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Effects of Ultraviolet Radiation on Attached Diatom Growth and Distribution

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EFFECTS OF ULTRAVIOLET RADIATION ON ATTACHED DIATOM GROWTH AND DISTRIBUTION

by

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Simulated solar irradiance was used to evaluate ultraviolet radiation (UVR = UVA + UVB) as a potential mechanism affecting the distribution of four dominant freshwater benthic diatoms from Lake McConaughy in western Nebraska. Clonal cultures of two large and two small dominant attached diatoms (periphyton) were isolated from upper and lower growth zones along the face of the dam. The large-celled diatom *Cymbella affinis* and the smaller-celled *Achnanthes lanceolata* var. *dubia* were isolated from the shallow upper growth zone, and the larger-celled *Synedra ulna* and the smaller-celled *Fragilaria construens* var. *venter* were isolated from the lower growth zone. Initial studies were conducted to determine optimal temperature and PAR light intensity for growth of each species. They were then randomly assigned to one of six light treatments: PAR + UVA + UVB under low light (~15 μmol m^{-2} s^{-1}), PAR + UVA + UVB under high light (~850 μmol m^{-2} s^{-1}), PAR + UVA (ultraviolet-A radiation, 320-400 nm) under low light, PAR + UVA under high light, PAR only under low light, and PAR only under high light, over a three-week period. Effects on growth were monitored via direct cell counts and chlorophyll fluorescence.

Clones under the high light treatments were photoinhibited, and exhibited dramatic variability and low to negative growth rates. Under UVR treatments, growth

rates of the upper zone diatoms were significantly greater than those of lower zone diatoms, indicating that PAR + UVR tolerance may be a primary mechanism governing periphyton vertical distribution. Diatom growth was significantly greater for the two larger-celled species than for the two smaller-celled species under PAR (400-700 nm) + UVA + UVB (ultraviolet-B radiation, 280-320 nm) treatments at two and three weeks. Only in week three was the diatom growth significantly greater under PAR + UVA than under PAR + UVA + UVB, indicating that although UVB is more detrimental than UVA, the relative effect of UVA on growth is similar over short time periods due to higher overall dosages.

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Introduction

Factors that influence attached algal populations (periphyton) and their vertical distribution (zonation) include light, temperature, water current, substratum, and grazing. Thus changes in periphyton community composition associated with depth are the result of a myriad of species-specific responses, such as wave disturbance, light quality and quantity, sedimentation load, and associated physicochemical factors (Stevenson and Stoermer 1981, Hoagland and Peterson 1990).

Ultraviolet radiation (UVR = UVA + UVB) (280-400 nm) has been shown to affect algal community composition. Previous research on phytoplankton has shown that on a photon basis, ultraviolet-B radiation (UVB; 280-320 nm) is more damaging than ultraviolet-A radiation (UVA; 320-400 nm). UVA has been found to induce photorepair and to cause photoinhibition (Borman 1989, Helbling et al. 1992, Smith et al. 1992), while UVB disrupts several photosynthetic processes, including the electron transport system (Noorudeen and Kulandaivelu 1982), photosystem II reaction centers (Iwanzik et al. 1983), and pigment stability (Dohler 1984, Roy et al. 1986). The extent of the photosynthetic damage depends on the duration and the magnitude of exposure (Cullen and Lesser 1991). UVB has also been found to damage algal DNA (Karentz et al. 1991) and reduce growth rates (Jokiel and York 1984, Behrenfeld et al. 1992, Thompson et al. 1980). However, extended exposure to UVR in natural communities may increase diatom biomass due to inhibitory impacts on grazers (Bothwell et al. 1993).

UVR effects on algae also appear to be cell-size dependent. For example, Garcia-Pichel (1994) noted that the amount of UVB able to penetrate a cell is dependent on cell

size. Karentz et al. (1991) also found that the sensitivity of Antarctic phytoplankton to UVR was greater in smaller cells than in larger cells.

Periphyton in shallow water is exposed to high levels of sustained UVR (DeNicola et al. 1992), and is often restricted to specific growth areas. Virtually all aquatic surfaces receiving light, from small streams to large lakes, sustain a periphyton community. Thus periphyton is ideal for studying the effects of UVR on community structure. In addition, since periphyton forms the base of the aquatic food chain, damage to periphyton caused from UVR exposure may also translate to ecosystem level impairment.

At the community level, there is a high degree of variation among algal tolerances to UVB, which complicates predictive models on the future impacts of increased UVR due to ozone depletion. There have been few previous studies to evaluate UVR effects on freshwater periphyton distribution at the community level. Bothwell et al. (1993) reported that UVR initially inhibited growth rates of freshwater periphytic diatoms, but this reversed after 3-4 weeks, and by week 5 total diatom abundance exposed to PAR + UVR was greater than in communities protected from UVR. Bothwell et al. (1993) suggested a suite of possible biochemical and morphological mechanisms to explain the differences in UVB tolerance among algal taxa, including: the efficiency of DNA damage repair mechanisms; the concentration of UV-protective compounds; cellular morphology and subcellular organelle arrangements that may protect the nucleus from UVB exposure; differences in cellular contents of UV-absorbing proteins, pigments, and nucleic acids; and differences in DNA base content and sequence. A subsequent study

found that this reversal was due to a reduction in herbivores by UVR, thus "the response of entire ecosystems to elevated UVB cannot be made on single trophic-level assessments" (Bothwell et al. 1994).

Santas et al. (1996) found reduced productivity and shifts in periphyton species composition, suggesting that communities in the upper euphotic zone may be capable of adjusting to stress caused by an increase in UVB. Vinebrooke and Leavitt (1996) provided the first evidence that naturally occurring levels of UVR significantly suppress periphyton development by inhibiting diatom production during the short ice-free season in an alpine lake. To understand UVR effects at multiple trophic levels and to make ecosystem-level assessments, the effects of UVR on individual dominant species must be delineated.

The present study investigated the effects of UVR on benthic diatoms, focusing on the effects on growth rates of two periphytic diatoms isolated from two different depths. The objectives of this study were to examine UVR as a potential mechanism affecting the distribution of individual diatom populations, to determine if UV tolerance differences were also based on cell size, and to differentiate the growth effects of UVA + UVB versus UVA versus no UVR.

Materials and Methods

Study Site

Lake McConaughy is a large eutrophic impoundment (surface area of 1.42×10^4 ha; volume of 2.4×10^9 m^3) located on the North Platte River at the southern edge of the

sandhills region of western Nebraska (Keith County, USA, 41°22'N, 101°56'W). Roemer and Hoagland (1979) reported mean annual chemical data for the reservoir as follows (near the present study site): pH = 8.6, total alkalinity =176 mg/L as $CaCO_3$: total hardness = 207 mg/L as $CaCO_3$, NH_3-N = 0.33 mg/L, NO_3-N = 0.52 mg/L, P as PO_4 = 0.32 mg/L, and Si =23.5 mg/L.

