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Environmental forcing versus endogenous control of photosynthesis in intertidal epilithic microalgae*

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ABSTRACT: Photoadaptation and photosynthetic capacity of an intertidal epilithic microflora were measured hourly over a period of 8 d. The algae were photoadapted to the dim light intensities experienced when submerged and showed little resistance to the extreme irradiances received when exposed. During neap tide when the community was permanently submerged, the photosynthetic response of algae collected hourly in the field followed a circadian cycle. The endogenous control of this cycle was demonstrated by isolation experiments. During spring tide, photosynthetic processes were photoinactivated at low tide when extreme irradiances reached the algae. These results lead to the general hypothesis that short-term variability in the photosynthetic capacity of intertidal epilithic microalgae is controlled primarily by endogenous phasing at neap tide and tidal forcing at spring tide. Time series of in situ production were reconstructed from solar irradiance measurements and hourly estimates of the parameters of the Photosynthesis-Irradiance curve. Despite the additional stress of low tide exposure, average daily production was comparable between spring and neap tides.

INTRODUCTION

Intertidal zones are highly dynamical ecosystems which are periodically destabilized by the action of auxiliary energies such as tides and waves. On some littorals, breaking waves may provide more energy per unit area than the sun (Leigh et al. 1987). However stressful to the organisms, the auxiliary mechanical energies of tides and waves could be responsible for the high productivity of some littoral zones by renewing limiting factors such as nutrients (Odum 1969, Steever et al. 1976), space (Lamontagne et al. 1986), or light (Leigh et al. 1987).

To profit from the energy subsidy provided by tides and waves, organisms living in the intertidal zone must develop adaptations to cope with the mechanical stresses caused by the input of these auxiliary energies (Odum 1969, Margalef 1985). An example of such adaptations is provided by the vertical migrations performed by epipelic (free-living) diatoms colonizing tidal muds or sand flats. These motile diatoms migrate to the surface of the substratum at or immediately after low tide, and back into the sediment at or immediately before flood (Callame & Debyser 1954, Pomeroy 1959, Perkins 1960, Taylor 1964, Palmer & Round 1967, Brown et al. 1972, Colijn & van Buurt 1975, Joint 1978, Holmes & Mahall 1982, Admiraal et al. 1984, Varela & Peñas 1985, Paterson 1986). The synchronization of the upward migration with exposure at low tide can be interpreted as an adaptation to limit resuspension by wave-induced turbulence when the diatoms are seeking higher light intensities at the surface of the sediment (Baillie & Welsh 1980, Riaux 1983, Heckman 1985). In addition, their motility presumably enables epipelic diatoms to select an optimum level along the light gradient found in the top millimetres of the sediments (Harper 1977, Admiraal 1984), therefore maximizing photosynthesis and avoiding desiccation or photodestruction of pigments by extreme irradiance. These last inferences are supported by observations: photosynthetic activity in epipelic diatoms is maximum during exposure (Pomeroy 1959, Brown et al. 1972, Joint 1978, Holmes & Mahall 1982, Varela & Peñas 1985), and photoinhibition cannot be induced when

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epipelic diatoms are incubated in their original substratum even at extreme irradiances (Taylor 1964, Cadée & Hegeman 1974, Colijn & van Buurt 1975, Joint 1978, Rasmussen et al. 1983, Revsbech et al. 1983, Mills & Wilkinson 1986). On the contrary, the few studies of epipsammic diatoms (attached to sand grains) indicate that the photosynthetic response of these non-motile organisms is maximum at high tide and minimum at low tide (Pamatmat 1968, Varela & Peñas 1985).

Epilithic diatoms colonizing hard substrata cannot avoid desiccation or extreme irradiation by burrowing. Whether intertidal epilithic diatoms are adapted to the low irradiances experienced when submerged or to the extreme irradiances received when exposed is unknown. In this study, we investigated the effects of exposure on the photosynthetic response and productivity of a natural epilithic microalgal mat dominated by diatoms. Isolation experiments were conducted simultaneously to detect endogenous variations in the photosynthetic response of the algae.

MATERIALS AND METHODS

The study was conducted at Pointe Mitis, Québec (68° 02.0' W, 48° 40.6' N) in the lower St Lawrence estuary, Canada. Tides in the area are semidiurnal lunar with a significant inequality of amplitude (Fig. 1). Tidal range at spring tide reaches 4 m. On May 31, 1983, artificial surfaces made of wooden panels covered with 350 ceramic platelets were installed in the intertidal zone of a semi-protected site for colonization. On July 5, one of the colonized surfaces was fixed to a system of pulleys and cables enabling retrieval at any tidal stage for the collection of samples (Lamontagne et al. 1986). A second artificial surface was isolated in a basin where filtered seawater pumped from the nearby sampling site was continuously renewed. Irradiance over the basin was kept constant at 90 μE m^{-2} s^{-1}. Sampling of the isolated community was delayed for 24 h to allow the pigment composition to adapt to the reduced irradiance (Harding et al. 1981). From 08:00 h on July 6 to 07:00 h on July 14, samples were collected hourly from each of the 2 surfaces. One sample consisted of 1 platelet taken randomly from the surface. The cells were delicately detached from the platelet and resuspended in 500 ml of filtered seawater. Of this suspension, 100 ml was reserved for enumeration and taxonomic identification, and 350 ml for the determination of chlorophyll *a* (chl *a*) concentration.

