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Photosynthate production in laboratory cultures (UV conditioned and unconditioned) of *Cryptomonas erosa* under simulated doses of UV radiation

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Abstract

We used a device called a Phototron to measure the effects of UV radiation on the cosmopolitan algae, *Cryptomonas erosa*, grown in continuous cultures. In the Phototron, we investigated changes in photosynthetic parameters (P_{max} – specific production rate at optimal light intensity; α – initial slope of the linear portion of the Photosynthesis-Irradiance curve; and θ – the convexity or rate of bending) and carbon allocation as a function of irradiance at three different environmentally-realistic doses of UV radiation in unconditioned (no prior UV exposure) and conditioned algae (15 d previous UV exposure). For unconditioned control algae, P_{max}-Total was lower, although not significantly, than the two highest UV treatments. For conditioned control algae, P_{max}-Total was higher, although not significantly, than all UV treatments. Our data suggest that short term (4 h) exposure to low levels of UV (8.09 W m^{-2} unweighted) does not affect P_{max}-Total in *C. erosa*, but does change the proportion of carbon allocated to lipids and proteins. Also, comparisons of lipids, polysaccharides and proteins as a percent of total carbon uptake between unconditioned and conditioned algae indicate that exposure history to UV radiation can have a negative impact on carbon allocation to lipids and proteins, in a wetland alga species that is crucial to the efficient transfer of energy through freshwater food webs.

Abbreviations: UV-B – 280–320 nm; UV-A – 320–400 nm, or UV radiation – 280–400 nm.

Introduction

Enhanced UV-B radiation (280–320 nm) from ozone depletion has become a major environmental concern (Madronich et al., 1995; Häder et al., 1995). Wetlands are a vital resource which may be affected by increased UV-B radiation due to a decline in the stratospheric ozone. Phytoplankton, at the base of aquatic food webs, in shallow lakes, rivers and wetlands, may be particularly vulnerable to increased UV-B levels (Häder et al., 1995; Smith & Cullen, 1995).

UV radiation impairs important physiological functions, such as photosynthesis, affects motility and orientation, and threatens aquatic organisms during their early life histories (Häder et al., 1989). In addition to causing DNA damage, UV radiation can impair enzymes and other proteins (Häder et al., 1989) as well as affect pigment composition (Döhler & Lohmann, 1995). UV radiation also affects various aspects of algal physiology, for example, the inhibition of inorganic nitrogen uptake (Braune & Döhler, 1994; Döhler, 1996) or the reduction of lipids and fatty acids (Döhler & Biermann, 1988; Goes et al., 1994; Wang & Chai, 1994).

Phytoplankton use solar radiation to fix inorganic carbon into the main cellular components of lipid, protein and carbohydrates. Light intensity (Cuhel & Lean, 1987), temperature (Thompson et al., 1992), pH (Guckert & Cooksey, 1990) and available nutrients

(Groeger & Kimmel, 1988) are the most important factors that determine the total amount of carbon fixed and how it is allocated to the major cellular pools. UV radiation has also been shown to affect total carbon uptake and carbon allocation in phytoplankton (Arts & Rai, 1997; Kasai & Arts, 1998).

Lipids, in particular, triglycerides and wax esters, are important energy reservoirs and a media for energy transfers through freshwater invertebrate food webs (Arts et al., 1992, 1993) such as those found in prairie wetlands. In addition, lipid composition is suggested to be an important factor in determining the nutritional quality of algae, specifically the levels of polyunsaturated fatty acids (PUFA) (Ahlgren et al., 1990). Of particular interest for food quality is the % of essential fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Ahlgren et al., 1992) which, compared to other algae, are found in high concentrations in *Cryptomonas* and *Rhodomonas* sp. and some diatoms (Ahlgren et al., 1990). Cryptophyceans contain large amounts of long chained PUFA, particularly EPA and DHA (Ahlgren et al., 1992). Ahlgren et al. (1990) indicated that the fatty acid composition of cryptomonads contributed to their characterization as an optimal food source for cladoceran zooplankters. Cryptophyte algae, often found in abundance in spring and fall, have been implicated as being crucial to lipid energy transfers to zooplankton in both field and laboratory studies (Chen & Folt, 1993; Hart & Santer, 1994; Santer & Van den Bosch, 1994).

Thus, if UV-B radiation affects the lipid levels of an alga such as *Cryptomonas*, which is known to contain high quality lipid reserves, there could be implications for the food quality and quantity available for herbivores. For example, if increased exposure to UV-B alters the allocation of carbon to the main cellular pools, there could be a decline in phytoplankton food quality (lipid energy stores or protein content) which could lead to declines in zooplankton abundance and diversity and ultimately effect organisms at higher levels in the food web. In our experiments, we were interested in observable changes in algal cellular components, such as lipids, polysaccharides and proteins, due to UV exposure.

Because organisms in nature may be exposed for long periods of time, and due to the limited number of chronic UV-B effects which have been measured (Behrenfeld et al., 1992), there is a need for more research into the chronic effects of UV radiation on algae. In our study, we maintained *Cryptomonas erosa* in an unconditioned (no UV exposure) and conditioned (15 d UV exposure) state in continuous cultures for a number of generations, prior to a short term (4 h) exposure in a temperature controlled light-gradient incubator known as the Phototron (Rai & Krambeck, 1992). We tested the null hypothesis that there is no effect of UV radiation on total carbon uptake nor on carbon allocation to the cell's main macromolecular components of lipid, protein, polysaccharide and low molecular weight compounds, in *C. erosa*, by measuring the acute effects of UV radiation on the Photosynthesis-Irradiance (P–I) curves of *C. erosa*. We investigated differences between UV exposure history for unconditioned and conditioned algae, as well as three UV exposure treatments compared to the controls. Our aim was to compare results on a short time scale for unconditioned algae to a longer time scale for the conditioned algae, in addition to noting responses due to differences in increased UV doses.

Materials and methods

Field investigation of light climate in wetlands at St. Denis

Natural solar spectral irradiance in air was determined using an Optronic Laboratories scanning spectroradiometer (OL-754, O-PMT). This instrument uses a double monochromator design and a temperature-controlled photomultiplier dectector. Before any series of measurements were taken, the instrument was calibrated (250–800 nm) against a NIST traceable 200 W tungsten-halogen standard lamp (OL752-10). We determined wavelength accuracy ($\pm$0.1 nm) and optical gainby using the OL752-159 Dual Calibration and Gain Check source Module. We used the Optronics submersible right angle teflon cosine receptor (Model OL-86-T-WP) spectroradiometer in the water at various sites at the St. Denis National Wildlife Refuge, Saskatchewan, to investigate solar irradiance (photosynthetically active radiation [PAR] and UV radiation) as a function of depth (Arts, unpublished data).

Algal cultures

C. erosa was maintained at 18 °C in the exponential growth phase in 2 L continuous cultures at a dilution rate of 0.19 d^{-1}. Freshwater media WC (Guillard & Lorenzen, 1972) was used to culture the algae. Phosphorus (in the form of K_2HPO_4), was the growth limiting factor in the continuous cultures. Photosynthetically active radiation (PAR=400–700 nm)

irradiance was supplied by four white fluorescent Durotest® bulbs on the outside of the continuous cultures. The light:dark photo regime was 12 h:12 h. PAR was ~70 μmol m^{-2} s^{-1} at the centre of each continuous culture with algae.

