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EFFECT OF LIGHT ON VERTICAL MIGRATION AND PHOTOSYNTHESIS OF *EUGLENA PROXIMA* (EUGLENOPHYTA)[1]

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During daytime low tides, sediment-inhabiting populations of *Euglena proxima* Dangeard migrate to the surface of intertidal sand flats to form large monospecific green patches near the Duke University Marine Laboratory in Beaufort, North Carolina. The effect of incident irradiance on the upward migration of *E. proxima* was studied by shading the sediment surface in the field with neutral density filters to 2, 20, 55, and 100% incident irradiance. The results of these *in situ* experiments revealed that *E. proxima*'s upward migration, as measured by population density or chl *a* concentration, was stimulated by decreasing incident irradiance: population densities and chl *a* concentrations on the sediment surface at 2% incident irradiance were greater statistically than at 100% full sunlight. *In situ* measurements of photosynthesis were performed by incubating 3-mL aliquots of a cell suspension in scintillation vials containing ^{14}C inorganic carbon beneath neutral density filters. These experiments revealed that *E. proxima* exhibited photoinhibition at 100% full sunlight, but this depression in photosynthetic performance at high light was absent when the cells were incubated in vials containing acid-cleaned sediment. This amelioration of photoinhibition in vials containing sediment is most likely due to the downward migration of the population away from photoinhibitory light levels. These results cannot be interpreted as strictly photic effects because the temperature of the vials varied with the light treatment. The results of this study suggest that the vertical migration behavior of *E. proxima* and its photosynthetic physiology are intimately linked. Vertical migration provides a mechanism to deal with short-term changes in irradiance and avoid photoinhibitory light levels.

Key index words: **Benthic microalgae; *Euglena proxima;* irradiance; North Carolina; photoinhibition; photosynthetic performance; vertical migration**

Abbreviations: **I_o, incident irradiance; P_{max}, maximum photosynthetic rate**

Dense patches of green and brown appear on the surface of intertidal sand flats around Beaufort, North Carolina, following aerial exposure by the receding tide. The formation of these patches results from the upward migration of unicellular algae from deeper layers in the sand. These patches disappear prior to submergence by the next flood tide, when an endogenous rhythm directs the microalgae to migrate down into the sediment (Fauré-Fremiet 1951, Callame and Debyser 1954, Palmer and Round 1965, 1967, Round and Happey 1965, Hopkins 1966a, b, Round and Palmer 1966, Happey-Wood and Jones 1988). Benthic microalgal assemblages are often composed of many species (Elliott and Bamforth 1975, Sullivan 1975, 1978, 1982, Cook and Whipple 1982, Sullivan and Moncreiff 1988), but monospecific patches also occur (Bracher 1919, 1938, Ganapati et al. 1959, Taylor 1967, Kingston 1990).

The vertical migration behavior of benthic microalgae on sand and mud flats was first reported by Fauvel and Bohn (1907) over 90 years ago. Since that time, vertical migration behavior has been observed in benthic species of chrysomonads (Fauré-Fremiet 1951), cyanobacteria (Palmer and Round 1967), dinoflagellates (Laurie 1913, Herdman 1924, Ganapati et al. 1959), euglenoids (Bracher 1919, 1929, 1938, Palmer and Round 1965, Round and Palmer 1966, Kingston 1990), and diatoms (Fauvel and Bohn 1907, Aleem 1950, Fauré-Fremiet 1951, Callame and Debyser 1954, Perkins 1960, Hopkins 1963, 1964, 1966a, b, Round and Happey 1965, Round and Palmer 1966, Harper 1969, 1976, Round 1978, 1979, Paterson 1986). The existence of vertical migration behavior in members of such diverse taxa, and from both tidal and nontidal habitats, suggests that this behavior confers some advantageous survival strategy on motile microalgae inhabiting soft-bottom habitats.

Numerous hypotheses have been formulated to explain the ecological significance of this behavior (Ganapati et al. 1959, Round and Happey 1965, Round and Eaton 1966, Heckman 1985, Happey-Wood and Jones 1988). Common to all these hypotheses is the idea that upward migration benefits the microalgae by placing them on the surface during low tide, when incident irradiance is highest. This study was undertaken to investigate this idea that upward migration is a feature that optimizes photosynthesis.

Much of the literature concerning the effect of light on the migration of microalgae deals with endogenous rhythms in phototaxis and not direct re-

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sponses to changes in irradiance (Round and Palmer 1966, Palmer and Round 1965, Round and Happey 1965, Hopkins 1964, 1966a). However, there are numerous anecdotal observations regarding ambient light conditions and the timing of migration that also suggest the importance of light for this behavior. Both Aleem (1950) and Perkins (1960) noted that downward migration of some diatom assemblages is delayed until there is sufficient water cover to reduce the irradiance below a critical level necessary to maintain the population at the surface. Hopkins (1966a) reported that the benthic diatom community in the River Ouse remains below the mud surface on bright summer days and even suggested that high light levels might have an inhibitory effect on upward migration. The importance of light is further supported by its overriding effect on the maintenance of the tidally induced migration behavior of some species; Hopkins (1963, 1966b) reported that diatoms surface out of phase with tidal behavior on bright days in winter when covered by calm, clear water.