The remaining 50 ml of the cell suspension was used for the determination of the photosynthetic response of the community following Lamontagne et al. (1986). The 50 ml aliquot was inoculated with 1 ml of a 50 μCi ml^{-1} solution of $NaH^{14}CO_3$ and 16 sub-aliquots of 1 ml were then incubated for 20 min at irradiances ranging from 0 to 3000 μE m^{-2} s^{-1}, in a photosynthetron-type incubator (Lewis & Smith 1983). Non-photosynthetic ^{14}C fixation was estimated by adding 50 μl of a 10^{-5} mol l^{-1} DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) solution to two of the vials, following Legendre et al. (1983). The average (non-photosynthetic) ^{14}C fixation in these vials was used as blanks and substracted from total fixation to estimate photosynthetic fixation. The incubation temperature was maintained at 12 °C ± 1 C° At the end of the incubation, the samples were added to 0.5 ml 6 *N* HCl and shaken for 40 min under a light vacuum to release inorganic carbon. The samples were then neutralized with 0.5 ml 6 *N* NaOH, and 10 ml of scintillation cocktail (Aquasol + 10 % methanol) was added to each vial. Radioactivity counts were converted to net production (mg C h^{-1}) following Strickland & Parsons (1972).

The Photosynthesis-Irradiance (P-I) response of the algae was described by fitting the empirical equation of Platt et al. (1980) to the production data normalized by photosynthetic biomass, B (chl *a*). For convenience, the superscript B, used to indicate the normalization of the parameters of the P-I curve to unit biomass of chl *a*, has been omitted throughout the text.

$$P = P_s (1-e^{-a}) e^{-b} \qquad \text{(Platt et al. 1980)} \quad (1)$$

where $a = \alpha I/P_s$ and $b = \beta I/P_s$. In this formulation, the 3 parameters α, P_s, and β are sufficient to describe the dome-shaped P-I curve. α = initial slope of the curve; P_s = maximum photosynthetic rate that would be achieved if no photoinhibition occurred; β = slope of the descending branch of the curve. P_m, the realized maximum photosynthetic rate, was also determined:

$$P_m = P_s (\alpha/[\alpha+\beta]) (\beta/[\alpha+\beta])^{\beta/\alpha} \qquad \text{(Platt et al. 1980)} \quad (2)$$

The derived parameters I_k ($I_k = P_m/\alpha$), an index of the light adaptation of the cells, I_m ($I_m = P_s/\alpha \ln(\alpha/[\alpha+\beta])$, the optimum irradiance for photosynthesis, and I_b ($I_b = P_s/\beta$), an index of the intensity of photoinhibition (Platt et al. 1980), were also calculated.

Time series of biological variables or photosynthetic parameters were smoothed to reduce high frequency noise associated with random variations and to accentuate low- and intermediate-frequency fluctuations representing interpretable ecological processes. The mathematical form of the filter applied to the series was:

$$F_t = (0.5\, V_{t-1} + V_t + 0.5\, V_{t+1})/2, \quad \text{for } t = 1, 2, \ldots, 190 \text{ h}$$

$$F_o = (V_o + 0.5\, V_1)/1.5, \quad \text{for } t = 0$$

and

$$F_{191} = (0.5\, V_{190} + V_{191})/1.5, \quad \text{for } t = 191 \text{ h.}$$