In order to characterize the light environment from which algal samples were later collected that day, spectral irradiance was measured on August 12, 1995 using an underwater spectroradiometer (Li-Cor, LI-1800 UW, Lincoln, NE). Measurements were taken under clear blue skies at 1309 CDT from the lower growth zone (8 m) and every half hour from 1130 until 1930 CDT from the upper growth zone, 91 cm below the water surface. A full daily dosage was simulated by extrapolating morning readings from afternoon readings due to intermittent cloudiness in the morning hours. Based on prior spectroradiometric readings in this reservoir (Hoagland and Roemer 1991), this appeared to be a reasonable approach.

Diatom Culturing

Periphyton samples were collected from the rocky dam, at the reservoir's deep, relatively clear, eastern end. Samples were collected on August 12, 1995 using SCUBA, collecting jars, and a brush to scrape algae from the rocks. Four samples were gathered from the lower growth zone at 8 m and four from the shallow upper zone at 1 m. These samples were stored in an ice cooler at approximately 15°C and transported to the laboratory in Lincoln. Samples from each growth zone were surveyed under a compound microscope, and one large and one small numerically dominate diatom species were

isolated from each growth zone. The larger-celled *Synedra ulna* (Nitz.) Ehr. var. *ulna* (biovolume = 2144 μ^3) and the smaller-celled *Fragilaria construens* var. *venter* (Ehr.) Grun. (biovolume = 75 μ^3) were isolated from the lower zone, and the larger-celled diatom *Cymbella affinis* Kütz. var. *affinis* (biovolume = 1890 μ^3) and the smaller-celled *Achnanthes lanceolata* var. *dubia* Grun. (biovolume = 155 μ^3) were isolated from the shallow upper zone, according to isolation procedures of Hoshaw and Rosowski (1973). Cultures were maintained in a growth chamber at 20°C on a 12:12 h light:dark cycle (PAR = 55.6 μmol m^{-2} s^{-1}). After cultures were established, isolates from each zone were transferred to 50 x 150 mm glass culture tubes containing WC-enriched soil-water medium and stored under the same conditions.

Optimal growth temperatures of the four species were determined through growth rates under a thermal gradient. One of seven constant temperatures (14, 17, 20, 23, and 26°C) was randomly assigned to seven growth chambers, each on a 12:12 h light-dark cycle. This range was based on previous studies conducted to determine optimal temperatures for other diatom species from the region. Incubation continued for six weeks, with cell growth monitored daily using a fluorometer (Turner Designs, model 10-AU-070, Sunnyvale, CA). Culture tubes were vortexed to obtain a uniform cell suspension before fluorescence was recorded; growth in cell doublings per day (dpd) was calculated using: ln (day1/day2)/1/ln (2) (Guillard 1975). Final fluorescence was recorded every ten days and a small aliquot was transferred, based on the fluorometer value, into fresh WC-enriched soil-water medium to maintain nutrient levels for optimal growth. On day 21, fluorescence was recorded, and sample aliquots were transferred to

smaller culture tubes (13 x 100 mm). The temperature gradient was then repeated for three weeks, with transfers every eight days, to verify that the previous fluorescence results were similar to those in the smaller tubes. These findings show that an acceptable temperature to achieve high growth rates for all four species was 20°C (Fig. 1); this temperature was used for all subsequent experiments.

The four diatom species were also grown along a light gradient (PAR only) to evaluate optimal light levels for growth. Growth was again monitored using a fluorometer. The four species were maintained in standard culture tubes and exposed to a light gradient (14, 51, 107, 515, and 855 μmol m^{-2} s^{-1}) in which maximum growth rate was based on the average daily upper growth zone irradiances and the minimum growth rate was based on the maximum PAR irradiance from the lower growth zone in Lake McConaughy. The light gradient experiment was conducted in the University of Nebraska stream microcosm facility; 1000-W metalarc lamps (Sylvania) were used as the light source, with the culture tube distance from the lamp varied to create different photon flux densities. The light gradient was maintained for 12 days, with cultures transferred every six days to avoid nutrient depletion. On the final transfer, aliquots were placed in 13 x 100 mm quartz test tubes (Ace Glass, #8683 TUBE Vineland, NJ) for later UVR exposure (Urbach and Gange 1986). Quartz test tubes were used, because unlike glass, quartz transmits full UVR. To mimic natural sunlight in the high and low light flux density treatments, average dosage and ratio findings from Lake McConaughy were mimicked.

Effects of UVR Exposure

The UVR response experiment also was conducted in the stream microcosm facility. UV fluorescent lamps (Q-Panel Co., model UVA-340 Cleveland, Ohio), and metalarc lamps combined with four filters (UF4 and OP4 Plexiglas, shade cloth, and Mylar) were used to establish six different light treatment combinations. UF4 Plexiglas filters out all UVR, OP4 Plexiglas is transparent and was the basis for comparison (transmits full UVR and PAR), Mylar filters out UVB and was used to determine the effects of PAR + UVA only, and shade cloth was used to produce low light intensities (Bothwell et. al 1994). Diatom species were randomly assigned to each of the six light treatments: PAR + UVR (OP4) at low light intensity, PAR + UVR (OP4) at high light intensity, PAR + UVA only (Mylar) at low light intensity, PAR + UVA only (Mylar) at high light intensity, PAR only (UF4) at low light intensity, and PAR only (UF4) at high light intensity.

Metalarc and UVR lamps were suspended above rectangular, plywood boxes (60.96 cm W x 91.44 cm L x 20.32 cm D) used to house culture tube racks, with the bottom of each box closed off and the inside divided into six equal sections coated with flat white paint. Quartz test tubes were placed into slanted racks to minimize internal reflection or shading from the edges or sides of the box. The boxes were designated as blocks (A, B, or C) according to their location on the table. Blocking was used to minimize the effects of possible temperature and light gradient differences within the aquatic microcosm facility. Each block represented a single replicate, with six plots randomly assigned a PAR light intensity and UVR light intensity combination treatment

using a randomization table; each rack (i.e., 8 tubes/rack) was randomly assigned to a plot within each block (Fig. 2). Each rack had two tubes of each cell size and growth zone of origin (i.e., a total of 8 = 2 x small, upper + 2 x small, lower + 2 x large, upper + 2 x large, lower). The experiment was conducted over a three-week period with cell densities monitored daily using a fluorometer to determine growth rates. In addition, cell densities were enumerated every six days (corresponding to transfer days) and used to corroborate fluorometric values.

Data Analysis

The experimental design was a split plot in which the main plot was the six light treatments and the sub-plot was the two cell sizes designated from the two growth zones. The treatment design was a 2x3x2x2 Factorial Arrangement Treatment design (i.e. 2 PAR light intensity treatments, 3 UVR light quality treatments, 2 growth zones, and 2 diatom cell sizes). Growth rates of the four diatom species were compared using an ANOVA, with the sums of squares associated with species partitioned into growth zones, cell size, and their interaction. All UVR light quality treatments were standardized to PAR-only treatments (i.e. controls) by calculating the difference between PAR only treatments and the other light quality treatments within each plot.