The necessity of light for the maintenance of vertical migration is supported by the observations that benthic microalgae do not migrate to the surface during nighttime low tides or during the day under an opaque canister (Palmer and Round 1965, 1967). The argument that light is simply a directional cue for upward movement out of the sediment is countered by the fact that microalgae already on the surface will reburrow if covered by an opaque canister (Palmer and Round 1965, 1967). It seems clear that changes in irradiance can have an overriding effect on the expression of the vertical migration behavior.

This study was undertaken to investigate the value of being on the surface during low tide for *Euglena proxima* Dangeard. To accomplish this task, the upward migration and photosynthetic response of this species to changes in the incident irradiance at the sediment surface was examined. *E. proxima* is a highly motile species that uses its anterior flagellum to swim in the interstitial pore water surrounding the sand grains of the tidal flat (Kingston 1990). This species forms monospecific patches on the intertidal sand flats of Beaufort, North Carolina. Initial field observations revealed that this species forms denser, more obvious patches on cloudy days than on sunny days. Anecdotal observations appear in the literature concerning the effect of especially bright (Hopkins 1966a) or overcast (Aleem 1950) days on diatom surfacing; however, no one has experimentally tested the effect of discrete changes in light on upward migration on a given day. These data are necessary if natural daily variation in population density, timing of migration, and physiological acclimatization to light are to be eliminated as confounding factors.

Three specific questions were addressed by this study. First, does *E. proxima* show similar behavioral and photosynthetic responses to changes in the quantity of incident irradiance on the sediment surface? Second, is the migration response of this species somehow related to its photosynthetic physiology? Finally, can this benthic species use vertical migration as a strategy that optimizes photosynthesis in the face of short-term changes in its light environment? These questions were investigated by using neutral density filters to examine the effect of shading on upward migration and photosynthesis of *E. proxima in situ*. Photosynthetic rates in the presence and absence of acid-washed sand were measured to determine whether vertical migration could have any effect on photosynthetic performance and to study whether downward migration might be used to escape photoinhibitory light levels at the sediment surface. In the present study, the traditional definition of photoinhibition as the light-dependent inhibition of photosynthesis at supersaturating irradiances is used. The actual mechanisms that cause this decline in photosynthetic rate, whether they be actual damage to photosystem II or mechanisms better classified as photoregulation or photoprotection (Krause 1988, Henley 1993), are not considered here.

MATERIALS AND METHODS

This study was conducted in the Newport River estuary on sand flats abutting the eastern seawall of the Duke University Marine Laboratory, which is located on Pivers Island, Beaufort, North Carolina. All vertical migration and *in situ* photosynthetic experiments were conducted on unialgal patches of *Euglena proxima* Dangeard located 0.6 m above mean low water in May 1988.

Vertical migration experiments. The upward migration of cells to the sediment surface was monitored by using the lens paper collection technique of Palmer and Round (1965), which was analyzed in detail by Eaton and Moss (1966). Following aerial exposure of the sand flat, sixteen 7 × 10-cm rectangles of lens paper (Filtration Coffee App. Co.; white chemical wood lens tissue) were placed on the sediment surface in a 4 × 4 arrangement prior to the appearance of the microalgal patch. An area within the patch with a relatively homogeneous distribution of *E. proxima* was located on the previous day to minimize the variability among treatments.

Two small lens paper squares (18 × 18 mm) were positioned near the center of each of the large lens paper rectangles. After 1 h, one lens paper square was removed and placed in a 20-mL scintillation vial on ice for chl *a* analysis. After removal of the baseline lens paper square, a neutral density filter was positioned over each lens paper rectangle. The neutral density filters consisted of a 9 × 12-cm wire frame covered with one or more layers of nylon window screening that attenuated the incident irradiance (I_o). A single wire support pushed into the sediment held the filter 2 cm above the sediment surface. Four filters were constructed with one layer of nylon screening (55% I_o), four filters with three layers of screening (20% I_o), and four filters with six layers of screening (2% I_o). Four frames without nylon screening served as controls (100% I_o). These 16 filters were arranged in a Latin square design so that none of the four light treatments appeared more than once in each of the four rows or columns of the square.

Continuous readings of ambient irradiance (400–700 nm) were collected with a spherical quantum sensor (Li-Cor; Model LI-193SA) and stored on a data logger (Li-Cor; Model LI-1000) during the course of all experiments. The incident irradiance transmitted to the sediment surface under the neutral density filters was later calculated with a correction factor that was obtained by simultaneously comparing the irradiance under the filters as measured by a cosine-corrected quantum sensor (Li-Cor; Model LI-

192SA) and the ambient irradiance measured by the spherical quantum sensor.

