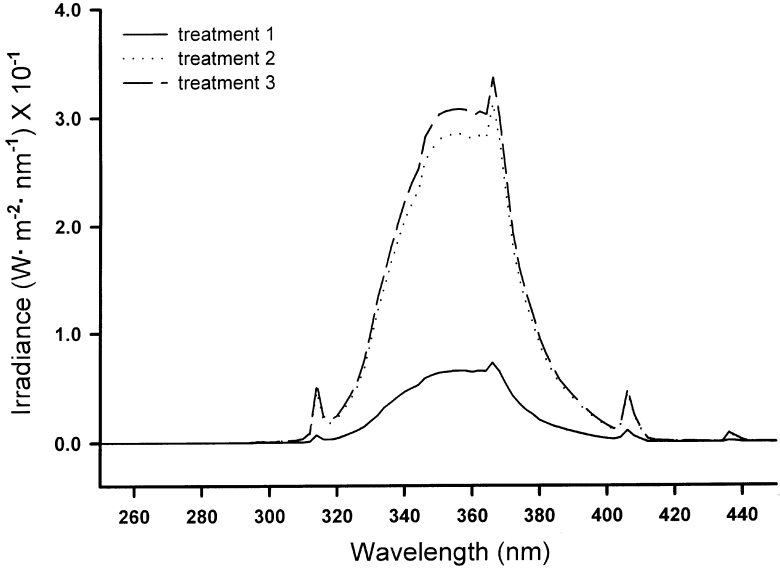


Fig. 1. Spectral irradiance of the three UV treatments that were used in the continuous culture experiment.

Pre-experiment conditions

Cryptomonas erosa was maintained at 16 ± 1°C in the exponential growth phase in 2 l continuous cultures at a dilution rate of 0.34 day^{-1}. Freshwater media WC (Guillard and Lorenzen, 1972) was used to culture the algae. In a pilot study, *C.erosa* was grown using the media outlined above at a dilution rate of 0.19 day^{-1}. However, at steady state, algal density was very high in these continuous cultures and therefore, we reduced the amount of nitrate ($NaNO_3$) and phosphate (K_2HPO_4) to 10% of the nominal Freshwater media concentration. This was done to minimize self-shading of the algal cells in the cultures from the UVR. The concentration of phosphorus, the limiting nutrient [c.f. (Redfield, 1958)], was 0.87 mg l^{-1}. Photosynthetically-active radiation (PAR) was supplied by four white fluorescent Durotest® bulbs. PAR was determined to be ~70 µE m^{-2} s^{-1} (inside the continuous cultures when filled with algae) using the Optronics cosine sensor. The light:dark PAR regime was 14 h:10 h. Algae were inoculated into 12 continuous cultures and allowed to reach steady state (this required about four turnovers of the culture, based on the dilution rate).

Continuous culture experiment

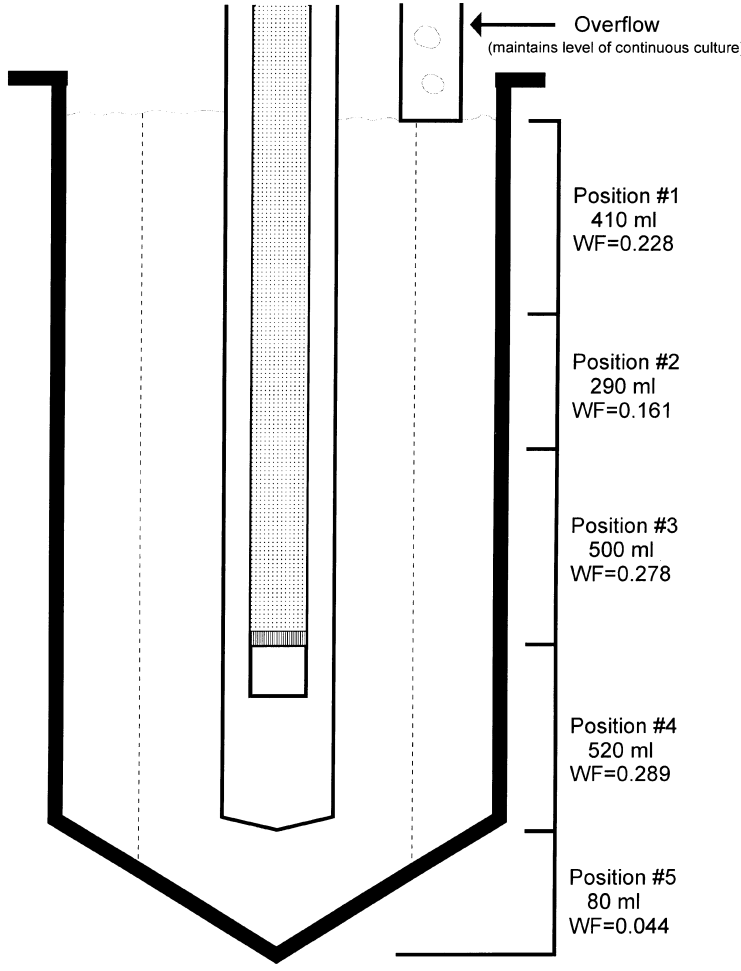
Three UV treatments were applied in this experiment (Table I, Figure 1). Treatment 1 (low) represents the average UVR irradiance found at 10 cm depth in prairie wetlands sampled at the St Denis sites. Treatment 2 (high) mimics the subsurface depth (0–3 cm) and treatment 3 represents a 14% UV enhanced