Results

Light Environment

The highest irradiance values in the shallow upper zone (1 m) for PAR, UVA and UVB were 1523, 61.27 and 0.76 μmol m^{-2} s^{-1}, respectively and occurred at 1230 CDT.

The average ratio of PAR to UVR (1:28) was derived from these measurements and later used as a basis for simulations of the light environment in the laboratory. Whereas, at 1309 CDT in the lower zone (8 m), below the maximum depths where UVR penetrates, PAR was 13.12 μmol m^{-2} s^{-1}. The maximum reading taken from the upper zone at 1230 CDT was compared with the lower zone reading at 1309 CDT to evaluate differences in light intensity of PAR and UVR penetration. These findings showed that the overall light intensity (PAR) was much greater in the upper growth zone than in the lower growth zone and that there was no UVA + UVB penetration into the lower growth zone (Fig 3).

The high (~850 μmol m^{-2} s^{-1}) and low (~15 μmol m^{-2} s^{-1}) light intensities for the main experiment (as determined from the daily dosage) were assigned at a 16:8 h light-dark cycle based on the optimal light intensity determination (Fig. 4) and the average dosage and ratio values from the upper and lower growth zone measurements in Lake McConaughy.

Cell density vs. Fluorescence

Cell densities under low light treatments for *Cymbella affinis*, *Achnanthes lanceolata* var. *dubia*, *Synedra ulna*, and *Fragilaria construens* var. *venter* were highly correlated (r = 0.72-0.97) with the fluorometer values from the three week experiment (Table 1), thus treatment comparisons using fluorescence and cell density resulted in similar trends. Cell densities were highly variable due to fewer time points [i.e., cell counts were performed on transfer days only (i.e., every six days)], therefore fluorescence values were used to compare growth among treatments.

Growth rates, in doublings per day (dpd), of high light treatments were low to negative (Fig 5) and the correlation between cell densities and fluorescence was highly variable for all species (r = ⁻0.72-0.82) (Table 2). Low to negative growth combined with the poor correlation between cell density and fluorescence, suggested that the high light levels may have caused photoinhibition. Consequently, fluorescence was not used as an estimate of cell density. In addition, significant differences among treatments were not detected and therefore only low light UVR treatment results are presented here.

Upper vs. Lower Growth Zone

Growth rates under low light PAR + UVR of the two upper zone species was significantly greater than growth rates of the two lower zone species at one (P = 0.0001), two (P = 0.0004) and three weeks (P = 0.0044) (Fig 6). Growth rates (dpd) of the two upper zone species, *C. affinis* and *A. lanceolata* var. *dubia,* ranged from 0.18 to 0.10 and 0.13 to ⁻0.04 respectively, whereas the two lower zone species, *S. ulna* and *F. construens* var. *venter*, had growth rates ranging from 0.05 to ⁻0.02 and 0.10 to ⁻0.12 respectively.

UVB vs. UVA

After one and two weeks, the combined growth rates of all species under PAR + UVA were not significantly different from those of all species under PAR + UVR (P = 0.54 for week one and P = 0.11 for week two). By week three, the growth rates of all species combined under PAR + UVA were significantly greater (P = 0.004) than those under PAR + UVR. Consequently, in week three the individual growth rates of *C. affinis* and *A. lanceolata* var. *dubia*, and *S. ulna* and *F. construens* var. *venter* were greater under PAR + UVA treatments than under PAR + UVR regardless of the growth zone of origin

(Fig. 7). Only the growth rate (dpd) of *F. construens* var. *venter*, the small lower growth zone diatom, had statistically significantly greater growth under PAR + UVA than under PAR + UVR.

Cell Size

Results of low light PAR + UVR treatments showed that the growth rates averaged over the two larger cells (*C. affinis* and *S. ulna*) were significantly greater than the growth averaged across the two small cells (*A. lanceolata* var. *dubia* and *F. construens* var. *venter*) at two ($P = 0.0013$) and three ($P = 0.0011$) weeks. There was no significant difference in growth rates between large and small celled diatoms at week one ($P = 0.3121$) under PAR + UVR (Fig. 8). Under PAR + UVA, no significant differences were detected in the growth rates of the averaged large-celled diatoms versus the averaged small-celled diatoms at one, two or three weeks (Fig. 9).

Discussion

Zonation

Growth rates of the two upper zone taxa (*C. affinis*, and *A. lanceolata* var. *dubia*) grown under PAR + UVR were significantly greater than the growth rates of the lower zone taxa (*S. ulna,* and *F. construens* var. *venter*) over the three-week experiment (Fig 6). This may be attributed to the prior UVR exposure, in their original collection site, of the upper growth zone taxa, *C. affinis* and *A. lanceolata* var. *dubia* as compared to the lower zone taxa, *S. ulna* and *F. construens* var. *venter,* which had never had UVA or UVB exposure. In addition, this indicates that UVA + UVB tolerance may be one of the

mechanisms influencing the vertical zonation of benthic diatoms in Lake McConaughy. Similar findings were reported by Dring et al. (1996) from a marine environment, where deep subtidal red algae were more sensitive to UVR exposure than the shallow subtidal red algal species. PAR as a primary mechanism dictating the vertical zonation of algae has been well documented (e.g., Evans and Stockner 1972), but species-specific light requirements, available substratum type, species growth form (Stevenson and Stoermer 1981, Jónsson 1987) and available nutrients also influence periphyton distribution (Oppenheim and Ellis-Evans 1989). In addition, Hoagland and Peterson (1990) reported that wave disturbance acts as a primary determinant of the distribution of epilithic diatoms such as *Cymbella affinis*.

The present study indicates that UVR affects periphyton zonation, although the role of PAR intensity is unclear. In this study, high light levels were photoinhibitive. A more complete understanding of UVR effects on algal distribution will require a determination of the depth at which UVR still inhibits algal growth, but photoinhibition caused by high light intensity no longer occurs. In more turbid water bodies, this may represent a very narrow, perhaps undetectable, zone of periphyton growth.

Photoinhibition

The relationship between light intensity and photosynthetic rate of benthic algae is a linear function at low irradiances. This response is essentially constant at moderate light intensities where light becomes saturating, then with increasing light intensities, the photosynthetic rate reaches a maximum or decreases due to photoinhibition (Hill 1996). Photoinhibition can be produced by light levels found at the surface of natural waters.

High light treatments under a 16:8 h light:dark cycle with PAR at ~850 μmol m^{-2} s^{-1}, representing light levels found in the shallow waters of Lake McConaughy, were apparently photoinhibited based on reduced growth rates.