Pre-phototron conditions

Four continuous cultures were used to grow algae, at the above mentioned conditions. Once steady state was achieved, two continuous cultures were used for unconditioned algae and the other two for conditioned algae. Conditioned algae received the same PAR as unconditioned algae. Algae were grown for 16 d before the acute UV experiments in the Phototron. We refer to this as the pre-Phototron period. During this period, conditioned algae were exposed to an unweighted UV dose of 0.28 W m^{-2} UV-B and 10.98 W m^{-2} UV-A. These values correspond to biologically weighted doses of 0.06 W m^{-2} UV-B and 0.44 W m^{-2} UV-A or 0.05 W m^{-2} UV-B and 0.24 W m^{-2} UV-A for *Phaeodactylum* sp. and *Prorocentrum micans*, respectively (Cullen et al., 1992). UV irradiance inside the continuous cultures was measured using the Optronics submersible cosine receptor. Conditioned algal cells were exposed to UV radiation for 8 h d^{-1} (between 10:00 and 18:00) from a UV bulb (The Southern New England Ultraviolet Company, Branford, Connecticut, RPR 350 nm) placed inside a central quartz sleeve within each continuous culture. In this design, algae were irradiated with UV light from the centre of the continuous culture where the algae were evenly distributed by the continuous air supply and hence the UV radiation exposure should be more uniform than is the case with exterior-mounted UV lamps. Unconditioned algae did not receive any UV radiation during the pre-Phototron period.

During the pre-Phototron period, 60 ml samples of algae were collected from the four continuous cultures for analysis of chlorophyll *a*, carbohydrate, lipid and protein content. Samples were collected prior to PAR irradiation, before daily UV exposure and just after the UV lights shut off but PAR was still provided. These samples were collected to ensure that there were no significant diurnal changes in photosynthetic uptake (Plante, unpublished data), so that two Phototron experiments could be performed on the same day (morning and afternoon). No significant differences were found in the measured parameters and therefore those results are not presented here.

Phototron experiments

We used a device called a Phototron which is a temperature controlled light-gradient incubator (Rai & Krambeck, 1992). The Phototron at the National Water Research Institute (NWRI) has been modified from the original design in that it has 36 incubation ports (instead of 24), computer control over PAR and the capacity for up to 13 UV lamps to be suspended above the incubation chamber. For these experiments, 30 of the incubation ports were used and the remaining were sealed. Using both unconditioned and conditioned algae, we measured ^{14}C uptake and incorporation to major end-products of photosynthesis after a 4 h incubation period under 12 different visible light and 3 environmentally-realistic UV doses, at an incubation temperature of $\bar{x} = 20.9 \pm 2\,^{\circ}C$.

PAR (Philips, Model 6434 FR Halogen bulbs) was applied from below, while UV radiation was applied from above. Twenty-six incubation ports were used for light measurements and four additional ports were reserved for dark measurements. Each port held a glass incubation vessel of 60 ml capacity. The 12 different nominal PAR intensities used were 20, 40, 60, 80, 100, 120, 140, 160, 200, 400, 600 and 900 μmol m^{-2} s^{-1}. The various PAR levels were attained by keeping the voltage constant at 11.5 V and using perforated metal sieves with different mesh sizes and/or neutral density filters (Schott NG 4, 5 or 11) and/or squares of Mylar-D placed in a tray located 3 cm above the PAR lights. PAR values were determined with a quantum scalar meter (Biospherical Model QSL-100) which could be placed in the centre of the cell suspension in each incubation vessel. The dark incubation chambers consisted of incubation bottles sealed with black tape.

UV radiation was generated from eight UV-A tubes (Q-Panel® UV340), held in a scaffolding above the Phototron. These Q-Panel bulbs have been recommended by Björn et al. (1996) for use as UV sources for algal photobiology experiments. The intensity of UV radiation was controlled by raising or lowering the UV tubes above the aluminum incubation chamber block via a pulley to decrease or increase the amount of UV irradiance, respectively and/or through the use of squares of Mylar-D film. Schott WG305 filters eliminated UV-C in all situations. Schott WG305 filters have a 50% cutoff at 305 nm. Mylar-D film has a 70% cutoff at 320 nm.

Of the UV doses applied in this experiment, Dose 3 (high) represents the average UV irradiance found at the subsurface (0–3 cm depth) of the 16 high DOC

prairie wetlands sampled at St. Denis. Dose 2 (low) and Dose 1 (very low) represent the average UV levels found at roughly 5 cm and 10 cm depths, respectively. For Dose 3, the bulbs were lowered 80 cm from the top of the Phototron and other than the Schott WG305 filters, no other filters were used. For Dose 2, bulbs were lowered 80 cm and a 5 cm $\times$ 5 cm square of Mylar-D film with 9 holes ($\sim$7 mm in diameter each) was placed on top of the Schott WG305 filter to reduce the UV-B dose. For Dose 1, bulbs were lowered 40 cm from the top and a square of Mylar-D film with 2 perforations ($\sim$7 mm in diameter each) was used. UV doses in the Phototron were measured with the Optronics cosine meter. A glass incubation chamber filled with 40 ml of algal cells with a cell density of $\sim 4.1 \times 10^4$ cells ml^{-1} was temporarily mounted over the sensor so as to mimic the optical geometry of the incubation chambers to which algae in the Phototron would be exposed.

In order to investigate the algal responses to UV radiation amongst the three different UV doses and control for both conditioned and unconditioned algae, it was necessary to conduct two experiments on one day (96/12/16) and two experiments the following day (96/12/17) for a total of four trials. In the morning (09:00 h), the UV-conditioned algae from one continuous culture were used in the Phototron to develop the P–I curve for control algae and algae exposed to Dose 1. In the afternoon (15:00 h), the second continuous culture of conditioned algae was used to expose algae to Dose 2 and Dose 3 levels of UV, over the range of 12 different PAR irradiances. Similarly on the next day, identical experiments were conducted but this time the two continuous cultures with unconditioned algae (no previous UV exposure) were used.

For each trial, before ^{14}C was added to the culture algae, two 10 ml aliquots of algae were filtered through GF/F filters (precombusted) for chlorophyll *a* analysis. Filters were stored at $-40\,^{\circ}$C until extraction in boiling 90% ethanol. A Turner Designs (Model 10-AU) fluorometer was used to measure the concentration of chlorophyll *a* as in Waiser and Robarts (1995). Chlorophyll concentration is a commonly used method for standardizing photosynthetic rates (Leverenz et al., 1990; Rai, 1995; Kasai & Arts, 1998; Arts & Rai, 1997). Although chlorophyll is a variable that may have been changed by the conditioning treatment, we used separate measurements for the unconditioned and conditioned algae, in order to standardize the results according to the pre-Phototron conditions. The average chlorophyll *a* concentration used in the chlorophyll specific uptake rate calculations was 274.2 μg L^{-1} and 243.2 μg L^{-1} for conditioned and unconditioned algae, respectively. In addition, a 60 ml sample of algae was collected and filtered for dissolved inorganic carbon (DIC) analysis which was estimated from total alkalinity measured by titration as in Robarts et al. (1992). The average DIC concentration used in uptake rate calculations for conditioned and unconditioned algae was 13.0 mg L^{-1}. Next, a 30 ml aliquot of unspiked algae was added to one dark (no PAR) and one light (PAR of 100 μmol m^{-2} s^{-1}) culture chamber for each of the four trials over the two day experiment, as a comparison to the beginning of the experiment. These were referred to as 'cold' samples.