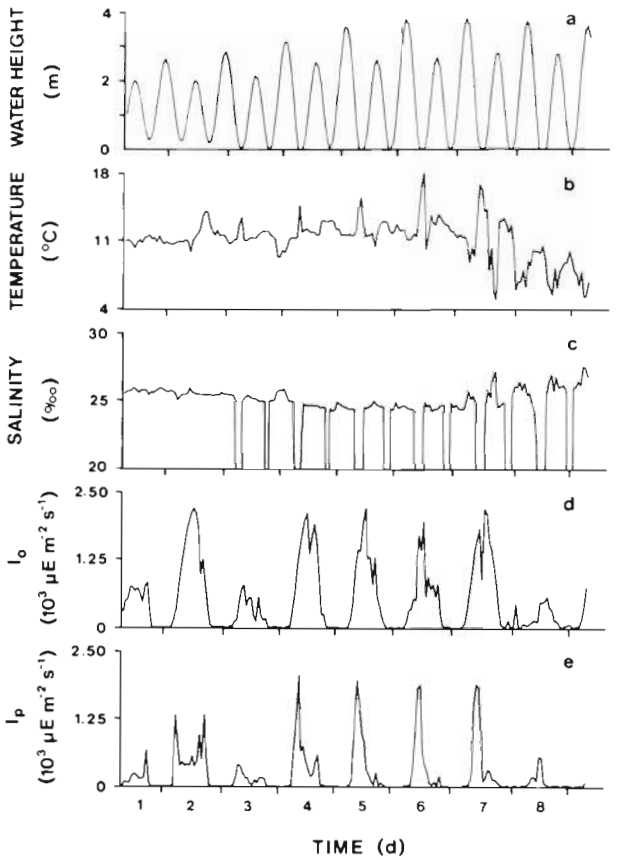

Fig. 1. Time series of the environmental variables measured at the level of the colonization panel in the intertidal zone of the St Lawrence lower estuary from 08:00 h on July 6 to 07:00 h on July 14, 1983. I_p: quantum scalar irradiance reaching the colonization panel; I_o: quantum scalar irradiance reaching the water surface

RESULTS

Environmental variability

Sampling began at the end of neap tide, when the amplitude of the semidiurnal tide was low, and ended 1 d after the apex of spring tide (Fig. 1a). During neap tide (Days 1 and 2), the artificial surface located in the intertidal zone remained continuously submerged. During spring tide, the algae were exposed for about 3 h at low tide. Sharp increases in temperature and decreases in salinity were associated with low-tide exposure (Fig. 1b, c). The incident irradiance at the surface of the water varied significantly from day to day with cloudiness (Fig. 1d). The diel cycle in the light available to the algae was strongly modulated by the semidiurnal tidal cycle in water height (Fig. 1e).

Table 1 Specific composition of the microalgal community. Average density (10^6 cells cm^{-2}) and average percentage of the total cell count for each taxon based on all samples (n = 192)

Taxon	Density	Percentage
Diatoms		
Achnanthes brevipes	0.6016	29.5
Navicula spp.	0.4372	20.2
Berkeleya rutilans	0.1186	3.9
Gomphonema spp.	0.1183	5.8
Fragilaria spp.	0.0853	4.5
Fragilaria striatula	0.0281	1.3
Cocconeis spp.	0.0265	1.4
Licmophora spp.	0.0192	1.4
Synedra spp.	0.0147	0.7
Nitzschia spp.	0.0121	0.7
Rhoicosphenia curvata	0.0051	0.3
Amphora spp.	0.0037	0.2
Centric	0.0013	0.2
Cylindrotheca sp.	0.0008	0.1
Rhabdonema spp.	0.0002	< 0.1
Gyrosygma spp.	0.0001	< 0.1
Chlorophyceae		
Ulothrix sp.	0.4491	29.9

Species composition and biomass

After 5 wk of colonization, the algal community was composed of diatoms (70 % of the total cell count) and the Chlorophyceae *Ulothrix* sp. (30 % of total count) (Table 1). The diatom assemblage was dominated by prostrate species attached to the substratum by means of a mucus pad (e.g. *Synedra* sp.), a mucilaginous stalk (e.g. *Achnanthes brevipes, Rhoicosphenia* sp.) or direct attachment of the valves (e.g. *Cocconeis* sp.) (Fig. 2). No arborescent layer was observed (Fig. 2).

Short-term variations in the concentration of chl *a* reflected small-scale heterogeneity in the intensity of colonization from one platelet to another (Fig. 3). Longer-term trends were rather weak, suggesting that the community studied was in a relatively steady state. In situ, the concentration of chl *a* generally increased over the first 4 d to reach a maximum of 90 mg m^{-2}, followed by a drop to a level of ca 40 mg m^{-2} until the end of the series (Fig. 3a). Due to the heterogeneity of the colonization from one sampling panel to the other, the initial concentration of chl *a* on the panel kept in the isolation basin was approximately half that on the panel left in situ. Except for a very slight decreasing trend, the concentration of chl *a* remained relatively constant over the duration of the isolation experiment (Fig. 3b).