Studies have shown that photoinhibition develops within a few minutes and can reverse after light intensity decreases in late afternoon (Neale and Richerson 1987). The dark period in our experiment perhaps was not long enough for recovery. Lake populations of *Asterionella* took 20 h to recover after a 6 h exposure to high levels of natural sunlight (Belay 1981). In our study, photoinhibition occurred under all high light treatments, but no significant pattern of photoinhibition was detected among treatments, due to high variability in cell doublings per day (Fig 5 & Table 2). Jokiel and York (1984) showed that some cultured marine algae were photoinhibited by UVA and by UVB or by PAR. The action spectrum of photoinhibition in spinach chloroplasts revealed that the main activity for photoinhibition was in the UVR region (Jones and Kok 1966).

Although photoinhibition of the lower growth zone diatoms was not surprising given the low light environment from which they were collected; photoinhibition of diatoms from the upper growth zone was unexpected. The high light intensity used (PAR = 850 μmol m^{-2} s^{-1}) was representative of the average daily dosage found in the upper growth zone in Lake McConaughy, but previous research by Hill (1996) reported that photoinhibition often occurs at irradiances $\geq$ 600 μmol m^{-2} s^{-1}. Moeller (1994) also reported photoinhibition, strong photobleaching of chlorophyll-*a* and depression of chlorophyll-specific photosynthesis in epilimnetic algae that had prior high light intensity

exposure history. Therefore, it is likely that these diatoms were photoinhibited in Lake McConaughy as well and that photosynthesis occurred at periods during the day prior to and after which light intensities had lessened. Findings of Belay (1981) reported that once photoinhibiting light was lessened or removed the cell could repair itself and begin to function normally. Apparently, at least some epilithic diatoms which occur in Lake McConaughy are not only relatively tolerant of UVR, but also capable of more rapid recovery from photoinhibition.

Photoreactivation

Photoreactivation, which occurs in diatoms, is accomplished by the enzyme photolyase, and is the photoenzymatic repair of DNA damage caused by exposure to UVB radiation (Karentz et al. 1991). Photoreactivation requires light at ~380-450 nm. Williams et al. (1979) found that lethal effects of far-UVR (254 nm) exposure to the blue-green alga, *Gloeocapsa alpicola*, could be repaired by immediate exposure to blue light (350-550 nm). If cells were not immediately exposed to blue-light after far UVR damage and instead were incubated in the dark or under non-photoreactivating growth conditions, their ability to photoreactivate was gradually lost. Subsequent research by O'Brien and Houghton (1982) confirmed that immediate exposure of this alga to photoreactivating light restored cells to pre-irradiation levels and that excision repair inhibitors (i.e. caffeine and acriflavin) delayed the gradual loss of photoreversibility during dark periods. The wavelengths required for photoreactivation include those involved in photosynthesis (Halldal and Taube 1972). Epibenthic and epiphytic algae survive at low flux densities, with photosynthetic production at PAR between 0 and 7

μmol m^{-2} s^{-1} (Jorgensen et al. 1987). Thus, the PAR + UVA level used in our low light experiment likely was sufficient for photoreactivation to occur.

Effects of UVR

Diatom growth under PAR + UVA was significantly greater than growth under PAR + UVR after the third week (Fig 7). Worrest et al. (1978) detected a 20% decrease in diatom growth under artificial UVB exposure after four weeks. Bothwell et al. (1993) found that the inhibitory effect of 90% of incident PAR + UVR on algal accumulation was reversed after 3-4 weeks, and by week 5 total diatom abundance exposed to PAR + UV was two to four-fold greater than in communities protected from UVR. A subsequent study (Bothwell et al. 1994) showed that this reversal was an indirect effect due to a reduction in herbivores by UVR. The results from the present research give further insight into these findings; they indicate that even in the absence of grazers there was a significant reduction after three weeks in the growth of the dominant species of large and small diatoms exposed to UVR. Furthermore, the effects of PAR + UVR were significantly greater than the effects of PAR + UVA alone which concurs with previous research on algae that has shown UVB to be more damaging than UVA at typical UVA:UVB ratios found in nature.

Cell Size

At two and three weeks into the experiment growth rates averaged across the two large-celled diatoms were significantly greater than the growth rates averaged across two small-celled diatoms under PAR + UVR treatments (Fig 8). In addition, this significant difference increased from week two to week three, indicating that longer exposure times

affected small cells more than large cells. Community-level studies on periphyton have revealed 30 to 42% reductions in algal growth due to UVR, as well as community dominance shifting from smaller to larger-celled diatoms (Bothwell et al. 1993). This may have resulted from increased damage to the DNA of the smaller-celled diatoms. Karentz et al. (1991) exposed Antarctic phytoplankton to UVB radiation with subsequent exposure to visible radiation, and found that because small cells have fewer UV-absorbing compounds than larger cells they were more susceptible to UVR than larger cells (especially under UVB exposure). Garcia-Pichel (1994) reported that the amount of UVB penetrating blue-green algal cells is also dependent on cell size and the efficiency of the UV-absorbing matter in the cell.

To better understand the zonation of periphytic algae with depth, a light gradient analysis should be conducted to ascertain the depth at which UVR is still inhibiting but photoinhibition, due to high light intensity, no longer occurs. This experiment should be repeated using a reduced high light level treatment (i.e., a light level which does not cause photoinhibition). Cell size distribution needs to be evaluated to examine whether there is a greater number of large-celled versus small-celled taxa located in the upper growth zone. Finally, an *in situ* experiment needs to be run to determine if there are differences, because of light attenuation, in UVR tolerance between upper and lower zone species within a biofilm located in the upper growth zone.

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TABLES

Table 1. Correlation coefficients for fluorescence versus cell density under low light PAR, PAR + UVA and PAR + UVA + UVB.

Species	PAR	PAR + UVA	PAR + UVA + UVB
Cymbella affinis	0.8	0.9	0.9
Achnanthes lanceolata* var. *dubia	0.97	0.86	0.84
Synedra ulna	0.93	0.95	0.73
Fragilaria construens* var. *venter	0.96	0.84	0.72

Table 2. Correlation coefficients for fluorescence versus cell density under high light PAR, PAR + UVA and PAR + UVA + UVB treatments.

Species	PAR	PAR + UVA	PAR + UVA + UVB
Cymbella affinis	0.49	0.67	0.82
Achnanthes lanceolata* var. *dubia	0.18	-0.72	-0.03
Synedra ulna	-0.04	-0.19	0.56
Fragilaria construens* var. *venter	-0.16	-0.1	0.16

FIGURES

Figure 1. Growth rates in cell doublings per day (dpd) of *Cymbella affinis*, *Achnanthes lanceolata* var. *dubia*, *Synedra ulna* and *Fragilaria construens* var. *venter* exposed to a temperature gradient under a 12:12 light:dark cycle (PAR = 55.62 μmol m^{-2} s^{-1}).