In addition to altering the irradiance incident on the sediment surface, the neutral density filters also were likely to affect sediment temperature. To assess this temperature effect, the sediment temperature under each neutral density filter was measured with a telethermometer (YSI; Model 44A) on a sunny afternoon while continuous irradiance measurements were collected using the spherical quantum sensor and data logger. All measurements were made during a 15-min interval when the mean irradiance measured by the spherical quantum sensor and data logger was 2562 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The highest and lowest recorded irradiances during this interval did not deviate from the mean by more than 1.5%. These data were analyzed using SigmaStat (Jandel, Version 1.01) employing a one-way analysis of variance (ANOVA) and Bonferroni *t*-tests of differences among means.

After another hour, the remaining lens paper square was removed and placed in a 20-mL scintillation vial on ice for laboratory analysis. The lens paper squares in the scintillation vials were extracted in 10 mL of 90% acetone at −5° C for 48 h. After extraction, the chl *a* content of each lens paper extract was measured with a fluorometer (Turner Designs; Model 10–005B) and the chl *a* density computed (μg chl $a \cdot m^{-2}$). The fluorometry methodology employed in this study is fully described by Parsons et al. (1984). Alternatively, the lens paper squares were mounted on glass microscope slides in the field with 0.1% phenol in Karo brand corn syrup when cell count data were desired. After preliminary experimentation, chl *a* extraction was favored over cell counts because of the high variability resulting from counting random fields of cells. The single chl *a* value for each lens paper square was a more reliable estimate of total cell biomass.

Final chl *a* density (μg chl $a \cdot m^{-2}$) was used in the analysis of these experiments because the baseline chl *a* values were extremely low and uniform. These baseline samples, which were collected 1 h after aerial exposure, confirmed that the light treatments were applied prior to the emergence of *E. proxima* on the surface of the sand flat. Consequently, the results of these migration experiments reveal the effect of shading on upward migration of interstitial cells. These results were evaluated with SAS statistical software using a one-way ANOVA and Bonferroni *t*-tests of differences among means (SAS Institute, Cary, North Carolina). When necessary, the data were logarithmically transformed to satisfy the assumptions of ANOVA.

Photosynthesis experiments. Photosynthesis measurements were conducted on 13 and 30 May 1988 in 20-mL glass scintillation vials using ^{14}C radioisotope techniques outlined in Parsons et al. (1984). Photosynthesis was measured concurrently with upward migration using the previously described procedure on 13 May 1988. During low tide, *E. proxima* cells were collected using the lens paper technique and then suspended in filtered estuary water (Millipore; HA type; 0.45 μm pore size). The density of this cell suspension was later measured in the lab with an improved Neubauer phase haemacytometer (American Optical Co.). As a result of natural changes in microalgal abundance, the cell suspension used on 30 May (26.3×10^4 cells·vial^{-1}) was more than twice the density of that used on 13 May 1988 (12.4×10^4 cells·vial^{-1}). Three-mL aliquots of cell suspension were transferred into 16 empty 20-mL glass scintillation vials and 16 vials containing a 5-mm depth of acid-washed sand (4 g). This sand had been previously collected at the experimental site and acid washed four times with concentrated hydrochloric acid followed by a 3-h soak to kill all living organisms and remove any bicarbonate. After the acid treatment, the sand was rinsed repeatedly with distilled water (until the rinse water was at a neutral pH), oven dried, and finally autoclaved. Each of the 16 vials containing sand (hereafter called the sediment treatment) was paired with a sediment-free vial under a 20 × 20-cm neutral density filter similar to those described previously. These larger filters attenuated the light to approximately the same degree as the smaller filters and were arranged in a Latin square spatial arrangement. After a 1-h preincubation period, each uncapped vial was inoculated with 9250 Bq $NaH^{14}CO_3$ (0.1 mL), swirled vigorously, and replaced under the light filter. After a 1.5-h incubation, three drops of buffered 5% formalin in seawater were added to each vial to kill the cells. The vials were capped, placed in a dark box, and brought into the laboratory for immediate processing.

The liquid contents of each vial were vacuum filtered through a glass fiber filter (Gelman Sciences; Type A/E; 1 μm nominal pore size). Each vial was rinsed with 3–5 mL of filtered Newport River estuary water (Millipore Corp.; Type HA; 0.45 μm pore size) twice, and these rinses were decanted onto the same filter disk. The sand in the sediment treatment vials was retained within the original incubation vial. The filter disks were placed in the bottom of 20-mL glass scintillation vials and acidified to pH 3 with three drops of dilute HCl to release any unfixed bicarbonate. In addition, the original scintillation vials containing sediment were acidified to pH 3 with dilute HCl and then air dried in a ventilation hood for 3 days.

A 10-mL aliquot of scintillation cocktail (DuPont; New England Nuclear Research Products; Aquasol-2) with 1% phenethylamine was added to the vials containing filter disks, and then the activity of each sample was counted in a liquid scintillation counter (Beckman Instruments; Model LS 8000) on the following day. A 10-mL aliquot of scintillation cocktail was then added to the vials containing the dried sediment, and these sediment samples were counted 3 days later to ensure total extraction of the sediment. The filter disk and sediment counts for the sediment treatment samples were summed to yield the total radioactivity incorporated into the cells in sediment. The radioactivity remaining in the sediment never exceeded 1% of that in the filter disks.