subsurface level. Three replicate continuous cultures were used for each treatment and the control (no UV exposure treatment). Treated algal cells were exposed to UVR for 8 h day^{-1} (between 09:00 and 17:00 h). We obtained these flux rates by placing a UV bulb (The Southern New England Ultraviolet Company, Branford, CT, USA, Model RPR #3500) inside a central quartz sleeve within each continuous culture. With this design, algae could be irradiated with UV light from within the continuous culture and hence, the UV exposure will be more uniform than with exterior-mounted UV lamps. Using several different weighting functions, we calculated biologically-weighted irradiances, primarily for the purpose of comparing our irradiances with those used in other studies (Table I). These include: an action spectrum for inhibition of electron transport in isolated chloroplasts (Jones and Kok, 1966); a generalized action spectrum for plants (Caldwell, 1971); an action spectrum for photosynthesis in a marine diatom, *Phaeodactylum* sp., and a marine dinoflagellate, *Prorocentrum micans* (Cullen *et al*., 1992); and the DNA action spectrum (Setlow, 1974), all normalized to 1 at 300 nm. PAR was supplied from 06:00 to 18:00 h.

Replicate irradiance measurements were made by placing the Optronics cosine sensor at five vertical positions along the midpoint between the quartz sleeve and the outer wall of the culture vessel to thoroughly characterize the UVR exposure (Figure 2). The volume at each vertical position (zones) was measured in order to calculate a weighting factor for that zone as a proportion of the total culture volume. This volume-weighting factor was then multiplied by the average irradiance for each zone to determine the weighted irradiance. The total irradiance was achieved by summing the weighted irradiance for each of the five zones. The three UV treatments for this experiment are shown in a table at the bottom of Figure 2; this table outlines the percentage of Mylar-D or aluminum foil wrapped around the bulb in positions 1, 2 or 3 to reduce the amount of UVR in order to achieve the particular treatment.

This experiment was conducted at 16 ± 1°C to approximate summer temperatures in shallow prairie wetlands, which were previously determined to be 20.5 ± 3.0°C (n = 154) for daytime measurements taken in 1995 from June through August (Environment Canada, unpublished data). However, the UV lamps unavoidably raised the temperature of the cultures by about 8°C over the course of a daily UV exposure period, such that the cultures reached a maximum daytime temperature of 24°C. Therefore, we placed a submersible aquarium heater (Hagen, Model ART.A-718 LR 52272) inside the quartz sleeve of each control (no UVR lamp) culture vessel in order to simultaneously raise the temperature by the same 8°C during the 8 h UVR exposure period.

Sampling was initiated from all 12 continuous cultures for the pre-exposure period (day –1 and day 0). UV exposure began on day 1 and continued until day 21. Samples were also collected throughout a 7 day recovery period (no UV exposure) and the experiment was completed on day 28. On selected days, samples were collected prior to PAR irradiation (05:45 h, during the dark respiratory period), before daily UV exposure (08:45 h) and just after the UV bulbs were shut off but the PAR bulbs were still on (18:05 h). On each occasion, 60 ml of algae were collected from the 12 continuous cultures for replicate samples of algal



Position	Dose 1	Dose 2	Dose 3
#1	full foil coverage	full Mylar, 50% foil	full Mylar, 50% foil
#2	full Mylar, 50% foil	60% Mylar, 50% of it foil	25% Mylar, 50% of it foil
#3	82% foil coverage	full bulb irradiance	full bulb irradiance
#4	full bulb irradiance	full bulb irradiance	full bulb irradiance
#5	full bulb irradiance	full bulb irradiance	full bulbirradiance

Fig. 2. Position of cosine meter used to determine UV treatments; irradiance × weighting factors (WF) for each position were summed for total irradiance values; volume measurements indicate the culture volume for each particular position (1–5); and WF = the proportion of culture relative to the total culture volume of 1800 ml.

and bacterial cell counts, chlorophyll *a*, carbohydrate and protein content, and percentage lipid. Because there were no large diurnal changes, and, for ease of comparison, we present only the post-UV measurement results.

Laboratory procedures and analyses

The relationship between *C.erosa* cell numbers (using a haemocytometer) and absorbance at 750 nm (optical density using a Milton Roy spectrophotometer Model = Spectronic 301) was determined to be: cell number*10^4 = 335.36 ($Abs_{750\ nm}$) – 4.6 (r^2 = 0.83). This equation was used to estimate cell numbers (density) at each sampling interval.

For chlorophyll *a*, carbohydrate and protein samples, 1.0 ml of algae with two replicates per culture vessel was filtered onto pre-combusted Whatman GF/C filters and stored at –40°C until analysis. The pore size of the GF/C filters (1.2 µm) would have allowed most bacteria to pass. However, we did not quantitatively determine the amount of bacterial contamination adhering to the GF/C filters. A Turner Designs (Model 10-AU) fluorometer was used to measure chlorophyll *a* concentration (Waiser and Robarts, 1995). We used the methods of Pick (Pick, 1987) and Peterson (Peterson, 1977) for carbohydrate and protein analyses, respectively. Protein concentrations were determined using a protein assay kit (P 5656) obtained from SIGMA Diagnostics® (St Louis, MO, USA).

For lipid analysis, 20 ml of algae were centrifuged in 60 ml plastic Falcon tubes in a Jouan centrifuge (Model C312) at ~1000 rpm for 5 min. This speed would not have been sufficient to pelletize the bacteria in the culture (S.Kaminskyj, University of Saskatchewan & G.Swerhone, NWRI, personal communication). The pellet was transferred to a 6 × 50 mm Kimble disposable borosilicate-glass culture tube and placed in a freeze-drier for 48 h. The freeze-dried material was sealed in glass tubes, purged with nitrogen gas and stored at –75°C until analysis. Total lipid (% dry wt) was measured using the micro-gravimetric technique (Gardner *et al*., 1985).