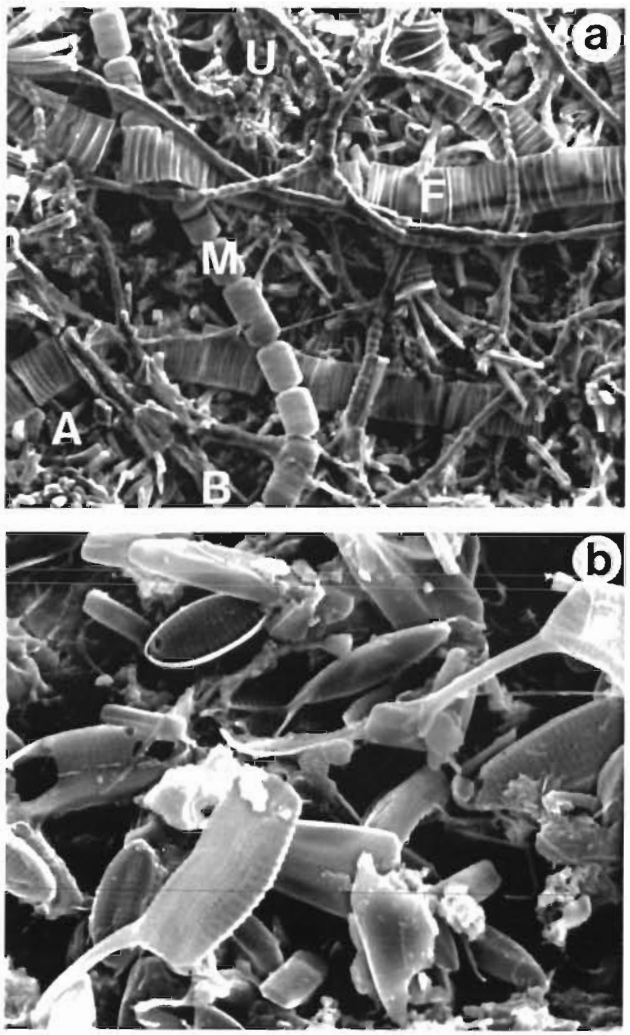

Fig. 2. Scanning electron micrographs of (a) the typical microalgal assemblage with the dominant genera (A = *Achnanthes brevipes*, B = *Berkeleya rutilans*, F = *Fragilaria striatula*, M = *Melosira nummuloides*, U = *Ulothrix* sp.), and (b) different modes of attachment of prostrate diatoms to the substratum

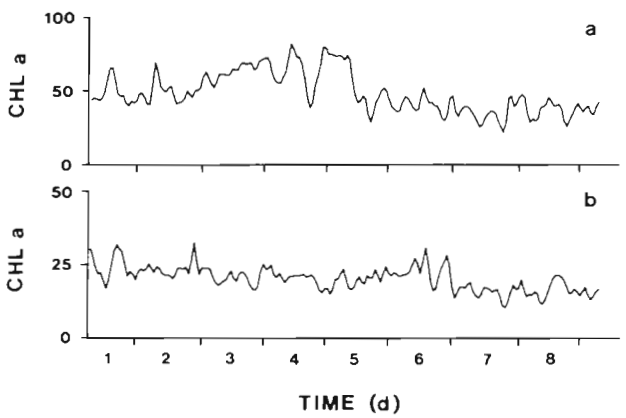

Fig. 3. Time series of chlorophyll *a* density (mg m^{-2}). (a) Colonization panel left in the intertidal zone. (b) Colonization panel isolated in the laboratory

Photoadaptation

Photoadaptation of the microalgae was studied by comparing the frequency distribution of the indices of light adaptation (I_k) and optimum irradiance for photosynthesis (I_m) as derived from the P-I curves, to the frequency distribution of the irradiance received on the colonization surface (I_p). I_p ranged from 0 to 2400 µE m^{-2} s^{-1} depending on the time of the day and water height (Fig. 1d). Null irradiances corresponding to darkness were experienced for 17 % of the time by the algae (Fig. 4). Irradiances ranging from > 0 to < 900 µE m^{-2} s^{-1} and corresponding to submergence in daytime were experienced during 71 % of the time. Within this range, frequency of occurrence decreased exponentially with irradiance (Fig. 4). Irradiances greater than 900 µE m^{-2} s^{-1} occurred solely during exposure at low tide (12 % of the time). I_k, an index of the irradiance to which the cells were photosynthetically adapted (Talling 1957), ranged from > 0 to 300 µE

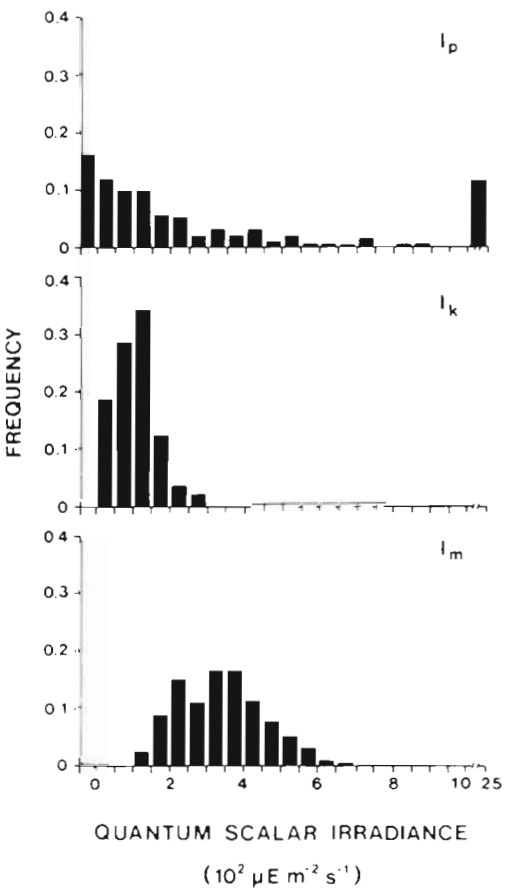

Fig. 4. Frequency distribution of I_p, the quantum scalar irradiance reaching the colonization panel in situ, I_k, the irradiance to which the microalgae were photoadapted, and I_m, the optimum irradiance for maximum photosynthetic output (n = 192 in all cases). The first class in the frequency distribution corresponds to null irradiances (darkness), the second class to irradiances > 0 but < 50 µE m^{-2} s^{-1}, etc.