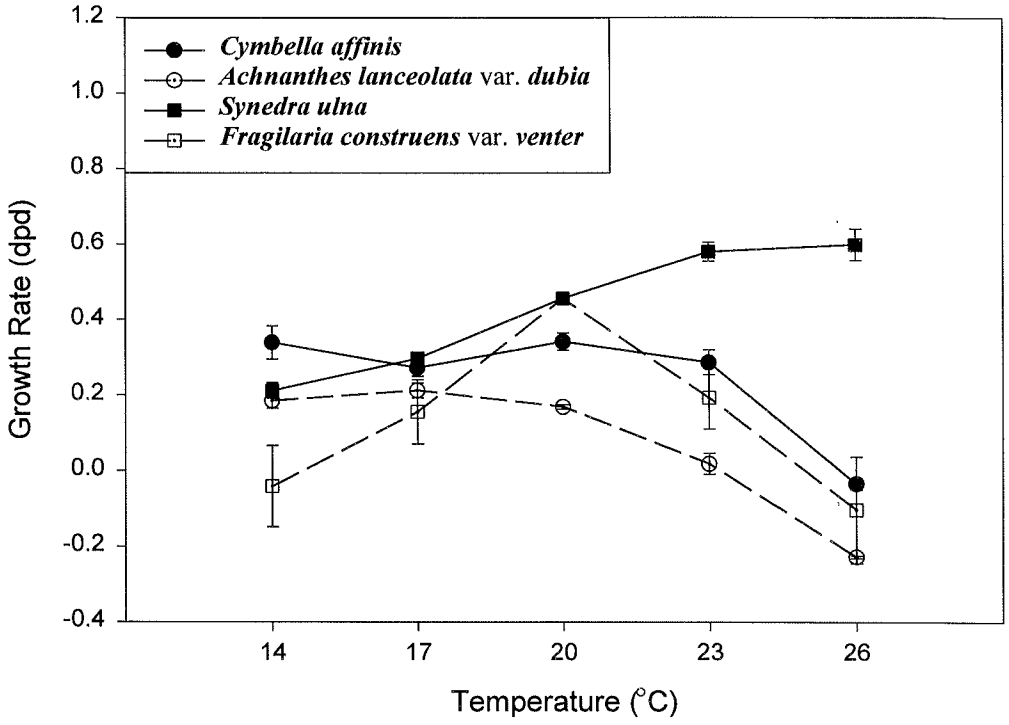

Cymbella affinis
Achnanthes lanceolata var. dubia
Synedra ulna
Fragilaria construens var. venter
Growth Rate (dpd)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
14
17
20
23
26
Temperature (°C)

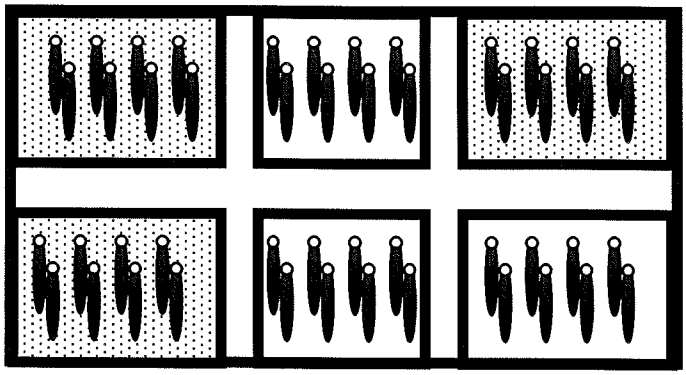
Figure 2. Diagrammatic representation of the experimental set-up and the assigned treatments for blocks A, B, & C. Each block contained six plots with eight test tubes in each plot.

B

6: PAR + UVA + UVB (low)

5: PAR + UVA (high)

4: PAR only (low)

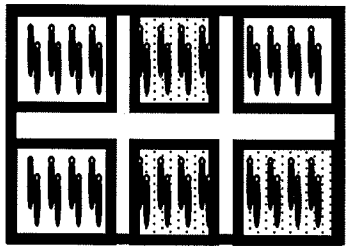


1: PAR + UVA (low)

2: PAR only (high)

3: PAR + UVA + UVB (high)

A



C

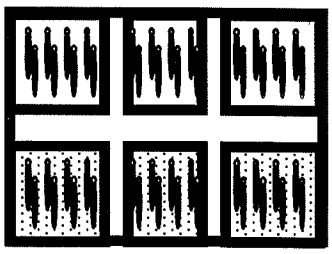


Figure 3. Spectral irradiance for upper (— ; 1 m) (right scale) and lower (---; 8 m) (left scale) growth zones recorded on August 12, 1995 at 1250 and 1300 CDT, respectively in Lake McConaughy.

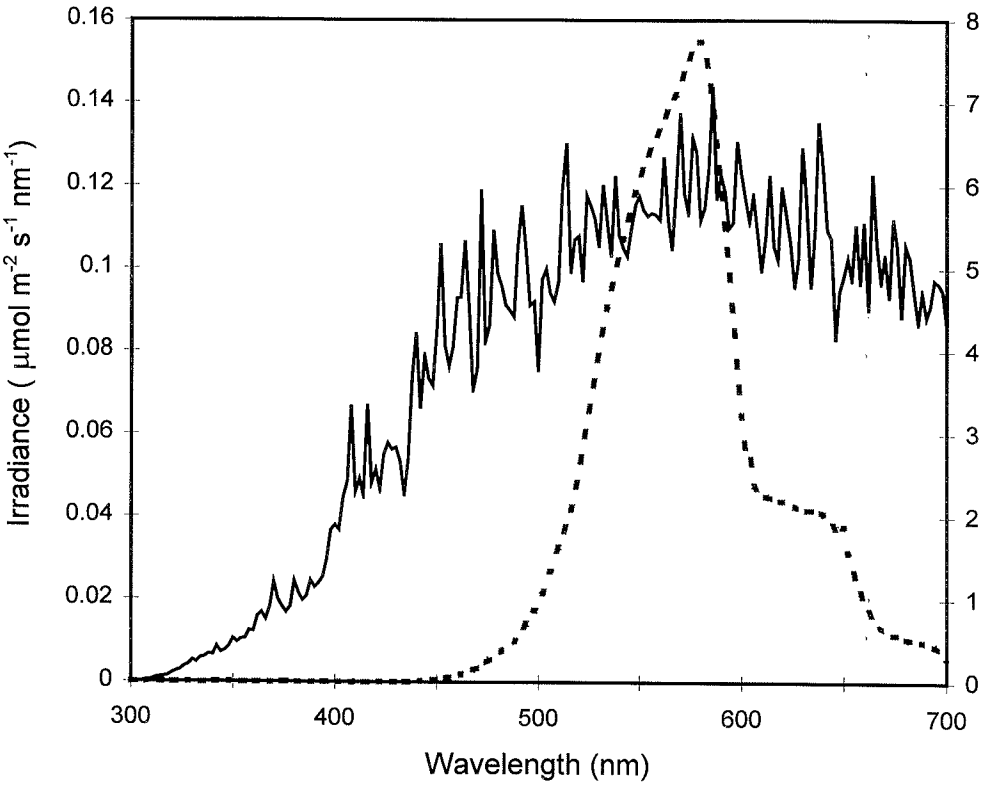

0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
8
7
6
5
4
3
2
1
0
300
400
500
600
700
Wavelength (nm)
Irradiance (µmol m-2 s-1 nm-1)

Figure 4. Growth rate in cell doublings per day (dpd), of *Cymbella affinis*, *Achnanthes lanceolata* var. *dubia*, *Synedra ulna* and *Fragilaria construens* var. *venter* across a light gradient (14, 51, 107, 515 and 855 μmol m^{-2} s^{-1}) at 20° C, on a 16:8 light:dark cycle for 12 days.