Bacteria were preserved by adding 30 µl Lugol's solution to 3.0 ml culture. Samples were placed in 5 ml Becton Dickinson sterile Vacutainer® tubes and stored at 4°C until analysis. We followed the method of Porter and Feig (Porter and Feig, 1980) to determine bacterial density. Samples were stained with DAPI, filtered through black Poretics® polycarbonate membrane filters (0.2 µm pore-size) and counted under epifluorescent light using an Ortholux II microscope.

Statistical analyses

Analyses were conducted using SigmaStat 2.0 (SPSS Inc., 1992). One-way ANOVAs were performed for algal and bacterial cell counts, chlorophyll *a*, carbohydrate and protein content, and percentage lipid, on all days for which samples were collected, to distinguish significant differences among treatments (control, treatment 1, treatment 2 and treatment 3). Similarly, two-way ANOVAs (treatment and day) were conducted to compare differences between the control and UV treatments on selected days throughout the experiment. Multiple two-way ANOVAs were carried out especially to compare results on days 0, 21 and

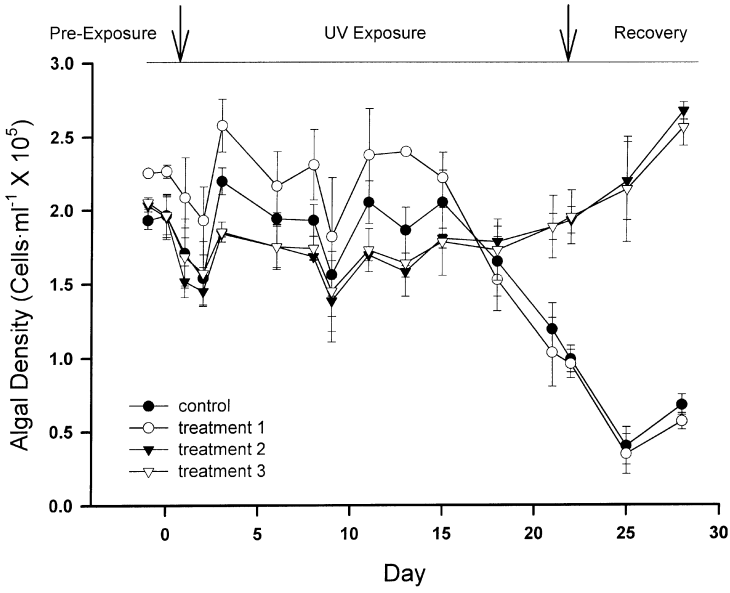


Fig. 3. Algal density (±S.E.) of *C.erosa* during the continuous culture experiment. Solid circle = control (no UV-R); open circle = treatment 1 (3.4 W m^{-2} UV-R unweighted); solid triangle = treatment 2 (14.9 W m^{-2} UV-R unweighted); open triangle = treatment 3 (16.2 W m^{-2} UV-R unweighted). Post-UV measurement.

28, and also to determine at which point during the study significant changes had occurred. For the two-way ANOVAs, in order to maintain a conservative error rate, a Bonferroni-corrected α of 0.05/(number of days) was employed for the *posteriori* comparisons. A Tukey test was performed for one-way ANOVAs to isolate which group(s) differed from the others if there was a significant difference ($P \leq 0.05$, or lower for corrected α) for the main effects.

Results

Algal density and chlorophyll a

For the first 15 days, algal density in the controls was slightly higher, although not significantly ($P = 0.089$), than in the two highest UV treatments (Figure 3). On day 18 there was no significant difference ($P = 0.797$) in algal density; by day 22, there was a significant difference ($P \leq 0.001$) between the control and treatment 1 compared with treatments 2 and 3, whereby the highest UV-treated cultures had substantially greater cell numbers than the control. This pattern continued during the recovery phase (day 28, $P \leq 0.001$). Algal density, for the control and treatment 1 was significantly lower on days 21 ($P \leq 0.001$) and 28 ($P \leq 0.001$) than at the beginning of the experiment (day –1). In contrast, there was no significant difference in algal density for treatments 2 and 3 ($P = 0.060$ and $P = 0.089$, respectively) on day 21 compared with the pre-exposure period (day 0), although they were significantly higher ($P \leq 0.001$) at the end of the recovery period.

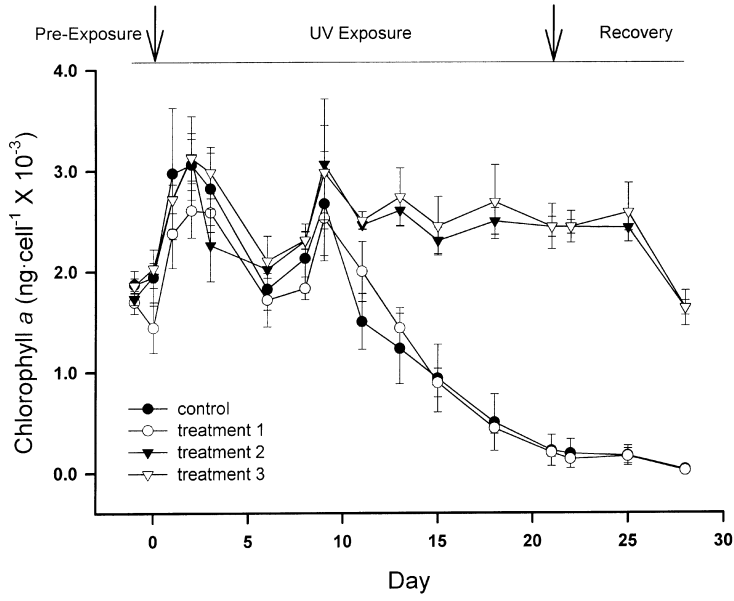


Fig. 4. Chlorophyll *a* content (±S.E.) of *C.erosa* during the continuous culture experiment (post-UV measurement); symbols as in Figure 3.