For the incubation, algal cultures were spiked with 109 μCi NaH^{14}CO$_3$ to achieve a specific activity of 1.5×10^5 DPM ml^{-1}. After mixing, two 1 ml aliquots were added to scintillation vials to confirm the concentration of ^{14}C added to the culture. A cell density of $\sim 4.1 \times 10^4$ cells ml^{-1} was used in all four trials. Forty ml aliquots were then rapidly dispensed using a Brinkmann Dispensette® into 26 of the glass incubation chambers in the Phototron (12 light chambers and one dark chamber for the control [or Dose 2] and 12 light chambers and one dark chamber for Dose 1 [or Dose 3]). These were referred to as 'hot' samples. The entire Phototron was mounted on an Orbital shaker table (Lab-Line Instruments Inc.). The shaker table prevented the cells from settling to the bottom and ensured that the UV radiation field passing through the perforated sieves was even.

At the end of the 4 h incubation, two 5 ml aliquots were collected for the light and dark unspiked algae from each of the four trials for chlorophyll *a* analysis. These were filtered onto precombusted Whatman GF/F filters and stored at $-40\,^{\circ}$C until analysis. From all the chambers containing spiked algae, one 5 ml and two 10 ml samples were collected to measure total uptake and ^{14}C incorporation into the end-products photosynthates, respectively. Samples were filtered onto glass-fibre filters (precombusted Whatman GF/F) and rinsed with Milli-Q water. Scintillation cocktail (3.5 ml of Filter Count, Packard Instruments) was added to the scintillation vials containing the filters for total uptake. A Liquid Scintillation Counter (Canberra-Packard Tri-Carb 1900 CA, Packard Instruments) was used to determine the DPM of ^{14}C in the samples. For macromolecular products, the GF/F filters were stored at $-40\,^{\circ}$C until analysis.

Macromolecular end products of photosynthesis

To determine the amount of ^{14}C fixed into the macromolecular end-products of photosynthesis, we used an extraction technique based on the methods originally developed by Li et al., (1980) and modified by Rai (1995) and Kasai & Arts (1998). Low molecular weight metabolites (LMW) consist of individual amino acids, fatty acids, sugars and nucleotides, etc. in the aqueous methanol-soluble fraction. A chloroform soluble, a hot 5% trichloroacetic acid (TCA) soluble and TCA insoluble fraction gave rise to the lipid, polysaccharide (including nucleic acids) and protein fractions, respectively.

Photosynthesis-Irradiance (P–I) curves

Photosynthesis-Irradiance curves were generated with the quadratic equation applied by Leverenz et al. (1990). This is a common model used by researchers (Leverenz & Jarvis, 1979; Marshall & Biscoe, 1980; Zhang, 1988; Johnson et al., 1989). In order to compare the P–I curves between the control and the three UV treatments for both unconditioned and conditioned algae, the original macromolecular data were applied to the equation:

$$\theta P^2 - (\alpha Q + P_{\max})P + \alpha Q P_{\max} = 0.$$

To solve for P, the equation was rearranged as follows:

$$P = 0.5\alpha Q + P_{\max} - (\alpha Q^2 + 2\alpha Q P_{\max} + P_{\max}^2 - 4\theta\alpha Q P_{\max})^{0.5}/\theta.$$

Here P relates the photosynthesis per unit chlorophyll biomass to irradiance (Q) measured in μg C μg Chl a^{-1} h^{-1}. $P_{\max}$ is the specific production rate at optimal light intensity. α is the initial slope of the linear portion of the P–I curve and θ is the convexity (curvature, or rate of bending). P–I curves were generated using SigmaPlot 4.1 (SPSS Inc.). This equation produced unreasonable values for $P_{\max}$ and α for conditioned Dose 1 and Dose 2 algae largely due to an apparent lack of photoinhibition resulting in very low θ values . This occurred because our highest light level was lower than that required for photoinhibition in this species. Although these $P_{\max}$, α and θ values were correctly generated using the P–I curve equation, biologically the values did not make sense, and would never have been achieved under natural light conditions. The P–I curves of ^{14}C uptake which are illustrated for unconditioned algae (Figure 2) and conditioned algae (Figure 3) are, however, indicative of the true algal response over the range of irradiance used in this study. Therefore to determine more reasonable values for α and θ, an average for $P_{\max}$ was calculated from the $P_{\max}$ at 700, 800 and 900 μmol m^{-2} s^{-1} for the control and each of the UV treatments for total uptake and all macromolecular components for both unconditioned and conditioned algae. These average $P_{\max}$ values were then substituted into the P–I curve regression equation ($R^2_{\text{adj}} \geq 0.82$ for all regressions) to achieve biologically reasonable α (Figure 4) and θ values (Table 3).

Statistical analysis

Analyses were conducted using SigmaStat (2.0 SPSS Inc.). Two-way ANOVAs were performed for total uptake and each of the macromolecular pools (lipid, polysaccharide and protein) to test for differences between exposure history (unconditioned algae and conditioned algae) and for differences among treatments (control, Dose, 1, Dose 2 and Dose 3) with respect to $P_{\max}$, α and θ. We used a balanced design with no interactions. A Tukey test was performed to isolate which group(s) differed from the others if there was a significant difference ($P < 0.05$) for the main effects.

We have not reported values for the low molecular weight metabolite fraction because the total carbon allocation to the LMW portion was less than 3% and consequently there was very little adherence to the P–I model ($R^2_{\text{adj}} \sim 0.19$).

Results and discussion

Light climate in wetlands at St. Denis and Phototron doses

The arithmetic mean UV doses (unweighted and biologically weighted for *Phaeodactylum* sp. and *Prorocentrum micans* (Cullen et al., 1992)) found at depths ranging from 0 cm to 75 cm for 16 ponds sampled between 10:00–16:00 h during the period July 22–August 30, 1996, at the St. Denis Wildlife Refuge, Saskatchewan, are listed in Table 1. Figure 1 shows the average UV irradiance spectra at four of these depths (subsurface, 5 cm, 10 cm and 20 cm) surveyed in the 16 wetlands at St. Denis. Figure 1 also shows the UV irradiance spectra delivered in the Phototron experiments as well as that delivered to condition the algae in the continuous cultures.

Using several different weighting functions, we calculated the biologically weighted Phototron doses

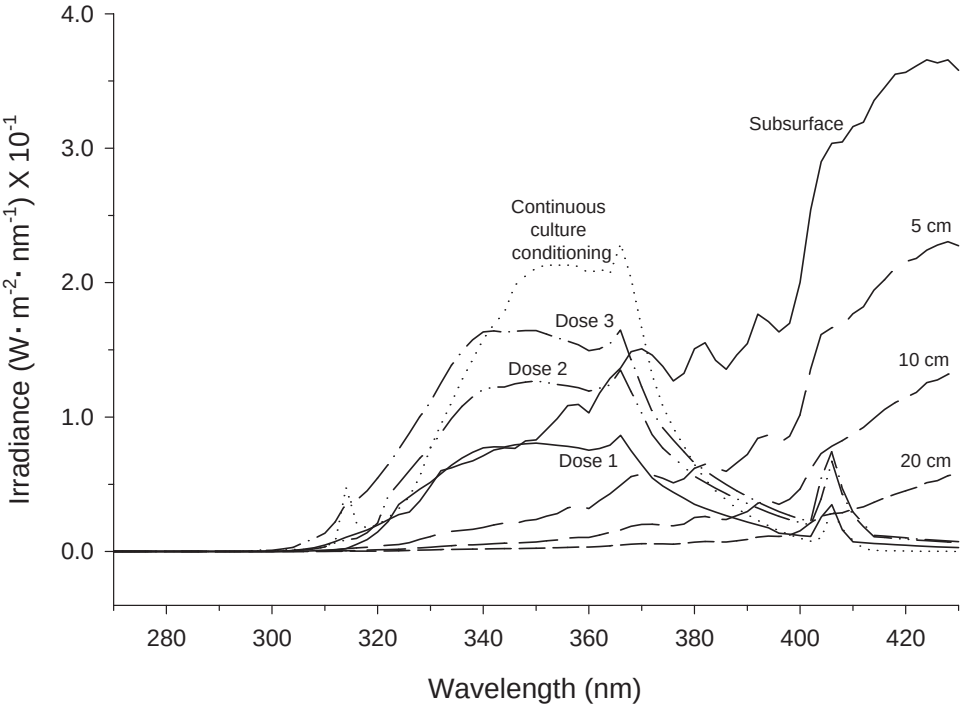

Figure 1. Average solar UV irradiance spectra at four different depths (subsurface, 5 cm, 10 cm and 20 cm depths) from UV measurements in 16 ponds surveyed at the St. Denis Wildlife Refuge, SK, in 1996. Also, UV irradiance spectra of the three Phototron doses and the dose used to condition algae in the continuous cultures prior to Phototron experiments.