m^{-2} s^{-1} with a mode of 100 to 150 µE m^{-2} s^{-1}. Thus, the microalgae were adapted to the lowest irradiances available to them (Fig. 4). Irradiances in the range covered by I_k prevailed on the colonization surface for 45 % of the time. I_m ranged from 150 to 600 µE m^{-2} s^{-1} with a mode of 300 to 400 µE m^{-2} s^{-1} (Fig. 4) Irradiances in this range prevailed on the colonization surface for 23 % of the time only, indicating that conditions for maximum production seldom occurred in the field.

Photosynthetic capacity and photoinhibition

The parameters of the P-I curve measured in the incubator reflect the photosynthetic status of the algae at the time of collection. α is a measure of the reactivity of the cells to light, P_m is the photosynthetic capacity, and β is a measure of the sensibility of the cells to photoinhibition at high irradiances.

The average photosynthetic response of the algae changed with the time of day and with the neap-spring tidal cycle (Fig. 5). At the end of neap tide (Days 1 to 3), all 3 parameters of the P-I curve (α, P_m and β) were minimum for samples collected at night, increased in the morning, and reached a maximum in the afternoon (Fig. 5). During spring tide, the photosynthetic response was stronger than during neap tide. Again, a minimum was observed at night, but the response was similar in the morning and the afternoon (Fig. 5). In all cases, a reduction of photosynthetic output was observed at irradiances exceeding 400 µE m^{-2} s^{-1}.

Short-term variations in the photosynthetic response of the community were best illustrated by plotting the time series of the photosynthetic parameters. At the end of neap tide (Days 1 to 3), a diel cycle was apparent in the relatively weak variations in the P_m of the algae maintained in situ (Fig. 6a). With spring tide, fluctuations in P_m became circatidal (Fig. 6a). Photosynthetic capacity reached a maximum value ranging from 5 to 9 mg C mg chl a^{-1} h^{-1}, 1 h after high tide on average (cross-correlation analysis, $r_{max} = 0.349$, $n = 192$, $p < 0.01$), and was drastically reduced at low tide on sunny days, when extreme irradiances hit the exposed algae (Fig. 6a). Variations in α, an index of the reactivity of the cells to light, followed closely the variations in P_m ($r = 0.520$, $n = 192$, $p < 0.01$, Fig. 6b). The derived parameter I_b is a measure of the resistance of the algae to photoinhibition. During neap tide, fluctuations in I_b followed a diel cycle, sensitivity to photoinhibition being maximum in algae collected at the end of the night (Fig. 6c). During spring tide, resistance to photoinhibition followed no clear pattern.

The comparison of the P-I curves of algae collected immediately before, during and after low tide during spring tide enables us to detail the impact of exposure on the photosynthetic response of the community. In the incubator, the photosynthetic response of algae that had experienced extreme irradiance and exposure in the field before or at the time of collection remained

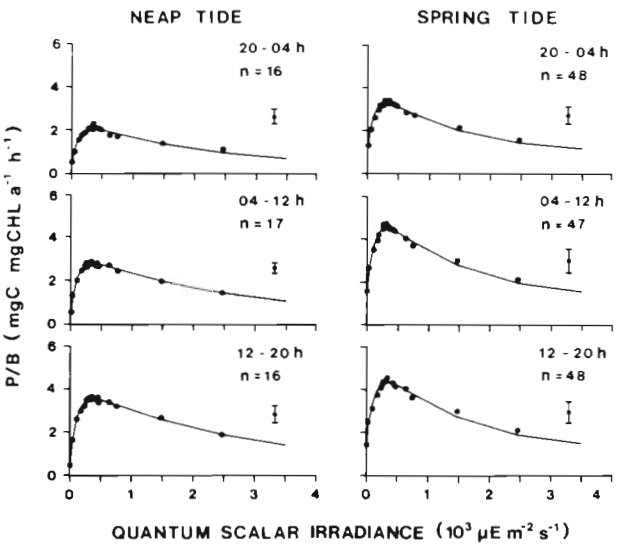

Fig. 5. Average Photosynthesis-Irradiance response of microalgae collected at night (20:00 to 04:00 h), in the morning (04:00 to 12:00 h) and in the afternoon (12:00 to 20:00 h) during neap tide and spring tide. Each point represents the mean P/B at a given irradiance averaged over the *n* P–I curves. Vertical range represents the average 95% confidence interval on these means