On day 0, there was no significant difference (P = 0.216) in chlorophyll *a* content (ng algal cell^{-1}) between the control and any UV treatment (Figure 4). There was still no significant difference (P = 0.868) in chlorophyll *a* content between the control and any UV treatment on day 9. However, on day 13 there was a significant difference (P = 0.030) between the control and treatments 2 and 3, whereby the two highest treatments had a higher chlorophyll *a* content than the control. This difference later became even more pronounced, with a 94% decrease in chlorophyll *a* content for the control and treatment 1 compared with treatments 2 and 3 by the end of the UV exposure (day 21). From day 21 onwards, the chlorophyll *a* content of both the control and treatment 1 was significantly lower ($P \leq 0.001$) than at the beginning of the experiment for those treatments.

Carbohydrate, protein and lipid

There was no significant difference (P = 0.618) in carbohydrate content (ng• algal cell^{-1}) in *C.erosa* between the control and UV treatments on days –1 and 0 (Figure 5). Carbohydrate content in treatments 2 and 3 was significantly higher ($P \leq 0.003$) than the control and treatment 1 at day 15. This difference remained throughout the rest of the UV exposure and the recovery phase. The control and all treatments had the lowest carbohydrate values on day 18. For treatments 2 and 3, there was no significant difference in carbohydrate content on day 0 compared with day 21 (P = 0.66). At the end of the recovery period, treatments

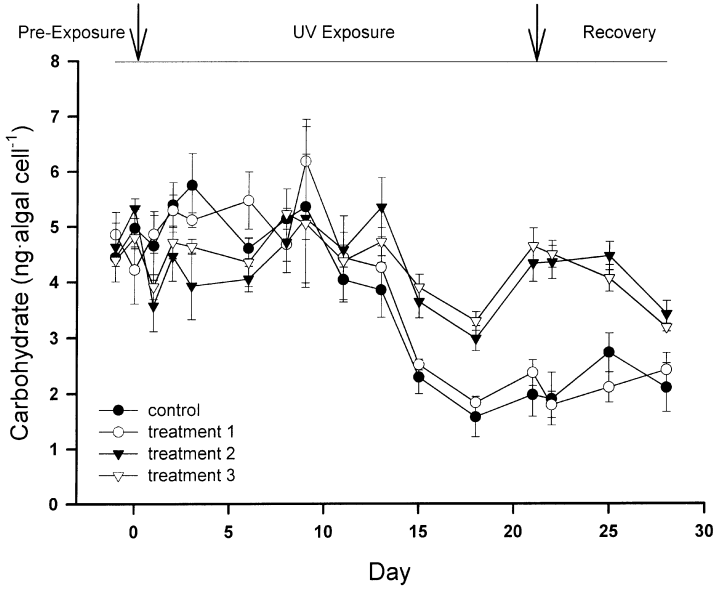


Fig. 5. Carbohydrate content (±S.E.) of *C.erosa* during the continuous culture experiment (post-UV measurement); symbols as in Figure 3.

2 and 3 had significantly lower ($P \leq 0.008$) carbohydrate content than at the beginning of the experiment, as did the control and treatment 1 ($P \leq 0.001$).

There was no significant difference in protein content (day –1 versus day 15, $P = 0.312$) between any of the treatments over the first two weeks of the UV exposure (Figure 6). These first two weeks showed large fluctuations in protein content. However, these were consistent for all treatments. On day 18, protein content in the control and treatment 1 had increased relative to treatments 2 and 3. At the end of the exposure period (day 21), protein content in the control and treatment 1 was significantly higher ($P \leq 0.037$) compared with treatments 2 and 3. The control and treatment 1 continued to increase until day 28, such that their final protein contents were significantly higher ($P \leq 0.001$) than the original contents on days –1 and 0. There was no change ($P = 0.115$) in protein content for treatments 2 and 3 on day 28 compared with the beginning of the experiment.

Lipid concentration (% dry wt) in *C.erosa* was comparable among all treatments until day 18 (Figure 7). On day 21, the control and treatment 1 had significantly lower ($P = 0.023$) lipid concentrations than treatments 2 and 3, and this trend continued to the end of the recovery phase (day 28, $P = 0.043$). For the control and treatment 1, there was very little algal material available from day 18 through day 28; and only one replicate was collected from those continuous cultures and the algae were pooled into a larger (40 ml) sample. Unfortunately, even this amount was insufficient to provide consistent values for the control and treatment 1 and as such, the standard error for these days was very high. The average percentage of lipid for the control and treatment 1 was not significantly lower ($P = 0.020$,