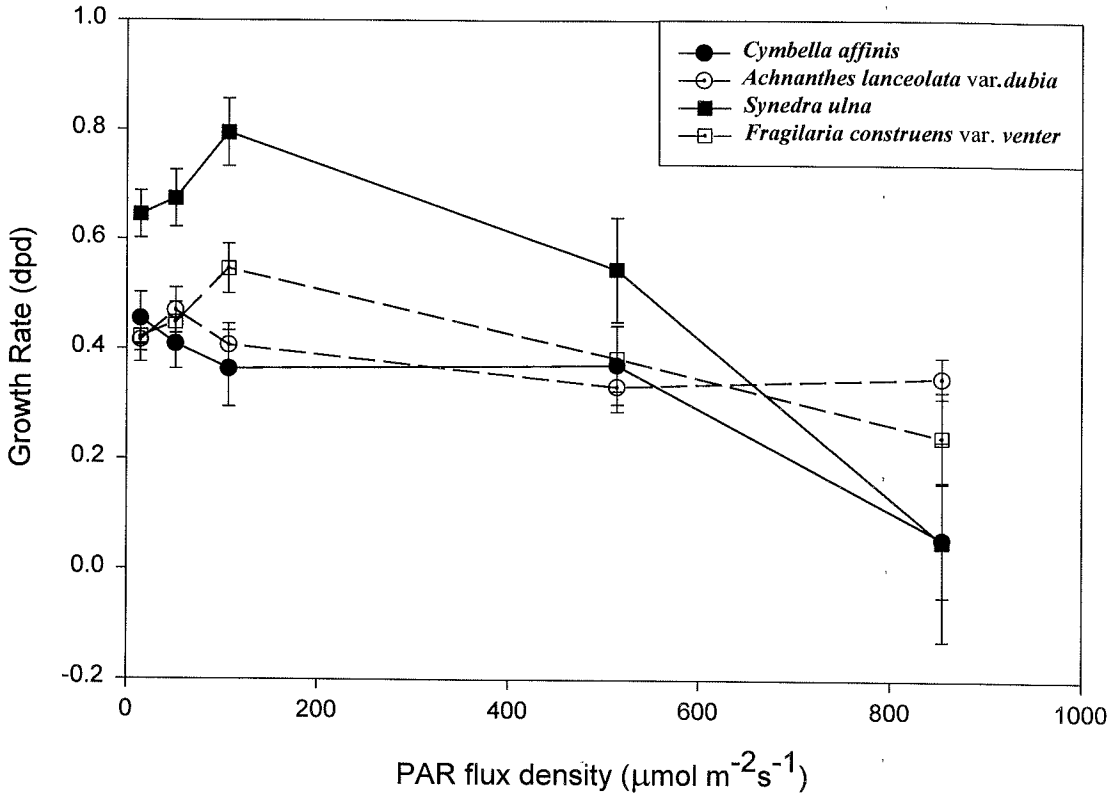

Cymbella affinis
Achnanthes lanceolata var.dubia
Synedra ulna
Fragilaria construens var. venter
Growth Rate (dpd)
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0
200
400
600
800
1000
PAR flux density (µmol m-2s-1)

Figure 5. Growth rates in cell doublings per day (dpd) of *Cymbella affinis(Ca)*, *Achnanthes lanceolata* var. *dubia (Al)*, *Synedra ulna (Su)* and *Fragilaria construens* var. *venter (Fc)* exposed to high light (PAR = 850 μmol m^{-2} s^{-1}) PAR + UVA, PAR + UVA + UVB and PAR only treatments.

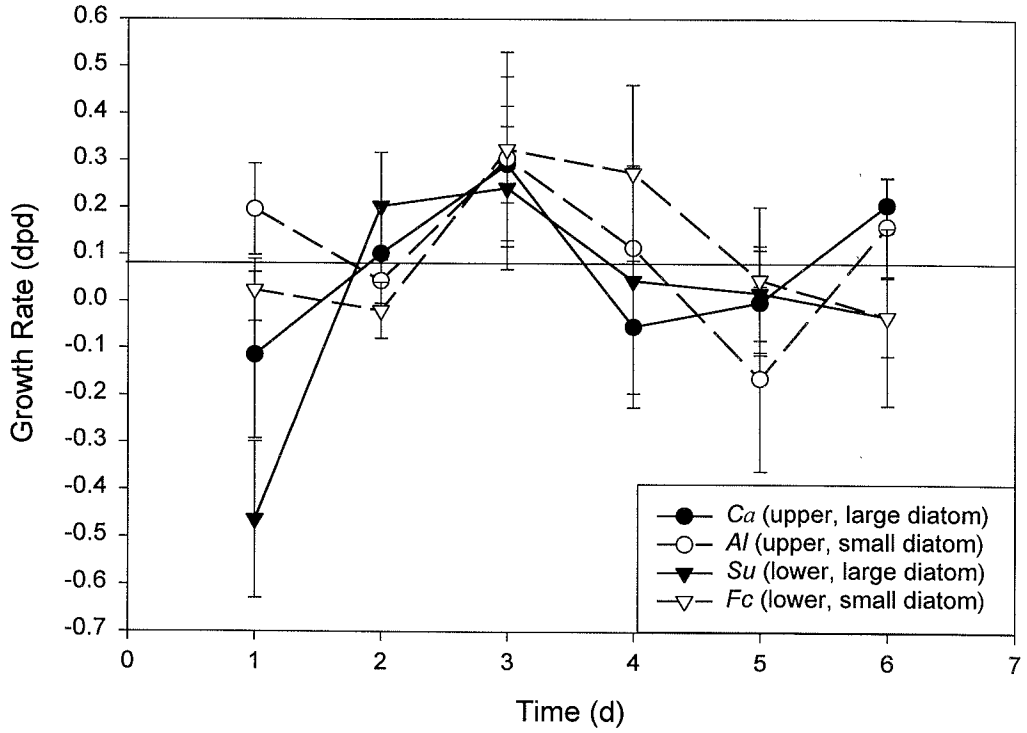

0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
Growth Rate (dpd)
0
1
2
3
4
5
6
7
Time (d)
Ca (upper, large diatom)
Al (upper, small diatom)
Su (lower, large diatom)
Fc (lower, small diatom)

Figure 6. Comparisons of upper *(Cymbella affinis* and *Achnanthes lanceolata* var. *dubia)* versus lower (*Synedra ulna* and *Fragilaria construens* var. *venter*) growth zone diatoms under PAR + UVA + UVB. Growth rates in cell doublings per day (dpd) standardized to PAR treatment (+1 S.E.).