Table 1. Mean values of unweighted and biologically weighted irradiance of solar radiation at the subsurface, 5 cm and 10 cm depths of 16 ponds at St. Denis Wildlife Refuge, Canada

UV-B (W m^{-2})	Subsurface[2]	5 cm depth	10 cm depth
Unweighted dose rate	0.169±0.12[3]	0.021±0.02	0.001±0.00
Biologically weighted dose rate[1]			
Phaeodactylum sp.	0.033±0.02	0.004±0.00	0.000±0.00
Prorocentrum micans	0.027±0.02	0.003±0.00	0.000±0.00
UV-A (W m^{-2})	Subsurface[2]	5 cm depth	10 cm depth
Unweighted dose rate	10.485±4.94[3]	3.609±2.79	0.997±0.87
Biologically weighted dose rate[1]			
Phaeodactylum sp.	0.296±0.16	0.080±0.07	0.016±0.02
Prorocentrum micans	0.134±0.08	0.030±0.03	0.005±0.00
UV-B + UV-A (W m^{-2})	Subsurface[2]	5 cm depth	10 cm depth
Unweighted dose rate	10.654±5.05[3]	3.630±2.81	0.998±0.87
Biologically weighted dose rate[1]			
Phaeodactylum sp.	0.329±0.18	0.084±0.07	0.017±0.02
Prorocentrum micans	0.161±0.009	0.033±0.03	0.005±0.00

[1]According to Cullen et al. (1992) normalized at 300 nm. [2]Average values of irradiance at subsurface and other depths of ponds measured during 10:00–16:00 from July 22 to August 30, 1996. [3]Mean±S.D.

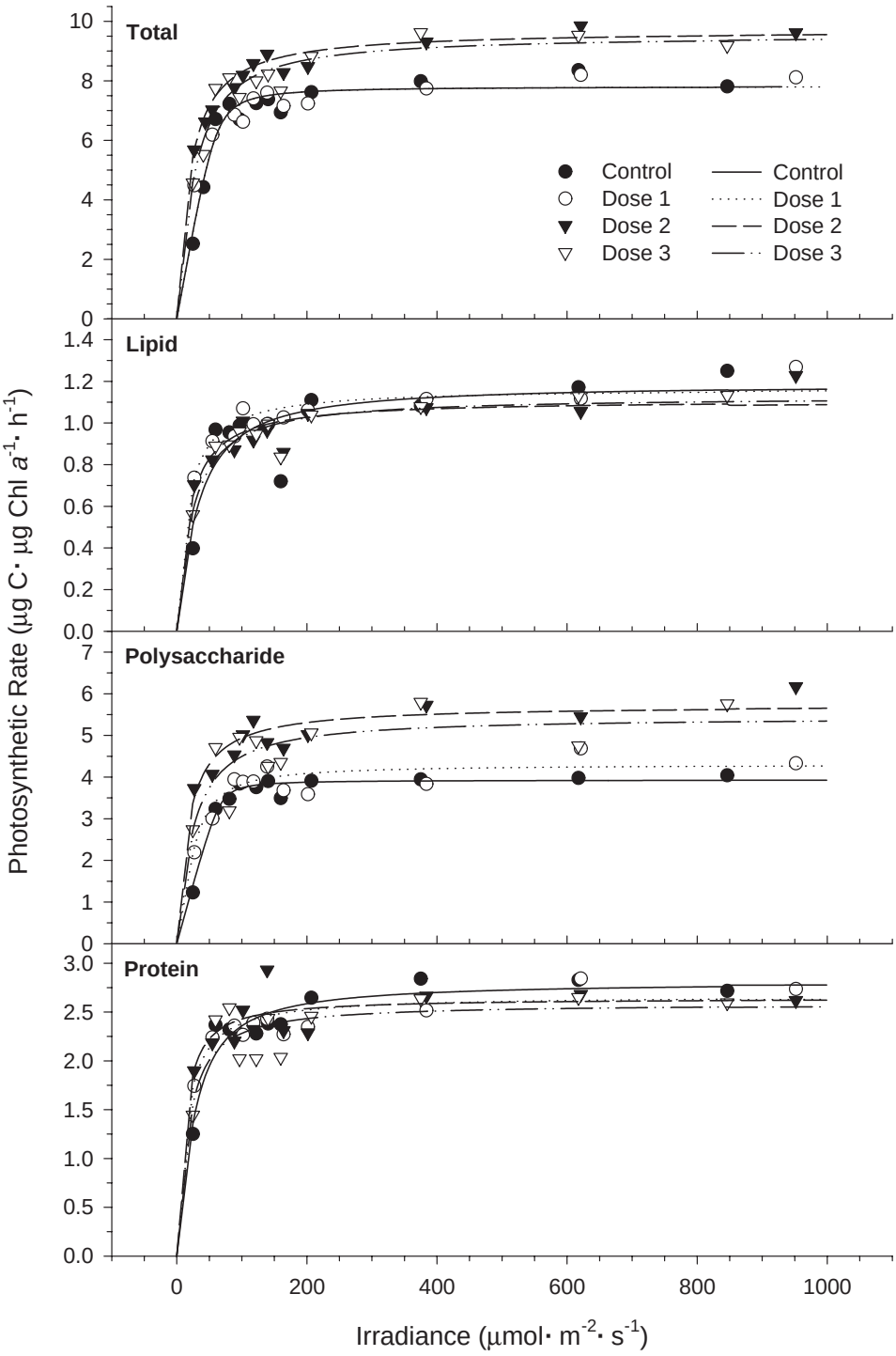

Figure 2. Total chlorophyll *a* specific uptake and ^{14}C allocation into the macromolecular end products of photosynthesis (lipid, polysaccharide and protein) for unconditioned algae (no prior UV exposure) after a 4 h incubation in the Phototron. Note: for total photosynthetic uptake, the control and Dose 1 algae responses are superimposed (i.e., having nearly the same P_{max} values).