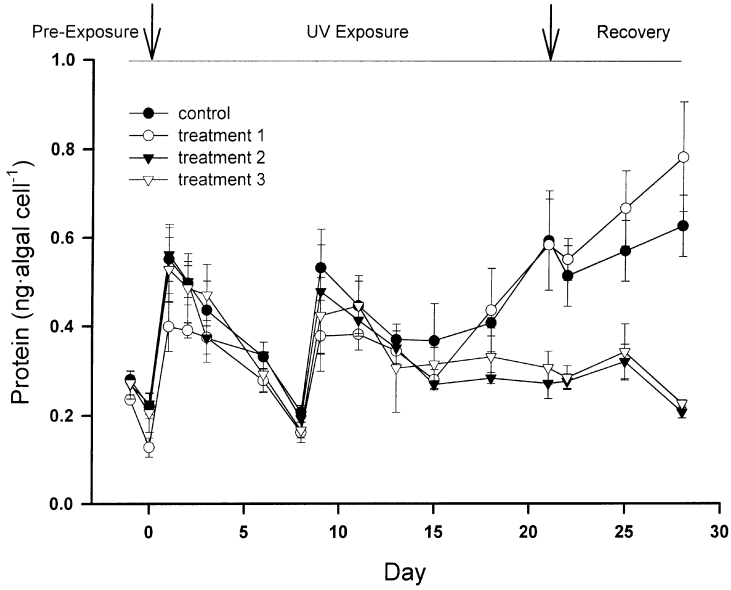


Fig. 6. Protein content (±S.E.) of *C.erosa* during the continuous culture experiment (post-UV measurement); symbols as in Figure 3.

$\alpha = 0.017$) on days 21 and 28 than on day 0, mainly due to the large amount of variation in the samples. The average percentage of lipid for treatments 2 and 3 on day 21, but not day 28, was significantly lower ($P = 0.010$, $\alpha = 0.017$) than on day 0.

Bacterial density

Bacterial densities for the control and all UV treatments are shown in Figure 8. There was no significant difference ($P = 0.427$) between the control and UV treatments on day 0 but on day 14, both the control and treatment 1 had a significantly higher ($P \leq 0.036$) bacterial density than either treatment 2 or 3. This higher bacterial density continued for the duration of the exposure period (day 21, $P \leq 0.001$) and recovery phase (day 28, $P \leq 0.001$). In contrast, for treatments 2 and 3, there was no significant difference in bacterial density on day 0 compared with days 21 and 28 ($P = 0.733$ and $P = 0.818$, respectively). The control ($\bar{x} = 7.8 \times 10^7$) and treatment 1 ($\bar{x} = 7.8 \times 10^7$) had bacterial densities nearly four times greater than treatments 2 ($\bar{x} = 2.0 \times 10^7$) or 3 ($\bar{x} = 2.48 \times 10^7$) by the end of the experiment. Unfortunately, no systematic measurements of bacterial cell size were obtained. However, no gross differences where observed using casual observation during the counting of the DAPI stained bacterial cells.

Discussion

Using an action spectrum for *Euglena gracilis*, which related the percentage of motile cells to exposure time at various monochromatic UV irradiances, Häder

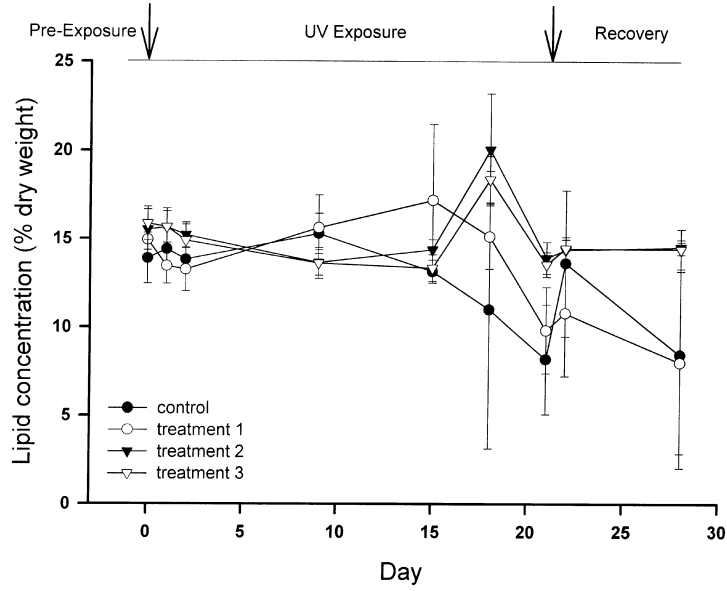


Fig. 7. Percentage of lipid (±S.E.) of dry weight in *C.erosa* during the continuous culture experiment (post-UV measurement); symbols as in Figure 3.

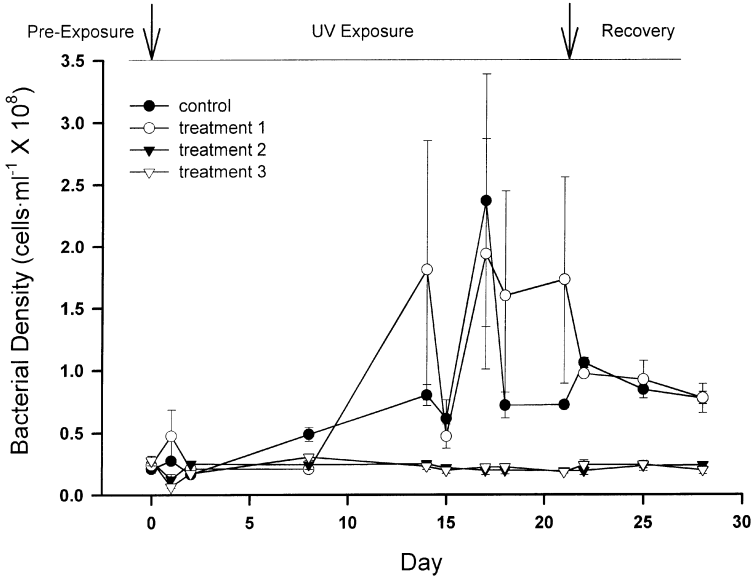


Fig. 8. Bacterial density (±S.E.) in continuous cultures during the long-term UV radiation exposure study (post-UV measurement); symbols as in Figure 3.