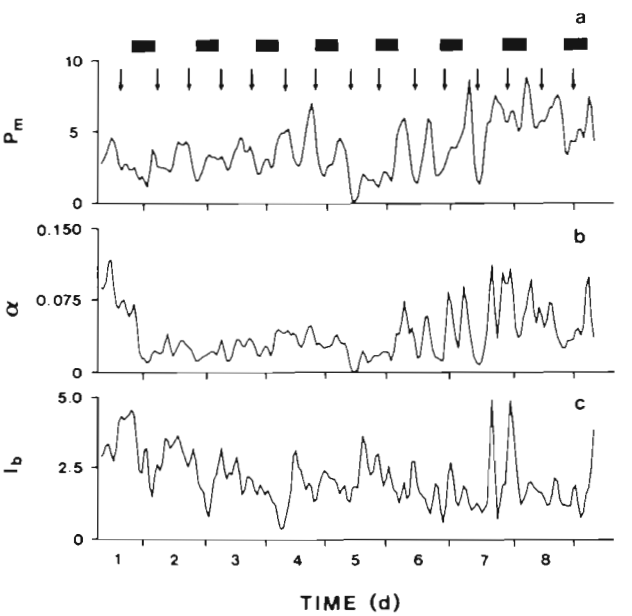

Fig. 6. Time series of the parameters of the P-I curve for microalgae collected hourly in situ. (a) P_m, the maximum photosynthetic rate per unit chlorophyll *a* (mg C mg chl a^{-1} h^{-1}); (b) α, the reactivity of the cells to light (mg C mg chl a^{-1} [µE m^{-2} s^{-1}]$^{-1}$ h^{-1}); and (c) I_b, an index of photoinhibition (10^3 µE m^{-2} s^{-1}) (a small value of I_b is indicative of a high sensitivity to photoinhibition). Heavy horizontal bars indicate darkness. Vertical arrows indicate low tide