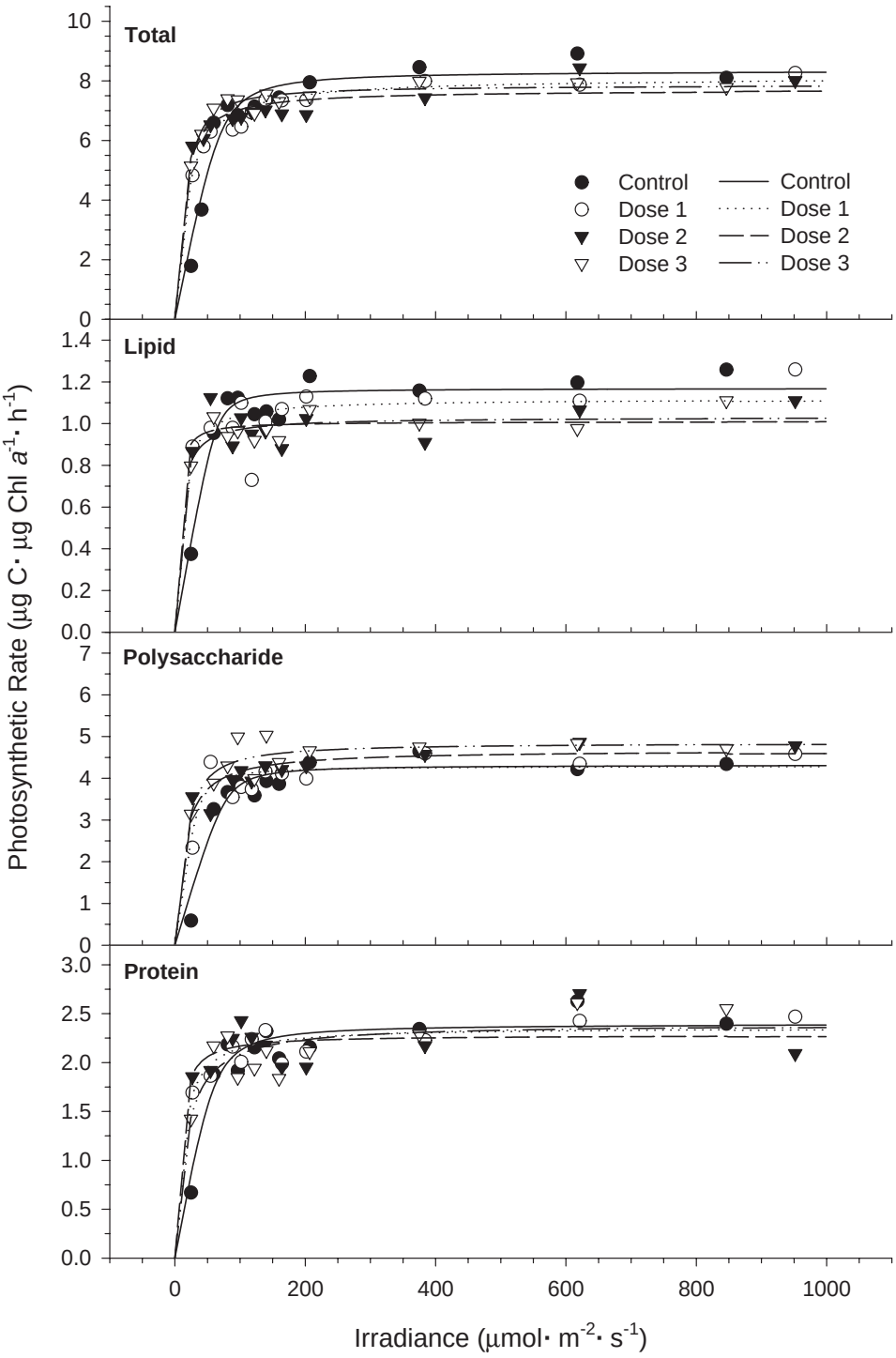

Figure 3. Total chlorophyll *a* specific uptake and ^{14}C allocation into the macromolecular end products of photosynthesis (lipid, polysaccharide and protein) for conditioned algae (15 d UV exposure) after a 4 h incubation in the Phototron.

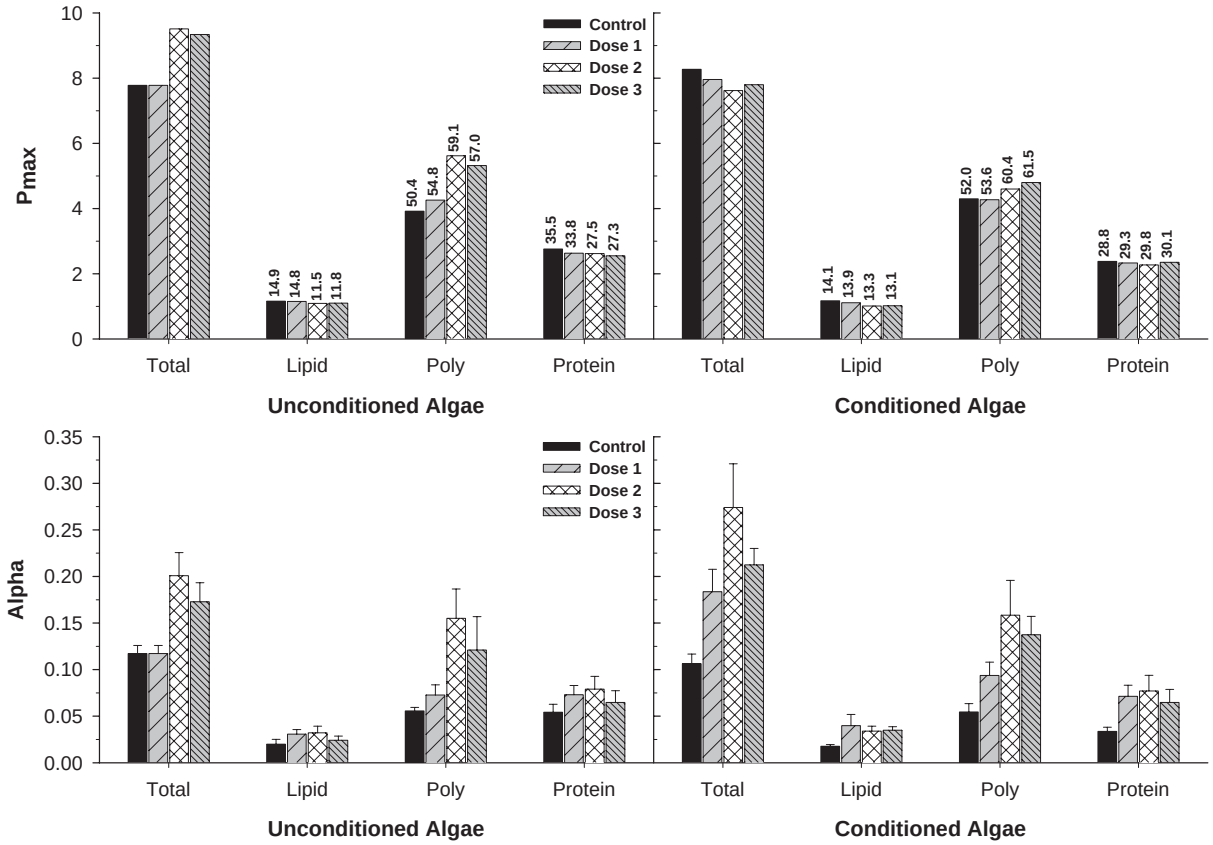

Figure 4. Comparison of $P_{\max}$ and alpha (α) values for total uptake and macromolecular components for unconditioned (no prior UV exposure) and conditioned (15 d UV exposure) algae in the Phototron experiments. $P_{\max}$ is the specific production rate at optimal light intensity measured in μg C μg Chl a^{-1} h^{-1}and α is the initial slope of the linear portion of the Photosynthesis-Irradiance curve. Note: Poly=Polysaccharide and the values above the macromolecular components for $P_{\max}$ provide the percent of total carbon uptake that is attributed to either lipid, polysaccharide or protein.

(Table 2). These include: an action spectrum for inhibition of electron transport in isolated chloroplasts (Jones & Kok, 1966); a generalized action spectrum for plants (Caldwell, 1971 cited in Smith et al., 1980); an action spectrum for photosynthesis in a marine diatom *Phaeodactylum* sp. (Cullen et al., 1992); a marine dinoflagellate *Prorocentrum micans* (Cullen et al., 1992); and the DNA action spectrum (Setlow, 1971 cited in Smith et al., 1980) (all normalized to 1 at 300 nm). We present these weighting functions in order to allow researchers to make comparisons between the doses we used and the effects observed in this study, and other experiments.