The specific activity of the ^{14}C-labeled bicarbonate incorporated into *E. proxima* cells over the course of the 1.5-h incubation was used to calculate the photosynthetic rate (pg C·cell^{-1}·h^{-1}). The total activity of ^{14}C fixed by *E. proxima* was divided by the specific activity of the inorganic carbon in the water in the incubation vials and then divided by the number of *E. proxima* cells and the incubation time (Parsons et al. 1984). The total inorganic carbon content of the water was calculated by titration with hydrochloric acid using the procedures described in Parsons et al. (1984). The presence of acid-washed sand did not affect the total carbon dioxide content in the treatment vials containing sediment. Statistical analysis of these experiments was similar to that described previously.

RESULTS

The results of the ANOVA indicate that temperature of the sediment surface was decreased significantly ($P < 0.0001$) when covered by neutral density filters. Bonferroni *t*-tests of differences among means revealed a linear decrease in temperature under the filters composed of zero (100% I_o), one (55% I_o), and three (20% I_o) layers of neutral density screening (Fig. 1). There was no statistical difference between the sediment temperatures at 20% I_o and 2% I_o.

Shading with neutral density filters affected the number of *E. proxima* on the sediment surface ($P = 0.001$). The Bonferroni *t*-tests for differences among means of the log-transformed cell densities revealed that the cell density under 2% I_o was significantly higher than under 55% I_o and 100% I_o (Fig. 2). In addition, this general trend of higher cell densities on the surface under the lowest prescribed light level was observed in experiments using chl *a* biomass as a measure of algal population density (Figs. 3, 4). Although a similar pattern was observed on different days, the chl *a* biomass on 13 May 1988 (Fig. 3) was approximately three times less than that on 30 May 1988 (Fig. 4). Both cell density and chl *a* biomass confirm that a decrease in the amount of light trans-

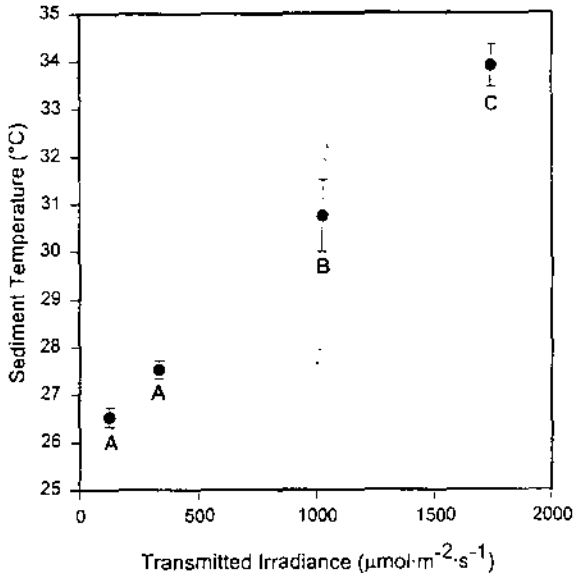

FIG. 1. Sediment temperature as a function of irradiance ($\mu mol \cdot m^{-2} \cdot s^{-1}$) under neutral density filters corresponding to 2% incident irradiance (I_o), 20% I_o, 55% I_o, and 100% I_o. Data represent the mean of four replicates, and the standard errors are indicated by error bars. The letters under the error bars represent the results of Bonferroni *t*-tests for differences among means ($P < 0.05$).

mitted to the sediment surface resulted in a concomitant increase in the *E. proxima* population located on the surface.

Investigations of vertical migration and photosyn-

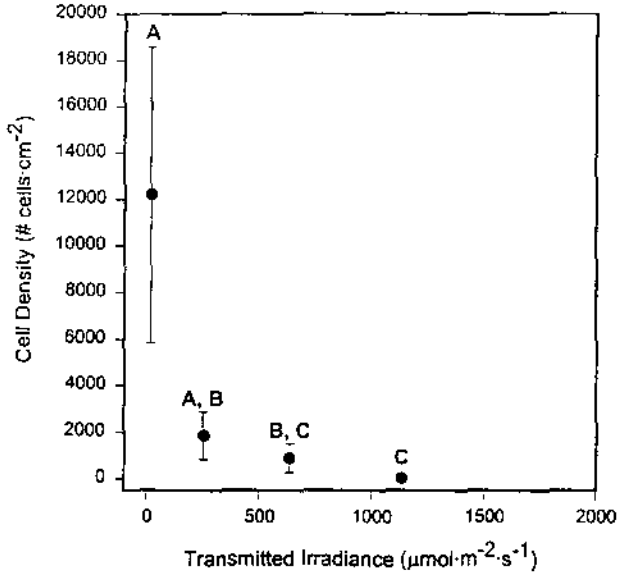

FIG. 2. Density of *Euglena proxima* (# cells·cm^{-2}) on the sediment surface as a function of irradiance ($\mu mol \cdot m^{-2} \cdot s^{-1}$) under neutral density filters corresponding to 2% incident irradiance (I_o), 20% I_o, 55% I_o, and 100% I_o on 1 May 1988. Data represent the mean of four replicates, and the standard errors are indicated by error bars. The letters above the error bars represent the results of Bonferroni *t*-tests for differences among log-transformed means ($P < 0.05$).