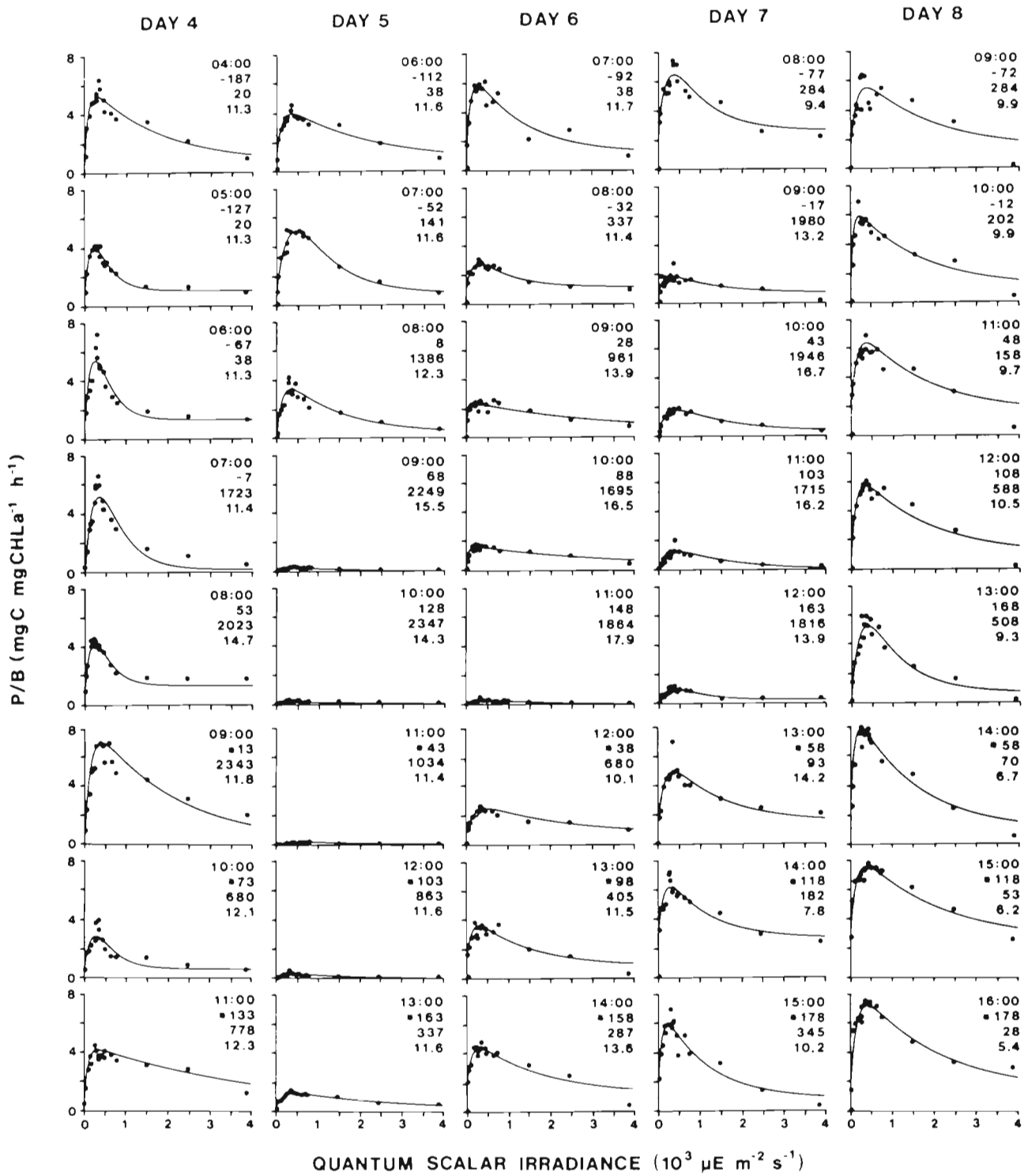

Fig. 7 Photosynthesis-Irradiance response of microalgae collected before, during, and after exposure at low tide during spring tide, for sampling Days 4 to 8. Values are: hour of collection, time (min) to emergence (negative values), since emergence (positive values) or since resubmergence (denoted by *), I_p, the quantum scalar irradiance at the time of collection (µE m^{-2} s^{-1}), and temperature (°C)

weak at any irradiance (Fig. 7). On Day 4 at the beginning of the spring tide period, the photon flux reaching the algae at low tide exceeded 1000 μE m^{-2} s^{-1}. Yet the sampling panel was only intermittently exposed, and waves and spray wetted the cells continuously. No severe desiccation occurred on that day and the photosynthetic response of the algae was little affected by exposure (Fig. 7). On Day 5, the sampling panel was completely exposed. An almost complete loss of photosynthetic capacity was observed within less than 1 h after irradiance exceeded 1500 μE m^{-2} s^{-1}. Irradiances higher than 1500 μE m^{-2} s^{-1} lasted for about 3 h, during which the photosynthetic response of the algae was totally depressed. Signs of recovery began to appear 2 h after flood had covered the sampling panel again. Although the irradiances experienced during exposure on Days 6 and 7 were only slightly less intense than on Day 5 (Fig. 1e), their impact on the photosynthetic response of the algae was less important (Fig. 7). On Day 6, complete inhibition of the response was observed only after 2 h of exposure to irradiances greater than 1000 μE m^{-2} s^{-1}, and algae collected 40 min after flooding of the panel exhibited a relatively strong photosynthetic response (Fig. 7). On Day 7, complete inhibition of the response was not observed even after 3 h of emersion and irradiances exceeding 1700 μE m^{-2} s^{-1}. Day 8 of sampling was cloudy (Fig. 1d) and irradiance at low tide did not exceed 600 μE m^{-2} s^{-1}. Even if emersion lasted for 3 h, no inhibition of the photosynthetic response occurred (Fig. 7).

Isolation experiment

The same P-I curve approach was used to monitor the photosynthetic status of the community isolated and maintained at a constant low irradiance (90 μE m^{-2} s^{-1}). The average level and variability of the photosynthetic parameters tended to decrease with time (Fig. 8). Initially, the photosynthetic capacity of the algae (P_m) presented a diel periodicity with maxima at noon (Fig. 8a). After 3 d, the periodicity faded away and P_m remained low and relatively invariable. Variations in α followed the same trend as variations in P_m except that the initial diel periodicity faded more quickly (Fig. 8b). Resistance to photoinhibition also presented a diel cycle during the first 3 d, and then stabilized at low values (indicative of high sensibility to photoinhibition) after 60 h of erratic variations (Fig. 8c).

Production rate in situ

Based on I_p, the irradiance reaching the sampling surface, and the hourly values of α, P_s and β, hourly estimates of the in situ production and production rate

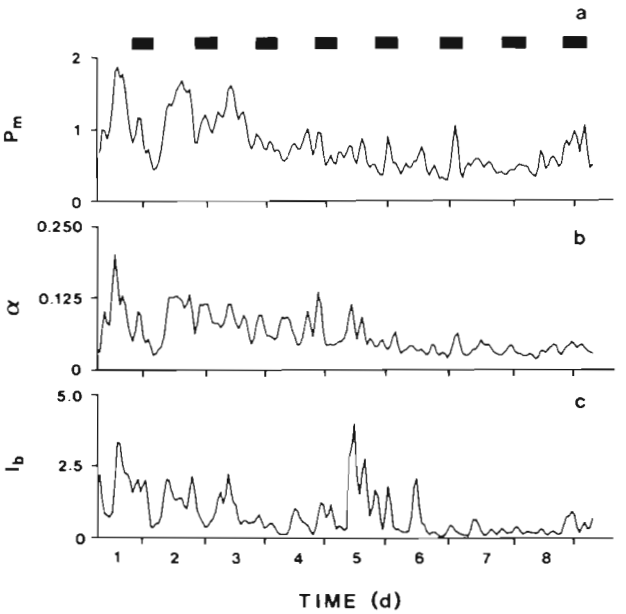

Fig. 8. Time series of the parameters of the P-I curve for microalgae collected hourly during the isolation experiment. (a) P_m, the maximum photosynthetic rate per unit chlorophyll *a* (mg C mg chl a^{-1} h^{-1}); (b) α, the reactivity of the cells to light (mg C mg chl a^{-1} [μE m^{-2} s^{-1}]$^{-1}$ h^{-1}); and (c) I_b, an index of photoinhibition (10^3 μE m^{-2} s^{-1}) (a small value of I_b is indicative of strong photoinhibition)