From Figure 1, the irradiance of the continuous culture conditioning and the three Phototron doses are higher in energy (lower wavelengths) than the average irradiance found in the 16 wetlands surveyed at St. Denis. However, a comparison between Tables 1 (natural pond data) and 2 (Phototron doses) shows that the overall UV doses (weighted and unweighted) are reasonably good comparisons between the natural environment versus a laboratory system. The relative proportion of UV-A to UV-B exposure is considerably higher in the ponds and our Phototron experiments, such that the results may be more indicative of UV-A effects rather than UV-B. However, since we did not specifically exclude one region or the other, the results we observed are indicated as UV radiation effects to encompass wavelengths from 305–400 nm (with Schott WG305 filter).

Photosynthetic rate

Maximum total uptake of ^{14}C for unconditioned and conditioned control algae were not significantly different ($P = 0.783$) than any of the UV treated algae

Table 2. Unweighted and biologically weighted doses of UV irradiation measured at the centre of the cell suspension in a simulated Phototron incubation chamber

	UV-B (W m^{-2})			UV-A (W m^{-2})			UV-B + UV-A (W m^{-2})		
	Dose 1	Dose 2	Dose 3	Dose 1	Dose 2	Dose 3	Dose 1	Dose 2	Dose 3
Unweighted dose rate	0.065	0.140	0.495	5.050	7.948	10.264	5.115	8.088	10.759
Biologically weighted dose rate									
Jones & Kok chloroplast (1966; normalized to 300 nm)	0.045	0.095	0.334	1.549	2.411	3.236	1.595	2.506	3.570
Caldwell Plant (1974; 300 nm)	0.002	0.008	0.037	0.000	0.000	0.000	0.002	0.037	0.037
Cullen et al. *Phaeodactylum* sp. (1992; 300 nm)	0.011	0.027	0.103	0.213	0.332	0.447	0.224	0.360	0.550
Cullen et al. *Prorcentrum micans* (1992; 300 nm)	0.008	0.023	0.086	0.118	0.184	0.251	0.126	0.206	0.338
Setlow DNA (1974; 300 nm)	0.001	0.003	0.016	0.000	0.000	0.000	0.001	0.003	0.016

Table 3. Convexity values (±s.e.) generated from the non-linear regression of the Photosynthesis-irradiance curves for total uptake and macromolecular components from unconditioned and conditioned algae in the Phototron experiments. θ is the convexity or rate of bending of a curve

θ	Control	Dose 1	Dose 2	Dose 3
Unconditioned Total	0.99 ± 0.007	0.99 ± 0.007	0.96 ± 0.01	0.96 ± 0.01
Lipid*	0.96 ± 0.02	0.96 ± 0.01	0.96 ± 0.02	0.96 ± 0.01
Polysaccharide	0.99 ± 0.005	0.97 ± 0.01	0.96 ± 0.01	0.96 ± 0.02
Protein	0.97 ± 0.01	0.97 ± 0.001	0.97 ± 0.01	0.97 ± 0.01
Conditioned				
Total	0.98 ± 0.001	0.96 ± 0.01	0.96 ± 0.01	0.98 ± 0.006
Lipid*	0.98 ± 0.008	0.97 ± 0.02	0.99 ± 0.01	0.99 ± 0.008
Polysaccharide	0.99 ± 0.01	0.99 ± 0.01	0.96 ± 0.01	0.98 ± 0.01
Protein	0.98 ± 0.01	0.97 ± 0.01	0.98 ± 0.01	0.97 ± 0.002

* indicates significantly different for exposure history (unconditioned versus conditioned algae).

(Doses 1, 2 or 3). For unconditioned algae, the two highest doses caused a 21% increase in P_{max}-Total, relative to the control over the 4 h period. This was largely due to carbon allocation to polysaccharide which on average accounted for 55% of total carbon uptake (Figure 4). In contrast, over the range of PAR irradiance in our experiment, conditioned control algae had marginally higher (∼5%) total uptake than all three UV treatments.

The α-Total values for unconditioned and conditioned control algae were lower, but not significantly different ($P = 0.060$), than the UV treatments. Note, however, that the values for α are tentative because generally only 1 or 2 data points were on the initial slope of the P–I curve. The θ-Total values for unconditioned and conditioned control algae were higher, but not significantly different ($P = 0.522$), than the UV treatments. There was no observable photoinhibition for any of the treatments.

Leverenz et al. (1990) indicate that low θ implies a reduced photosynthetic efficiency beginning at very low light and continuing through full sunlight. For algal cells to operate efficiently they must achieve both a high maximal quantum yield (high α) and a high convexity (θ approaching 1.00) (Leverenz et al., 1990). In our study, the control algae had θ closest to 1.00, suggesting greater photosynthetic efficiency for algal cells not exposed to UV radiation (Table 3).

Algae from the conditioned continuous cultures did not have significantly different P_{max}-Total ($P = 0.333$), α-Total ($P = 0.115$) or θ-Total ($P = 0.703$) values than algae which had no UV exposure prior to the Phototron incubation. Thus, it would appear that UV exposure history of *C. erosa* does not have

an effect on the algae's response to UV radiation for total carbon uptake, at least at these low UV radiation levels.

In the NWRI Phototron, Kasai & Arts (1998) exposed two genetically different strains (herbicide susceptible and herbicide tolerant) of the green alga *Scenedesmus gutwinskii* to a herbicide (Simetryn) and to UV-B levels which were comparable to Doses 1 and 3 used in this study. They observed that total uptake rates of *Scenedesmus* were not reduced by UV-B radiation alone, even at their highest dose. Gerber & Häder (1995) through careful fluorescence measurements demonstrated that *Scenedesmus* cf *quadricanada* is less sensitive to solar radiation than *Cryptomonas maculata*. If *Cryptomonas* sp. are in general more sensitive to UV radiation than *Scenedesmus* sp. then one would expect to first see negative impacts on the cryptoflagellate at lower dose rates. Our conditioned algae showed roughly a 5% depression in $P_{\max}$ (although not significant) for the total uptake curves relative to the control after exposure at the highest UV dose.

^{14}C *allocation to end-products of photosynthesis*

$P_{\max}$ for lipids ($P = 0.076$) and proteins ($P = 0.202$) for unconditioned and conditioned control algae was slightly higher than that of Doses 1, 2 and 3, but not significantly different (Figure 4). Polysaccharide for pooled control algae was lower, but not significantly ($P = 0.272$), than all three UV treatments. Comparing exposure history, pooled $P_{\max}$ values for the control and all UV doses revealed no significant difference for lipids ($P = 0.113$) or polysaccharides ($P = 0.417$), however for protein, unconditioned algae had a significantly higher $P_{\max}$ ($P = 0.004$) than conditioned algae.

As indicated by Arts & Rai (1997), UV-B exposure has the potential to produce subtle, species-specific, effects on the relative allocation of recently fixed carbon to lipid, polysaccharide, protein and LMW compounds in algae. Effects on the overall photosynthetic rate were observed at low UV-B dose rates for *Cryptomonas* sp. (e.g., 0.033 mW cm^{-2} UV-B weighted, Jones and Kok). They found a negative effect on the production of photosynthates in a dose-dependent and exposure-duration dependent manner, even at very low dose rates. However, it was noted, that at high PAR levels ($\sim$1000 μmol m^{-2} s^{-2}), UV-B weighted rates of 0.093 mW cm^{-2} and 0.061 mW cm^{-2} actually enhanced the rate of photosynthesis. At these high PAR levels, the lowest UV-B dose rates stimulated the production of photosynthate to lipid, polysaccharide and protein relative to their control algae with no UV exposure (Arts & Rai, 1997). This was similar to the effect we observed in this study with unconditioned algae where there was an enhancement of photosynthesis for the two highest doses.