and Liu (Häder and Liu, 1990) demonstrated that an intrinsic component of the motility apparatus, flagellar protein, is the main targert of UVR for motility inhibition. In the second week of our experiment, we observed *C.erosa* under an Ortholux II light microscope at ×1250 and noticed a lack of motility (movement) in algal cells from the two highest UV treatments, despite the fact that algal density (Figure 3) did not decrease over the course of the experiment. Preserved samples, analyzed at a later date with scanning confocal laser microscopy, confirmed this initial observation. In addition, a change in shape (becoming more spherical) was observed for *C.erosa* exposed to the two highest UV treatments. Although qualitative, our observations on the loss of flagella and changes in cell shape in *C.erosa* are consistent with studies on other algae (Häder, 1986; Häder and Häder, 1990a, b; Häder and Liu, 1990; Donkor and Häder, 1991; Gerber and Häder, 1995a).

In order to protect themselves from UVR, phytoplankton can increase or modify their pigmentation or enhance their repair mechanisms (Calkins and Thordardottir, 1980). Some species can also avoid UV exposure by downward vertical migration, although this may reduce their ability to harvest light for photosynthesis (Häder, 1986; Worrest and Häder, 1989), and in very shallow prairie wetlands (<30 cm depth in some ponds at St Denis), this may not be an available option. Thus, loss of motility could have significant effects for algae if the cells cannot orient themselves properly in the water column to avoid excess UV exposure, or become trapped at lower depths and cannot swim back to the surface for sufficient levels of PAR irradiance for photosynthesis. In turn, the diminution of susceptible species (e.g. through a loss of flagella) could promote a change in algal community structure with possible ramifications for the rest of the aquatic food web (Worrest and Häder, 1989).

Chlorophyll a

Many studies have demonstrated UV effects on photosynthetic pigments (Häder and Häder, 1990b; Donkor and Häder, 1991; Döhler and Haas, 1995; Döhler and Lohmann, 1995). A few studies have specifically examined the response of pigments to UVR in the genus *Cryptomonas* (Häder and Häder, 1990a; Zündorf and Häder, 1991, Gerber and Häder, 1995b). These studies indicate that if UVR is to have an effect on the pigments in phytoplankton, the accessory pigments are likely to be damaged first, and chlorophyll *a* will remain less affected for a longer period. In this study, the applied UVR fluence rates were insufficient to cause a decline in chlorophyll *a* content in the two highest UV treatments (Figure 4). However, due to the absence of a true control for the latter part of the exposure period, this comparison can only be for the first two weeks (i.e. during the period when bacterial numbers were more similar amongst the treatments). The unweighted UV-B fluence rates in the studies on cryptomonads referenced above were nearly an order of magnitude higher than those used in this study, and may explain why we did not observe a gross change in chlorophyll *a* content. If the two highest UV treatments were insufficient to cause a marked decrease in chlorophyll *a*, then one might expect *a priori* that the chlorophyll *a* content for

the lowest UV treatment would be more similar to the control. This, however, was not the case. The chlorophyll *a* content in the control and lowest UV treatment cultures decreased significantly over the course of the experiment. The prolific growth of bacteria in the control and treatment 1 likely reflected their ability to out-compete *C.erosa* for the culture's limiting nutrient. In these cultures, *C.erosa* cells declined and became stressed (as suggested by their shrivelled appearance) and this may have led to the reduced chlorophyll *a* contents we observed.

Carbohydrate

In this experiment, there was no longer a true control after the second week due to the proliferation of bacterial growth. It should be noted, however, that relative to the beginning of the experiment, for treatments 2 and 3, the carbohydrate content declined during the third week of UV exposure (Figure 5). This result was similar to that observed by Plante and Arts (Plante and Arts, 1998) when they exposed *C.erosa* for 15 days to a UVR irradiance of 11.26 W m^{-2} (unweighted) prior to a 4 h incubation in a Phototron at three different UV fluence rates. In that experiment, UV-conditioned algae demonstrated a slight depression in P_{max}-Total and the main macromolecular components, including polysaccharides, as a consequence of UV exposure history (Plante and Arts, 1998).

Arts and Rai (Arts and Rai, 1997) suggested that exposing algae to UV-B has the potential to produce subtle, species-specific effects on the relative allocation of recently fixed carbon to lipid, polysaccharide, protein and low molecular weight compounds. Even at very low fluence rates, they found negative effects of UV-B radiation on the production of photosynthates that were both dose- and exposure duration-dependent. For *Cryptomonas* sp., effects on the overall photosynthetic rate were observed at relatively low UV-B irradiances [e.g. 0.33 W m^{-2} weighted (Jones and Kok, 1966), normalized at 300 nm] after a 4 h incubation period in a Phototron. In this experiment, the two highest fluence rates were 0.195 and 0.226 W m^{-2} [weighted (Jones and Kok, 1966), normalized at 300 nm], similar to the lowest irradiance used by Arts and Rai (Arts and Rai, 1997) in their experiment (0.18 W m^{-2}, weighted), but substantially lower than the irradiances which caused the greatest effects (0.47 W m^{-2}, weighted).