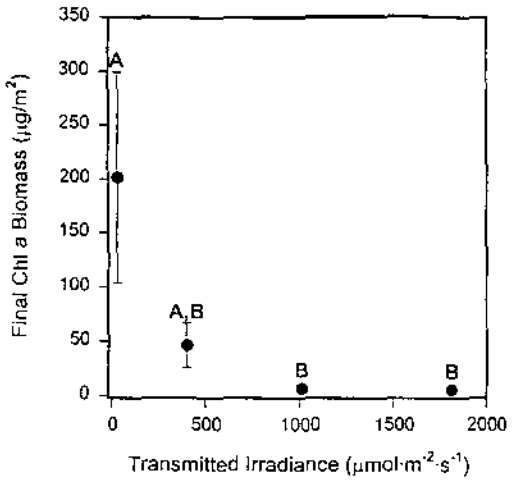

FIG. 3. Chlorophyll *a* biomass (μg chl $a \cdot m^{-2}$) as a function of irradiance ($\mu mol \cdot m^{-2} \cdot s^{-1}$) under neutral density filters corresponding to 2% incident irradiance (I_o), 20% I_o, 55% I_o, and 100% I_o on 13 May 1988. This experiment was run concurrently with the photosynthetic experiment of 13 May 1988 (Fig. 5). Data represent the mean of four replicates, and the standard errors are indicated by error bars. The letters above the error bars represent the results of Bonferroni *t*-tests for differences among log-transformed means ($P < 0.05$).

thesis of *E. proxima* on 13 May 1988 revealed a correspondence between the light levels that resulted in greater upward migration and those that supported higher photosynthetic rates (Figs. 3, 5A). The chl *a* biomass at 100% I_o was significantly less than that at 2% I_o (Fig. 3). This inverse trend was also observed in the ^{14}C photosynthetic incubations, in which the mean carbon fixation rate at 100% I_o was significantly lower than the other three light lev-

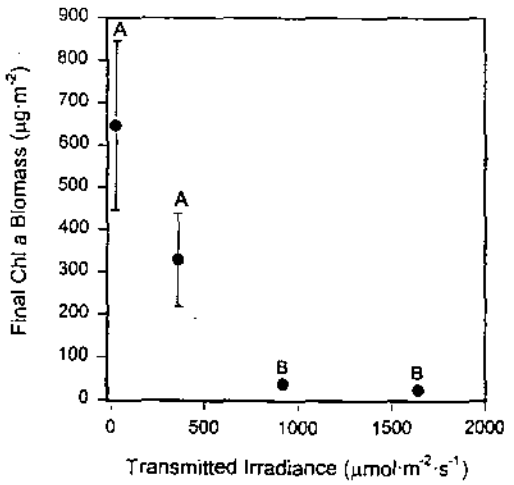

FIG. 4. Chlorophyll *a* biomass (μg chl $a \cdot m^{-2}$) as a function of irradiance ($\mu mol \cdot m^{-2} \cdot s^{-1}$) under neutral density filters corresponding to 2% I_o, 20% I_o, 55% I_o, and 100% I_o on 30 May 1988. Data represent the mean of four replicates, and the standard errors are indicated by error bars. The letters above the error bars represent the results of Bonferroni *t*-tests for differences among log-transformed means ($P < 0.05$).