Calculated α values for lipid, polysaccharide and protein components were lowest for the control algae compared to all UV treatments. Comparisons among values for α are presented in Figure 4. For lipids, α values were not significantly different among any treatments ($P = 0.093$). For polysaccharides, α values for the control and Dose 1 algae were significantly lower ($P = 0.003$) than Doses 2 and 3, and for proteins, the α values for the control algae were significantly lower ($P = 0.049$) than for Dose 2 algae. There was no significant difference in α values for lipid ($P = 0.212$), polysaccharide ($P = 0.157$) or protein (P=0.300) for unconditioned versus conditioned algae.

Convexity values associated with the $P_{\max}$ and α values computed from each regression are provided in Table 3. Convexity values were $\geq$0.96 for all macromolecular fractions, and there was no significant difference between the control and all UV treatments for lipid ($P = 0.773$), polysaccharide ($P = 0.082$) or protein ($P = 0.232$) θ values. Comparing exposure history, lipid θ values for unconditioned algae were significantly lower ($P = 0.023$) than conditioned algae, but there was no significant difference for polysaccharide ($P = 0.196$) or protein ($P = 0.159$) θ values. For all cellular fractions, no photoinhibition was observed for the control or any UV treatments.

Figure 5 compares the amount of lipid, polysaccharide and protein as a percent of total uptake over the range of PAR irradiances used in this study. Also, the percent of total uptake calculated from the $P_{\max}$ values is shown in Figure 4 on the top of the bars for lipid, polysaccharide and protein, as a means of comparing relative changes in carbon allocation for unconditioned and conditioned alga. As a percent of total uptake, the lipid, polysaccharide and protein values do not add to 100%, either because low molecular weight compounds are not included or because of over/under-estimation of the calculated $P_{\max}$ values from the curve fitting model, relative to the actual data points. For unconditioned algae, all three UV doses decreased in % carbon allocation to lipid (28% decrease for Doses 2 and 3) and protein (29% decrease for Doses 2 and 3) over the 4 h incubation period (Figure 5). For Doses 2 and 3, there was a 15% increase in

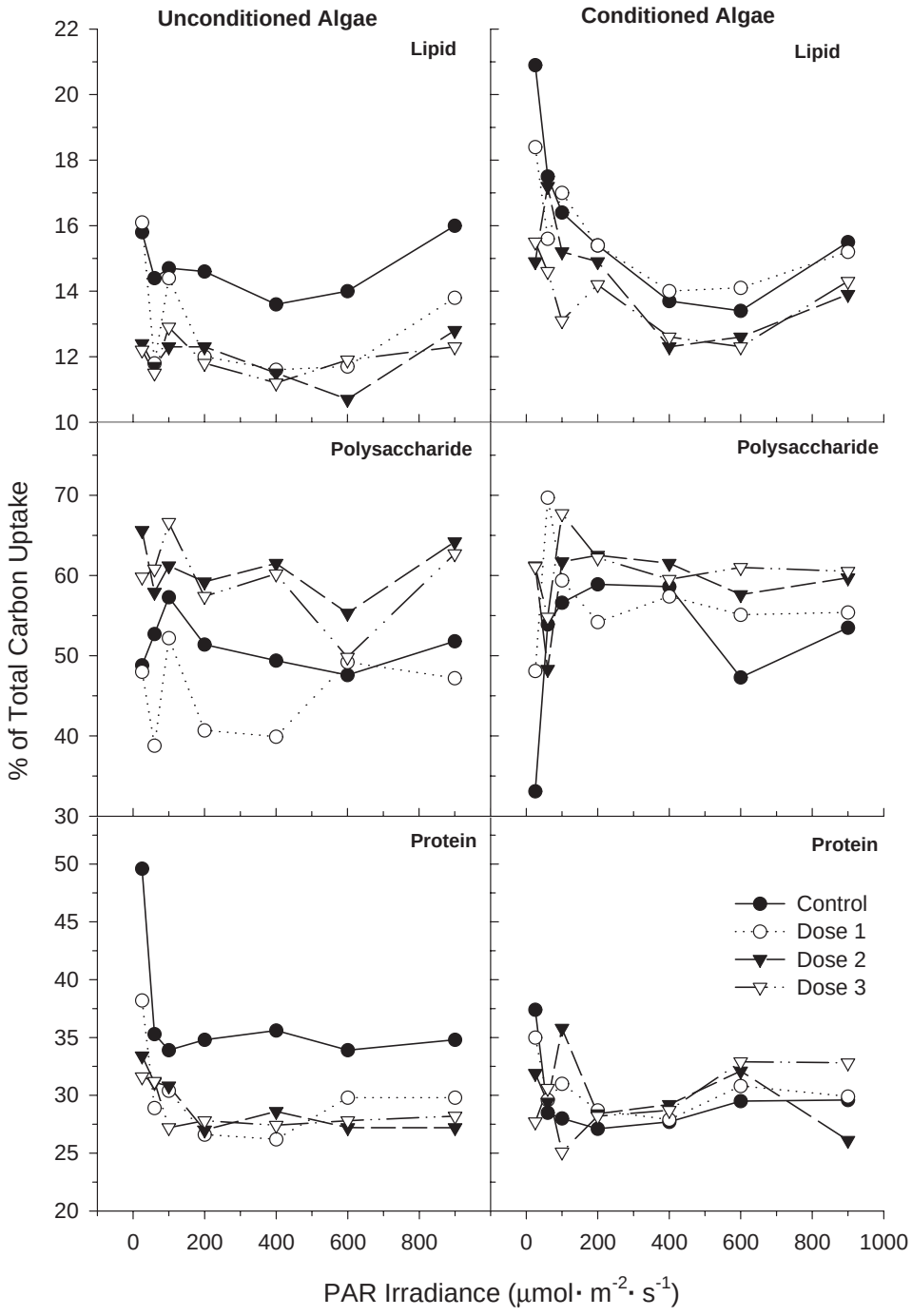

Figure 5. Carbon allocation to lipid, polysaccharide and protein as a percent of total carbon uptake for unconditioned (no prior UV exposure) and conditioned (15 d UV exposure) over the range of irradiance used in the 4 h Phototron incubation.

polysaccharide as a % of total uptake relative to the unconditioned control algae. For conditioned algae, there was not nearly the same acute effect as that observed for unconditioned algae. The % of total uptake to lipid and protein only decreased 7% and 4%, respectively, for Doses 2 and 3 relative to the conditioned control (Figure 5). However, similar to the unconditioned algae, for Doses 2 and 3, there was a 17% increase in polysaccharide as a % of total uptake relative to the conditioned control algae. Comparing controls, conditioned algae were lower than unconditioned algae for lipids and proteins, by 6% and 23%, respectively, but were 3% higher for polysaccharides. Hence, UV exposure appeared to change the proportions of lipid, polysaccharide and protein relative to total uptake, especially for unconditioned algae. This indicates that acute exposure decreases the amounts of lipid and protein while increasing polysaccharides and that chronic UV exposure exacerbates this reduction of lipid and protein.