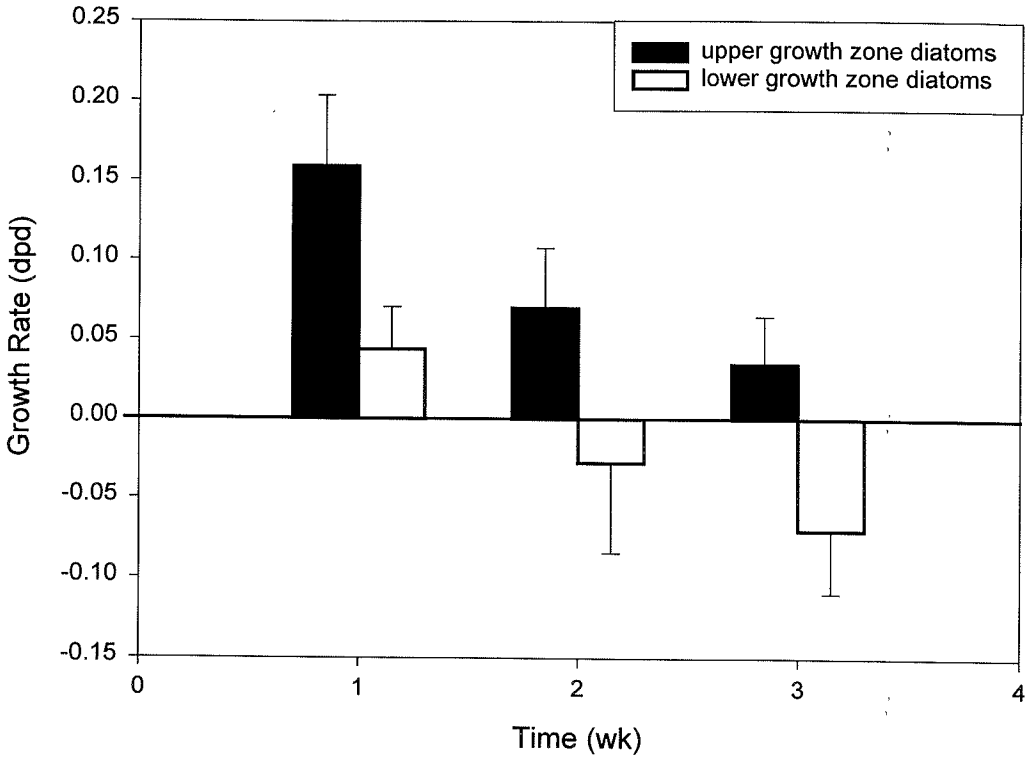

upper growth zone diatoms
lower growth zone diatoms
Growth Rate (dpd)
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
0
1
2
3
4
Time (wk)

Figure 7. Growth rate in cell doublings per day (dpd) of *Cymbella affinis* (*Ca*), *Achnanthes lanceolata* var. *dubia* (*Al*), *Synedra ulna* (*Su*) and *Fragilaria construens* var. *venter* (*Fc*) under low light, standardized to PAR treatment (+1 S.E.).

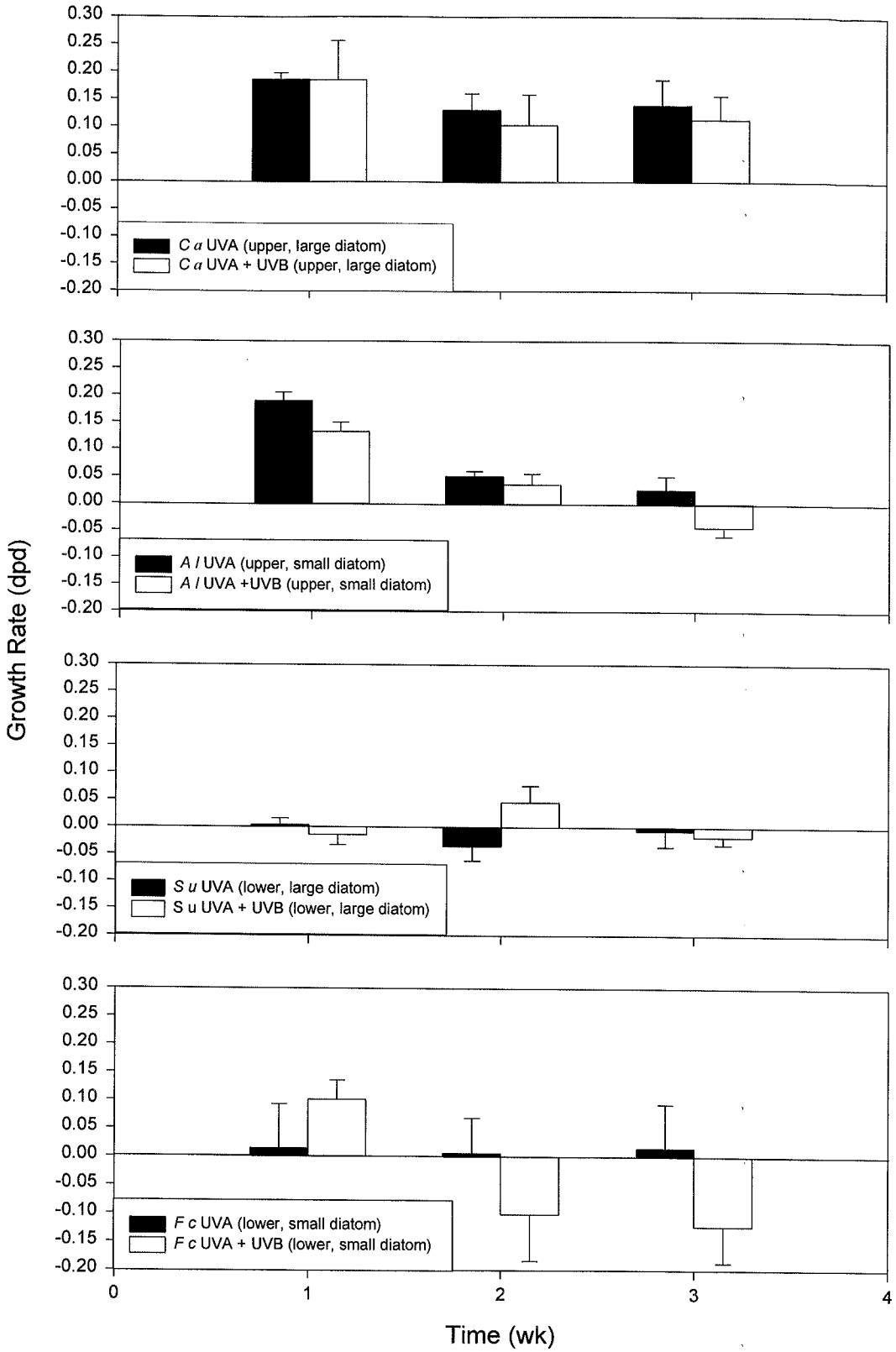

0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
C a UVA (upper, large diatom)
C a UVA + UVB (upper, large diatom)
A l UVA (upper, small diatom)
A l UVA +UVB (upper, small diatom)
S u UVA (lower, large diatom)
S u UVA + UVB (lower, large diatom)
F c UVA (lower, small diatom)
F c UVA + UVB (lower, small diatom)
Growth Rate (dpd)
0
1
2
3
4
Time (wk)