Protein

Döhler observed significant reductions in protein content in five marine diatoms after a 2-day exposure to UV-B radiation (1230 J m^{-2} day^{-1}) (Döhler, 1995). Plante and Arts showed that there was no significant difference between the overall photosynthesis-irradiance curves for *C.erosa* in unconditioned (no previous UV exposure) and conditioned (15 day UV exposure) treatments (Plante and Arts, 1998). However, as a percentage of total uptake, carbon allocation to protein for unconditioned and conditioned algae decreased by 24 and 4%, respectively, for the two highest UV treatments relative to the controls. Here, negative effects due to UV exposure on protein content compared with the

beginning of the study could not be distinguished. Levels of UV exposure in the Plante and Arts (Plante and Arts, 1998) study were comparable with this study and thus, we suggest that the increase in per cell protein content for *C.erosa* in the control and treatment 1 (Figure 6) occurred as a result of some effect which the high numbers of bacteria had on the algae.

Lipid

The mean lipid fraction of carbon fixation (LFCF) of phytoplankton from a number of freshwater lakes ranged from 15.8 to 24.7% (Shifrin and Chisholm, 1981; Fahnenstiel *et al*., 1989; Wainman and Lean, 1992; Wainman *et al*., 1993). These values approximate the average percentage of lipid of *C.erosa* on day 0 for the control and all UV treatments (15.1%, Figure 7). The declining trend in lipid content of *C.erosa* for treatments 2 and 3 at the end of the experiment was consistent with the findings from other studies, in which a decrease in lipid content was observed due to UV exposure (Arts and Rai, 1997; Plante and Arts, 1998).

Bacterial density

As the continuous cultures initially contained the same bacterial numbers, we suggest that the increase in bacteria in the control and lowest UV treatment was due to their ability to out-compete *C.erosa* for the limiting nutrient, phosphorus, while the suppression of bacteria in the higher UV treatment cultures was primarily due to the UV exposure effect. Treatment 1 and, to a lesser degree, the control cultures, demonstrated a great deal of variance throughout weeks 2 and 3 of the experiment (Figure 8). Biofilm growth along the inner walls of the culture chambers, media inflow, sample port and outflow glass tubes likely contributed to the observed variance in bacterial numbers if, for example, parts of the biofilm sloughed off during sample collection. In both the control and treatment 1, a substantial biofilm growth, not evident in the two highest UV treatments, was observed. The appearance of biofilms during the latter part of the experiment would have heightened competition for phosphorus.

Brussaard and Riegman investigated the effects of bacteria on the mortality of the diatom, *Ditylum brightwellii*, when phosphorus or nitrogen was limiting algal growth (Brussaard and Riegman, 1998). Death rates of the diatom increased after bacterial inoculation when phosphorus was limiting algal growth, likely due to competition for the limiting phosphorus. In contrast, the presence of bacteria had either no pronounced effect, or caused a reduction in the specific death rates of *D.brightwellii*, when nitrogen was the limiting nutrient. It was suggested that remineralized ammonium from the bacteria was utilized by the N-starved algal cells, thereby reducing the cells' death rates (Brussaard and Riegman, 1998). In this study, the medium N:P ratio was 20:1. According to Redfield (Redfield, 1958), there was sufficient nitrogen in the system and therefore, the limiting nutrient in the medium was phosphorus. Given the bacterial growth in our cultures, competition for the limiting nutrient, phosphorus, increased algal cell mortality compared with bacteria in the control and lowest UV treatment continuous

cultures. In the highest UV treatment, we suggest that the effect of UVR on the bacteria was greater than the competition effect for phosphorus, so that the algal populations were not as affected by the bacteria. Brussaard and Riegman noted that the degree of bacterial influence on algal death kinetics depends mainly on the nutrient limiting algal growth and on culture conditions (Brussaard and Riegman, 1998). Here, our UV treatments (culture conditions) clearly had a dramatic effect on the bacteria that were competing with *C.erosa*.

Ferreyra *et al*. studied the physiological responses of natural plankton communities to UV-B radiation in Redberry Lake, Saskatchewan (Ferreyra *et al*., 1997). They measured primary productivity, chlorophyll *a*, phytoplankton size, bacterial density and the electron transport functioning under natural UV-B fluxes at the lake's surface. For both phytoplankton carbon assimilation and chlorophyll *a*, no obvious effects of UV-B were discovered, due perhaps to the synthesis of UV-B-absorbing substances, carotenoids and enzymatic antioxidants, and/or, possibly, the high concentrations of DOC in Redberry Lake (Ferreyra *et al*., 1997). They did, however, find a clear reduction in bacterial cell numbers under the highest UV-B irradiance levels, which suggests a high sensitivity of Redberry Lake bacteria to UV-B exposure (Ferreyra *et al.*, 1997). Ferguson and Palumbo (Ferguson and Palumbo, 1979) and Thomson *et al*. (Thomson *et al*., 1980) described similar sensitivity to UV radiation for bacteria from neritic and estuarine waters, respectively.

Relative proportion of algal to bacterial biomass

In order to determine the relative proportion of algal to bacterial biomass, estimates of both algal carbon (in pg C · cell^{-1}) and bacterial carbon (in fg · cell^{-1}) were used. A value of 5.4 pg C · cell^{-1} was used as an estimate of algal carbon content, which was determined in the marine diatom, *Thalassiosira pseudonana*, grown in P-limited continuous cultures at a dilution rate of 0.42 day^{-1} [data from (Perry, 1976), presented in (Darley, 1982)]. A value of 24.1 fg C · cell^{-1} was calculated from data in the study by Simon and Azam (Simon and Azam, 1989), where the average carbon content was determined from marine bacteria ranging in size from 0.02 to 0.40 μm^3.