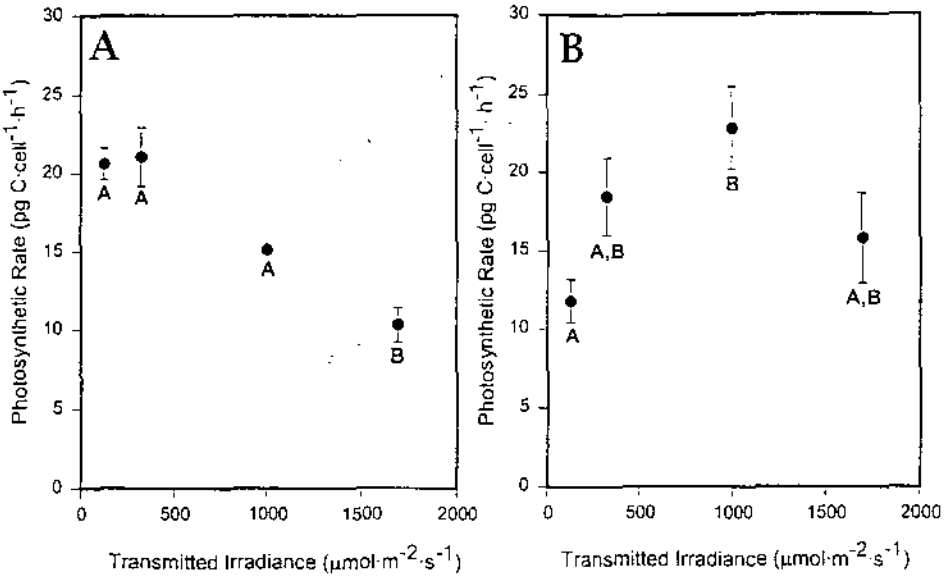

FIG. 5. Photosynthetic rate (pg C·cell^{-1}·h^{-1}) of *Euglena proxima* as a function of irradiance (μmol·m^{-2}·s^{-1}) under neutral density filters corresponding to 2% I_o, 20% I_o, 55% I_o, and 100% I_o on 13 May 1988. (A) *E. proxima* cells in scintillation vials without acid-washed sand. (B) *E. proxima* cells in scintillation vials containing a 4-mm depth of acid-washed sand. Data represent the mean of four replicates, and the standard errors are indicated by error bars. The letters under the error bars represent the results of Bonferroni *t*-tests for differences among log-transformed means ($P < 0.05$). The absence of error bars signifies that the standard error is smaller than the filled-in symbol. The cell density in all replicates was 12.4×10^4 cells·vial^{-1}.

els and roughly half that of 20% I_o and 2% I_o (Fig. 5A).

A comparison of the photosynthetic rates in the sediment-free and sediment treatments on 13 May 1988 revealed that different irradiance treatments resulted in statistically significant effects on photosynthetic rate in both sediment treatments ($P = 0.0001$ in the sediment-free treatment and $P = 0.045$ in the treatment containing sediment) (Fig. 5). In addition, the two sediment treatments were similar with maximal photosynthetic rates (P_{max}) of between 21 and 23 pg C·cell^{-1}·h^{-1}. However, the photosynthetic response to irradiance differed between the two sediment treatments. In the sediment-free treatment, the photosynthetic rate at 100% I_o was statistically lower than that at 55% I_o, but measurements of these two irradiance values were not different statistically in the treatment containing sediment. Additionally, the 2% I_o photosynthetic rates were not different statistically from that at 55% I_o in the sediment-free treatment but were statistically significant in the treatment containing sediment.

A second experiment, on 30 May 1988, confirmed that the photosynthetic rate at 100% I_o was significantly lower than at 55% I_o in the sediment-free treatment but not in the sediment treatment (Fig. 6). On this day, irradiance again resulted in a statistically significant effect on photosynthetic rate in both the sediment-free treatment ($P = 0.009$) and the sediment treatment ($P = 0.044$). One striking difference between the two sediment treatments on this day concerned the maximal photosynthetic rate: P_{max} was 20 pg C·cell^{-1}·h^{-1} in the sediment-free treatment and 30 pg C·cell^{-1}·h^{-1} in the treatment containing sediment.

DISCUSSION

Measurements of cell density and chl *a* biomass revealed that shading the surface of the sand flat artificially during low tide increased the upward migration of *E. proxima* to the surface of the sediment. This result might indicate that bright sunlight inhibits the upward migration of the population to the surface. Because artificial shading of the sediment surface resulted in a concomitant decrease in sediment temperature, the inhibitory effects of temperature and irradiance cannot be separated. The three-fold increase in chl *a* biomass between 13 and 30 May 1988 might be explained by natural population growth, although some of this change might have resulted from greater intracellular concentrations of pigment as a response to self-shading, which increases with population density.

Round and Palmer (1966) reported the appearance of a midday depression in cell numbers of *E. mutabilis* Schmitz on the sediment surface on the day after collection in the lab as well as on the day of collection *in situ*. They suggest that such a drop in phototactic sensitivity at about midday might explain the small downward migrations during the "up" phase of the tidal rhythm. Pohl (1948) found a similar decrease in the phototactic response of *E. gracilis* corresponding to the time of highest photosensitivity. The existence of a midday decrease in numbers of cells at the surface of the sediment and its continuation the following day in the laboratory might represent a response that avoids prolonged exposure to bright light.

Evidence for avoidance of high irradiances in benthic microalgae is not restricted to euglenoids. The diatoms *Cylindrotheca signata* Reimann & Lewin and *Nitzschia tryblionella* Hantzsch undergo decreases in cell numbers at midday at the sediment surface similar to that of *E. mutabilis* (Round and Palmer 1966). Hopkins (1966a) noted that the number of cells of the diatom *Navicula crucigera* (W. Smith) Cleve is 10-fold greater on sediment surfaces in shaded areas