Figure 8. Comparisons of large *(Cymbella affinis* and *Synedra ulna)* versus small-celled (*Achnanthes lanceolata* var. *dubia* and *Fragilaria construens* var. *venter*) diatoms under PAR + UVA + UVB. Growth rates in cell doublings per day (dpd) standardized to PAR treatment (+1 S.E.).

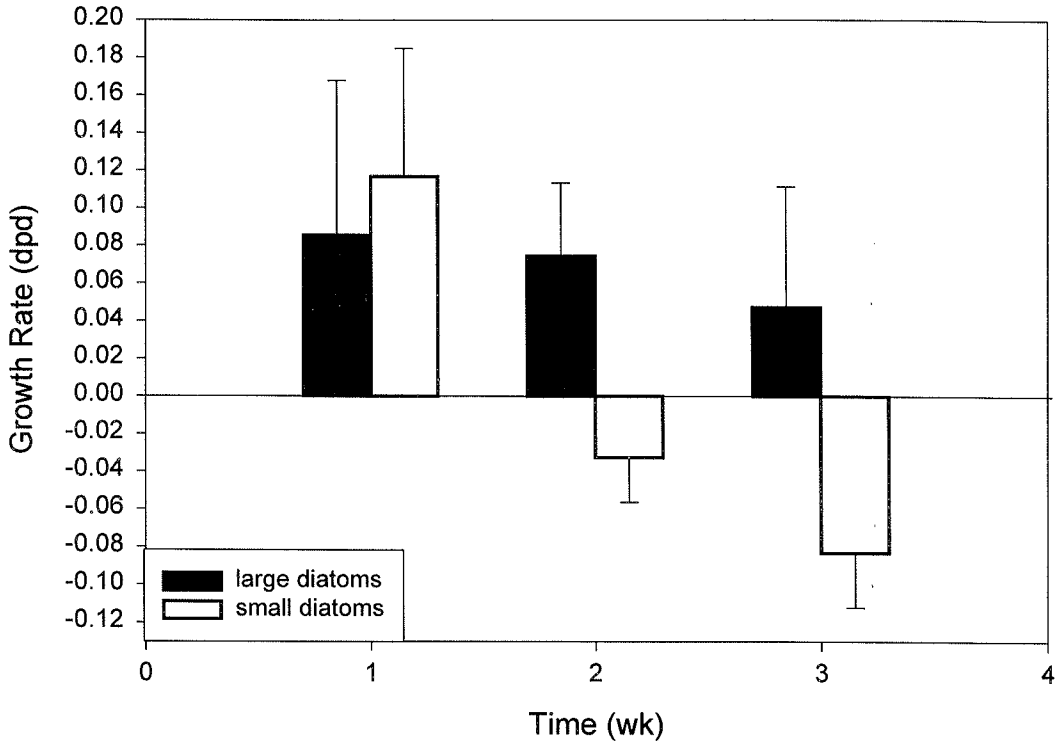
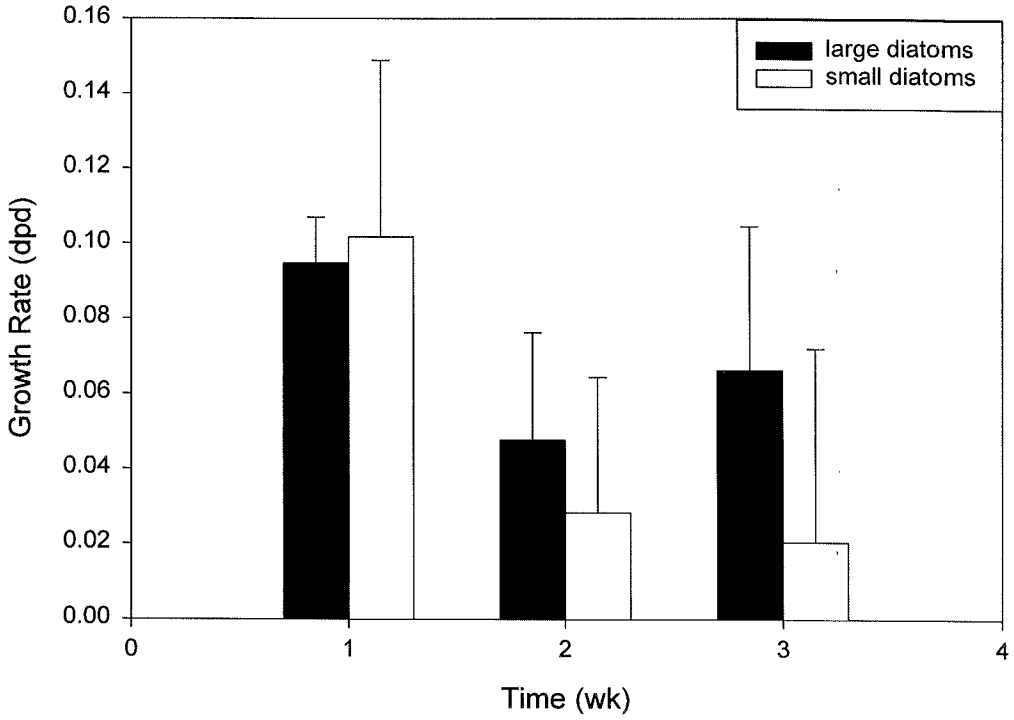

0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
-0.10
-0.12
Growth Rate (dpd)
large diatoms
small diatoms
0
1
2
3
4
Time (wk)

Figure 9. Comparisons of large *(Cymbella affinis* and *Synedra ulna)* versus small-celled (*Achnanthes lanceolata* var. *dubia* and *Fragilaria construens* var. *venter*) diatoms under PAR + UVA. Growth rates in cell doublings per day (dpd) standardized to PAR treatment (+1 S.E.).


0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Growth Rate (dpd)
0
1
2
3
4
Time (wk)
large diatoms
small diatoms