The algal to bacterial biomass ratio on day 0 was 1.8:1, so *C.erosa* accounted for nearly twice as much 'living' carbon in the culture system as bacteria. However, in the control and treatment 1 cultures on day 15, the ratio was 0.8:1. At the end of the UV exposure, on day 21, for the control and treatment 1, there was a significant increase in bacterial density compared with the beginning of the experiment, and the algal to bacterial biomass reversed, with the ratio being 0.20:1. At this point, bacteria were contributing more carbon to the system than *C.erosa*. This trend continued until the end of the recovery period, on day 28, where the algal to bacterial biomass ratio was then 0.18:1. For the two highest UV treatments, there was no dramatic change in algal to bacterial biomass; on days 15, 21 and 28, the relative proportions of algal to bacterial biomass were 1.95:1, 2.25:1 and 2.72:1, respectively. This increase was due to a rise in algal density, with no significant change in bacterial density over the course of the experiment.

If bacteria were retained on the GF/C filters, they might have made a contribution to the carbohydrate and protein measurements but would not have affected the chlorophyll *a* results. With an increase in bacterial numbers in the controls and treatment 1 over the last 2 weeks of the experiment, there was a corresponding decrease in carbohydrate content and an increase in protein content; these effects may have been an artifact due to high bacterial numbers (with high protein and lower carbohydrate content) which were analysed with the algae. To verify whether bacterial contamination on the GF/C filters had occurred in sufficient amounts to significantly alter the results, we would have had to determine the relative abundance of bacteria versus algae that the filter retained by counting the bacteria that pass through a filter compared with those that were retained. Unfortunately, these comparisons were not made during our experiment. However, we suggest that even with the significant change in algal to bacterial biomass ratio for the control and treatment 1, samples analysed for chlorophyll *a*, protein and carbohydrate, and lipid, reflected the algal composition and not the bacteria, due to the relatively coarse filters used and the slow centrifugation speed. The switch in the relative proportion of carbon biomass is, however, very important in terms of the bacterial–phytoplankton interactions that occurred within these cultures.

Bacteria–phytoplankton interactions

Bacterial uptake of inorganic phosphorus and nitrogen places bacteria and phytoplankton in competition for growth-limiting nutrients, rather than their traditionally perceived roles as the receptive 'sources' and 'sinks' for these nutrients (Caron, 1994). Bacteria were more successful at competing for phosphorus in this experiment because of their higher surface area-to-volume ratios and higher affinities for phosphorus (Currie and Kalff, 1984). Also, the concentration of phosphorus was reduced to 0.87 mg l^{-1} (10% of the amount in the standard FW media), and this created a situation conducive for competition between bacteria and *C.erosa*.

Thingstad *et al*. proposed a mechanism by which bacterial carbon consumption is restricted by food web dynamics controlling both growth and biomass, such that the phytoplankton growth rate is kept low due to bacteria–phytoplankton competition for mineral nutrients, and bacterial biomass is kept low by bacterial predators (Thingstad *et al*., 1997). Bacterial predators were, however, absent in our continuous culture systems. Pace *et al*. indicate that the fate of planktonic bacterial production depends on the planktonic community structure, which also suggests that the lack of a bacterial grazer could have offset the bacteria–phytoplankton interactions in our culture systems (Pace *et al*., 1990). These findings are also supported by Weisse and Scheffel-Moeser who showed that bacterial production was balanced by grazing protozoans (Weisse and Scheffel-Moeser, 1991), and that the removal of heterotrophic nanoflagellates led to a pronounced bacterial peak.

Bacteria can have a direct effect on the size, biomass and species composition of the phytoplankton community (Kirchman, 1994). In this experiment, UVR

played a significant role in determining the overall algal and bacterial biomass in the continuous cultures, thereby influencing the competitive relationship between *C.erosa* and bacteria for phosphorus.

UV radiation and the microbial loop

More detailed investigation is required in order to begin to understand the effects of UVR on the microbial loop and on the coupling between carbon and mineral nutrients in prairie wetlands [see (Azam *et al*., 1983)]. The levels of UVR used in our study did not appear to greatly alter carbon allocation in *C.erosa*, perhaps due to adaptation. However, this study suggests a competitive advantage of phytoplankton over bacteria in the face of UVR stress, such that bacteria may be more influenced by increased levels of UVR than algae.

If UVR causes bacterial numbers to decline relative to phytoplankton, there may be changes in (i) the utilization of dissolved organic matter by bacteria produced by phytoplankton, (ii) competition between phytoplankton and bacteria for nutrient minerals and (iii) predation rates by micro-flagellates on bacteria. The direct effect of UVR on the growth and metabolism of microbial communities in prairie wetlands has not been measured (Robarts and Waiser, 1998), and such studies will be integral to gaining an understanding of the potential impacts and implications of UVR on prairie wetland ecosystems.

In addition to UVR, phytoplankton may be simultaneously exposed to many natural and/or anthropogenic stressors. On the prairies, lakes and sloughs surrounded by agricultural land are often exposed to increased loadings of nutrients, pesticides and herbicides. Herbicides, for example, can directly inhibit algal growth, and the standing crop of phytoplankton is generally lowered (deNoyelles *et al*., 1982). In herbicide-affected communities, the species and/or genetic composition may also change from susceptible to tolerant species (deNoyelles *et al*., 1982). Experiments investigating the interactions between UVR and anthropogenic or natural stressors are quite rare [e.g. salinity (Döhler, 1984); copper (Rai *et al*., 1995); herbicide (Kasai and Arts, 1998)]. Thus, future research efforts are encouraged to conduct multi-species, multi-level and multi-stressor experiments so that important interactions can be identified and understood in relation to the effects of UVR on aquatic organisms.

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