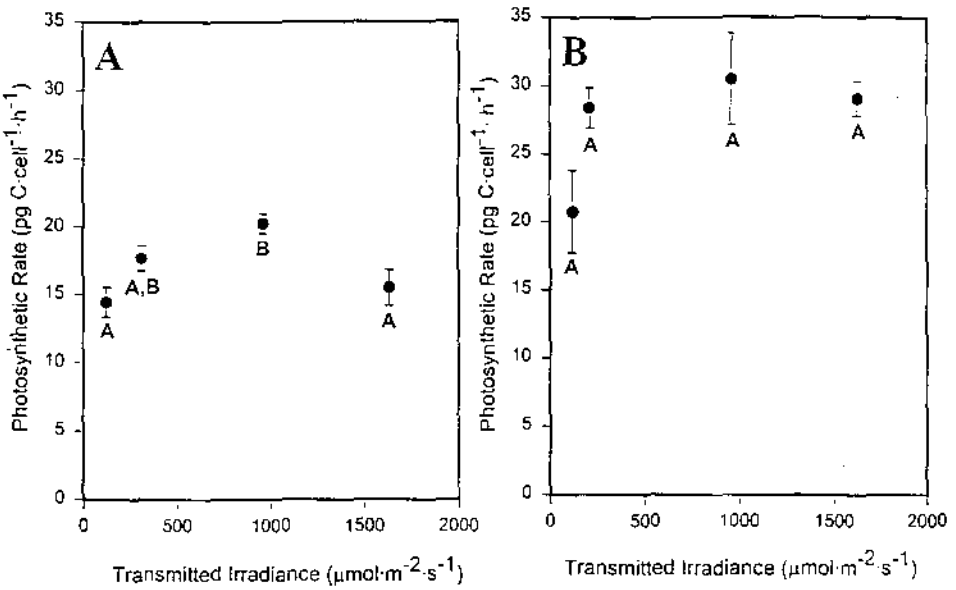

FIG. 6. Photosynthetic rate (pg C·cell^{-1}·h^{-1}) of *Euglena proxima* as a function of irradiance (μmol·m^{-2}·s^{-1}) under neutral density filters corresponding to 2% I_o, 20% I_o, 55% I_o, and 100% I_o on 30 May 1988. (A) *E. proxima* cells in scintillation vials without acid-washed sand. (B) *E. proxima* cells in scintillation vials containing a 4-mm depth of acid-washed sand. Data represent the mean of four replicates, and the standard errors are indicated by error bars. The letters under the error bars represent the results of Bonferroni *t*-tests for differences among log-transformed means ($P < 0.05$). The cell density in all replicates was 26.3 × 10^4 cells·vial^{-1}.

than in areas of bright sunlight. Laboratory experiments on *Surirella gemma* (Ehrenberg) Kützing indicate that upward migration is inhibited by light and that this diatom migrates upward only until the conditions for photosynthesis are satisfied (Hopkins 1966a). The results of the present study provide experimental evidence that upward migration of *E. proxima*, like *S. gemma*, is inhibited by high light.

Concurrent studies of vertical migration and photosynthetic experiments on 13 May 1988 revealed that the migration response of *E. proxima* to shading had a physiological basis. The upward migration of *E. proxima* was dependent on light and was related to the inhibitory effect of high light on photosynthesis. The photosynthetic rates of *E. proxima* in the sediment-free treatment revealed a strong effect of photoinhibition; the photosynthetic rate at 100% I_o was lower statistically than at 55% I_o, 20% I_o, or 2% I_o. The cell surfacing experiment showed a similar inverse trend with fewer cells on the surface in the highest light treatment (100% I_o). The *E. proxima* cells in the sediment-free vials were precluded from any movement out of the prescribed light treatment, and consequently photoinhibition occurred at the 100% I_o level.

Inhibition of photosynthesis by high light has rarely been demonstrated in benthic microalgal communities (Taylor 1964, Gargas 1971, Gallagher and Daiber 1973, Cadée and Hegeman 1974, Colijn and van Buurt 1975, Rasmussen et al. 1983, Mills and Wilkinson 1986, Pinckney and Zingmark 1991, 1993). The use of intact sediment cores in these studies means that partial shading of cells by the sediment, as mentioned by Colijin and Van Buurt (1975), might explain the rarity of photoinhibition even at incident surface irradiance. One notable exception to this trend was a study using intact sediment cores that demonstrated strong seasonal photoinhibition in the edaphic communities living on salt marsh sediments (Whitney and Darley 1983). Photoinhibition in these diatom-dominated assemblages seemed to be influenced by their illumination history. In winter, photoinhibition might have resulted from acclimatization to several days of cloudy weather preceding experimentation. Acclimatization, as it is used here, refers to an organism's adjustment to natural changes in its environment, such as a seasonal temperature change, whereas acclimation is an adjustment to an artificially imposed condition, such as a change in the treatment temperature of a laboratory experiment (Schmidt-Nielsen 1984). In summer, photoinhibition was exhibited by algae in the portions of the marsh where the vegetation was clipped immediately prior to experimentation, exposing the benthic community to light levels five times greater than before clipping. Thus, it appears that photoinhibition might be a temporary anomaly that is eliminated by acclimatization over a period of days. Indeed, even suspensions of natural benthic diatom assemblages from environments characterized by high irradiance have only slight inhibition of photosynthesis even at high light levels (Williams 1962, Taylor 1964).