Environmental significance

The contrast between the study of Arts & Rai (1997) and this study may be indicative of the species-specific effect of UV radiation. *C. erosa* in our study was less sensitive to the natural levels of UV radiation which were mimicked in the Phototron compared to the study by Arts & Rai (1997) where they observed substantial declines in photosynthetic uptake and carbon allocation in *Cryptomonas* sp. This highlights how difficult it may be to predict changes in ecosystem structure and function when there is likely great variability in the responses between individual species.

Another possible explanation for this contrast may be the light systems which were used in the studies. Arts & Rai (1997) noted that they were not trying to duplicate the solar spectra but were more interested in determining changes due to the most energetic and damaging of the wavelengths reaching the earth from UV-B radiation. They used UV-B bulbs which had a peak at 312 nm (compared to our 340 nm UV-A bulbs). Hence, their UV-B:UV-A (or UV-B:Total UV+PAR) ratios were much higher than was applied in our study. The ratio of UV-B to UV-A to PAR used in laboratory systems may be of great importance in explaining the effects observed in these systems and is an important consideration in extrapolating laboratory results to natural environments. For example, while investigating the chronic effects (36 d) of UV radiation on growth and cell volume of *Phaeodactylum tricornutum*, Behrenfeld et al. (1992) showed growth rates decreased with increasing ration of UV-B:UV-A+PAR.

Other researchers have also suggested that individual phytoplankton species demonstrate differential sensitivity to UV-B (Calkins & Thordardottir, 1980; Worrest & Häder, 1989; Karentz et al., 1991). Smith & Cullen (1995) indicate that while there has been general agreement with this hypothesis of differential sensitivity, there is little agreement over possible consequences. Due to seasonal and geographical changes in species composition, there are uncertainties associated with the ecological consequences of UV-B on communities and ecosystems. Bothwell et al. (1993; 1994) for instance, have demonstrated the complexity of trophic level response to UV-B radiation and they emphasize the need for long-term (multigenerational times) ecological studies to assess ecosystem structure.

Short term effects due to UV radiation on Cryptomonads have been observed. After $\leq$2 h of exposure to UV radiation ($\sim$2.9 WA m^{-2} solar UV-B radiation or $\sim$1.0 W m^{-2} artificial UV radiation), the accessory photosynthetic pigment, phycoerythrin, was bleached, photosynthetic oxygen production decreased and also, membrane proteins showed a significant decrease in chromoproteins in the marine alga *Cryptomonas maculata* (Zundörf & Häder, 1991). Häder and Häder (1991) observed that in *C. maculata*, the percentage of motile cells decreased dramatically after a 20 min exposure to solar radiation and the velocity of movement decreased after 100 min. These results were almost entirely attributable to UV-B radiation. Solar radiation and UV radiation induced massive bleaching of all major photosynthetic pigments in the cells (Häder & Häder, 1991).

Imposing a UV radiation limitation on a nutrient-limited chemostat culture could potentially decrease it to a lower concentration, and thereby provide the UV exposed algae with a higher nutrient supply per cell. This would involve some confounding effect of different levels of nutrient limitation with UV radiation effects during our pre-Phototron phase, which would be carried over to our Phototron experiment (R. Moeller, personal comm., 1998). However, if such confounding was occurring, it could only mean that our experiment was in fact made more conservative with respect to identifying effects due to UV radiation. Also, Furgal & Smith (1997) investigated P–I curves of phytoplankton from Georgian Bay to determine the influence of UV-B radiation and the interacting effects

of natural changes in nutrient status and photoadaptive state. They found that the widely varying nutrient status of the algae had no significant influence on their sensitivity to UV-B radiation. We suggest therefore, that the effects we observed were due mainly to differences in UV, including those between unconditioned and conditioned algae.

Our study implies that, in the short term, *C. erosa* which have never been exposed to UV radiation may exhibit an increased rate of photosynthesis, mainly due to an increase in polysaccharides which could be an adaptive mechanism to protect the algal cells from UV radiation. Over time, however, UV radiation may harm the cells such that their carbon uptake becomes reduced. Exposure to levels of UV radiation found in subsurface depths of wetlands may therefore affect the photodynamics of photosynthesis. In shallow wetlands, it is likely that algae are exposed to UV radiation for a significant periods during the day. From this study, it would appear that *C. erosa* may be negatively affected by UV radiation which penetrates to depths of up to 1.0 m and 20–40 cm in the high DOC water of prairie lakes and prairie wetlands, respectively, by a change in carbon allocation, especially in terms of their lipid and protein production, perhaps due to allocating more energy to repair.

We also suggest that drastic changes in biological responses do not have to be observed for there to be an effect on the organism. Subtle responses may occur at low levels of UV radiation, like those used in this study, and as shown in Figure 5, where there are obvious differences in the trends between unconditioned algae and algae with a UV exposure history. For instance, cryptomonads are composed of a high % of essential fatty acids and are considered to be a nutritious algae for zooplankton. We propose that if their lipid stores (specifically EPA and DHA) decline as a result of an effect of UV radiation, as has been shown for marine algae (Wang & Chai, 1994; Goes et al., 1994), there may be profound effects on zooplankton which rely on *C. erosa* as a valuable food source. For example, Chen & Folt (1993) found that *C. erosa* was of higher quality food (compared to *Chlamydomonas reinhardtii*) Chen & Folt (1993), when investigating survivorship and reproduction of two copepod species, found that *C. erosa* was a higher quality food than *Chlamydomonas reinhardtii*. Hence, any small changes that occur in the fatty acid composition of *C. erosa* may lead to an alteration in the nutritional value of the algae to zooplankton. UV effects on carbon allocation may therefore influence the nutritional quality of algae and subsequently, the transfer of energy to higher trophic levels through the food web (Arts et al., 1992, 1993, 1997).

In this study, we observed subtle changes in the photosynthetic rate and carbon allocation of *C. erosa* caused by the UV radiation levels that were mimicked using the Phototron. In combination with other stressors, significant and more pronounced negative impacts may occur. For example, Kasai & Arts (1998) studied the interactive effects of UV-B radiation and a herbicide, Simetryn, on two strains of *Scenedesmus*. The UV levels alone did not affect total uptake rates in either strain, however, the UV levels did reduce the uptake rates in the presence of the herbicide in the herbicide-susceptible strain. Thus, for future studies, it will be important to emphasize holistic approaches as a means of integrating the effects of UV radiation with natural and/or anthropogenic stressors, to further understand and anticipate the responses of organisms in aquatic ecosystems.

Conclusions

Short term effects of UV radiation, relative to control algae, did not negatively affect P–I curves for unconditioned (no previous UV exposure) *C. erosa*. In fact, there was an increase, although insignificant, in P_{max}-Total by 21% for the two highest UV doses, relative to the control algae. However, acute UV exposure decreased the allocation of ^{14}C to the cell's main macromolecular components of lipid and protein as a % of total uptake. Although insignificant, after the 4 h Phototron incubation, conditioned (previous 15 d UV exposure) *C. erosa* showed a depression of 7% in P_{max}–Total, as well as lower amounts of ^{14}C allocated to macromolecular compounds, especially lipids ($\sim$11% decrease in P_{max}–Lipid), relative to the control algae. In addition, the % of total carbon attributed to lipids and proteins was lower in all conditioned alga relative to the unconditioned controls. Thus, we conclude that the environmentally realistic doses of UV-B and UV-A used in this study can have a negative impact on *C. erosa*.

Our study details some of the impacts of UV radiation on a wetland alga species that is crucial to the efficient transfer of energy up the food web. Further to this, it will be interesting to study the effects of UV radiation on changes in fatty acid composition or quantity and how this relates to a food chain affect between phytoplankton and zooplankton. More broadly,

it will be important to investigate UV radiation in conjunction with multiple stressors to determine what will be the overall effects on various processes and interactions in wetland systems.

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