Unlike benthic diatom assemblages, suspensions of *E. proxima* in the sediment-free vials did exhibit strong photoinhibition of photosynthesis at 100% I_o. However, the *E. proxima* cells in the vials containing acid-washed sand did not exhibit photoinhibition on either 13 May or 30 May 1988; the mean photosynthetic rate at 100% I_o in the sediment treatment was not statistically different from 55% I_o, 20% I_o, or 2% I_o. After ^{14}C inoculation and vigorous swirling of the vials, the *E. proxima* in the sediment-free vials would have settled onto the glass bottom of the vial, and the microalgae in the vials containing acid-washed sand would have settled onto a 5-mm-deep layer of

sand grains. Unlike the *E. proxima* cells in the sediment-free vials, the cells in the vials containing acid-washed sand were free to undergo vertical migration and seek out light levels lower than those prescribed in the experimental design. These results point to the fact that vertical migration of microalgae provides a mechanism for avoiding photoinhibitory light levels and maintaining the cells in conditions for optimal photosynthesis. The ability to respond to changes in light behaviorally through migration rather than physiologically would permit a faster response to short-term changes in irradiance by an organism; such changes are common in the natural environment. In addition, it should be pointed out that the photoinhibition was much greater on 13 May than on 30 May; on 13 May the photosynthetic rate at 100% I_o was approximately 50% of P_{max}, whereas on 30 May it was 80% of P_{max}. This drop in photoinhibition might be the result of physiological acclimatization to the high light and temperature conditions of summer.

Another striking difference between the experiments conducted on 13 and 30 May is seen in the comparison of the P_{max} for the sediment-free and sediment treatments. On 13 May, P_{max} was similar under both treatment conditions, but on 30 May the P_{max} in the sediment treatment was approximately 50% higher than in the sediment-free treatment. This difference in P_{max} might be related to the fact that the density of the cell suspension used on 30 May was more than twice the density used on 13 May and that the cells in the sediment-free treatment on 30 May appeared to be clumped in the center of the bottom of the vials. Clumping was not visibly apparent on 13 May. This aggregation of cells might have resulted in self-shading and increased CO_2 limitation, resulting in a lower total population productivity. The cells in the sediment treatment vials were not visibly aggregated and so were probably not subjected to light or CO_2 limitation. During experiments conducted in a 1-m vertical phototaxis chamber immersed in a pond, *E. gracilis* cells clustered into dense aggregations at the bottom of the chamber and obtained protection from bright white and UV-B irradiation by mutual shading (Häder and Griebenow 1988). This behavior is similar to that observed in this study, suggesting that the clumping observed in the vials might have been a behavioral response to high light.

Another factor that might partially explain the lower P_{max} in the sediment treatment vials is that prolonged exposure to surface irradiance during low tide inhibits motility in a proportion of the *E. proxima* population (Kingston 1990). After reaching the sediment surface during low tide, many *E. proxima* cells experience a change in morphology from a teardrop swimming shape to become nonmotile spheres with no obvious anterior-posterior axis. Bracher (1919) describes these rounded-off cells for *E. obtusa* (mistakenly identified as *E. deses*) and noted their abundance at night. Brinkman (1976) has also noted this morphology in *E. gracilis*, in which it represents the shape of cells in the nonmotile period of a circadian rhythm. Loss of motility would also result in a larger boundary layer around the cells, resulting in lower photosynthetic rates. However, the overall effect on photosynthesis of this change to a nonmotile state might be small because active swimming of phytoplankton does reduce diffusion transport limitation but does not eliminate it completely (Gavis 1976). A portion of the population in the sediment treatment vials was shaded partially by the sediment; this might mean that a smaller proportion of the population adopted the nonmotile spherical morphology.

Past studies on laboratory strains of *E. gracilis* have demonstrated that even short exposure (90 min) to the UV-B component of solar radiation (280–320 nm) results in drastic inhibition of both photo-orientation and motility (Häder 1985, Häder and Häder 1988, 1989). Under these conditions, when the cells lose the ability to photo-orient, the cells are eventually killed not by UV-B irradiation but by photo-oxidation of their photosynthetic pigments by strong irradiance at the surface (Häder 1985).

The results of *in situ* vertical migration experiments revealed that *E. proxima* avoided the photoinhibitory irradiance of the sediment surface by adjusting the extent of upward migration during low tide. The *in situ* photosynthetic experiments demonstrated that once on the sediment surface, *E. proxima* ameliorated the photoinhibitory effects of increased light by migrating downward. Consequently, the vertical migration behavior of *E. proxima* during low tide is consistent with the hypothesis that vertical migration provides a mechanism that optimizes photosynthesis in the face of short-term changes in irradiance imposed by passing clouds. The time scale required for this behavioral response in benthic microalgae is probably much shorter than that needed for physiological acclimatization attained through changing the size or number of the photosynthetic units (Prézelin 1981, Richardson et al. 1983, Pinckney and Zingmark 1993).

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