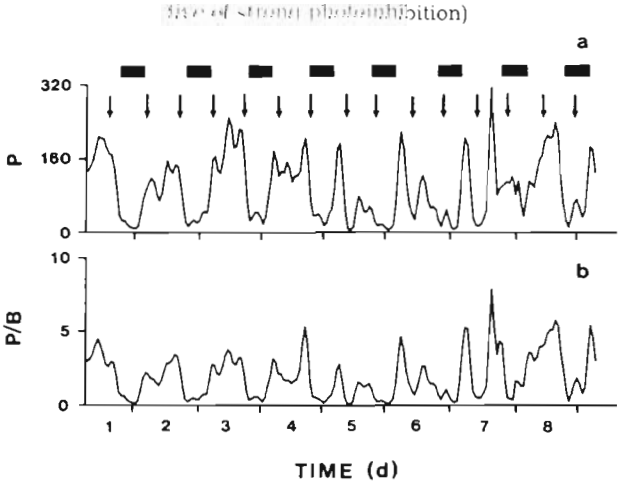

Fig. 9. Time series of in situ production (P, mg c m^{-2} h^{-1}) and production per unit biomass (P/B: mg C mg chl a^{-1} m^{-2} h^{-1}) as reconstructed from the hourly estimates of the Photosynthesis-Irradiance response of the community and the measurement of I_p, the quantum scalar irradiance reaching the colonization panel. Heavy horizontal bars indicate darkness. Vertical arrows indicate low tide

per unit biomass of the community were computed using Eq. 1. Production in situ ranged from 0 to 300 mg C m^{-2} h^{-1} and variations followed primarily the day-night cycle in light availability (Fig. 9a). Production per unit biomass (P/B) ranged from 0 to 8.5 mg C mg chl a^{-1} h^{-1}, and, given the relative constancy of biomass (Fig. 3a), followed the same day-night cycle in light

availability (Fig. 9b). The semidiurnal tide modulated the dominant circadian cycle by affecting the thickness of the water layer that filtered the light available to the algae. During neap tide, a slight increase in production rate was associated with low tide, consistent with an increase in light availability due to the reduced thickness of the water layer above the algae (Fig. 9). During spring tide, peaks in production occurred just before exposure, when the photon flux reaching the still submerged substrate was maximum. Thus, until the algae were fully exposed, peaks in production were proportional to the thinning of the covering layer, being minimum in neap tide and maximum in spring tide (Fig. 9). Production was drastically reduced when low tide occurred in daytime and the microalgae were exposed to extreme irradiances.

DISCUSSION

Species composition and structure of the microalgal mat

The algal assemblage studied was dominated by prostrate diatoms. The arborescent layer that often develops on hard substrates of the type used (Hudon & Bourget 1983, Lamontagne et al. 1986) was conspicuously absent. This lack of stratification after 1 mo of colonization is indicative of a strong grazing pressure (Hudon & Bourget 1983) and/or wave-induced erosion of the upper canopy (Lamontagne et al. 1986), which favour the development of the underlaying prostrate forms. The dominance of diatoms such as *Navicula* spp. and *Achnanthes brevipes* is consistent with the reported tolerance of these species to environmental extremes (Castenholz 1963, McIntire & Reimer 1974, McIntire & Moore 1977). Thus, the photosynthetic response and light adaptation measured in this study may be expected to be typical of communities adapted to the stresses of the intertidal zone.

Photoadaptation and photoinhibition

Plants tend to adapt their photosynthetic apparatus to the average level of irradiance received by increasing or decreasing chloroplast density when the photon flux density is low or high respectively (shade versus sun adaptation). The photosynthetic apparatus of intertidal algae may adapt to 2 different levels of irradiance: the more frequent low irradiances experienced during submergence or the less frequent high irradiances received during low tide exposure (Fig. 4). Our results clearly show that the microalgal community was adapting to the low irradiances (0 to 300 $\mu E\ m^{-2}\ s^{-1}$) prevailing during submergence (Fig. 4). Thus, the photoadaptive strategy of the microalgae favoured the optimization of production at low irradiance during submergence rather than the development of a high maximum photosynthetic capacity (P_m) for the exploitation of the short burst of high irradiance during exposure at low tide. This is consistent with the conclusions of Prézelin & Sweeney (1978) who showed that the photoadaptive strategy of the dinoflagellate *Gonyaulax polyedra* tended to optimize photosynthetic efficiency rather than photosynthetic capacity.

In intertidal macroalgae, the photoadaptive strategy selected depends on the vertical distribution of the organisms. Species growing in the higher reaches of the intertidal zone tend to be more productive during emergence, whereas species growing in the lower reaches are more productive during submergence (Quadir et al. 1979). In the present study, the experimental microalgal community was developed in the lower reaches of the intertidal zone and was productive when submerged. Whether a vertical zonation of the photoadaptive strategies exists for different species of intertidal epilithic diatoms, as in macroalgae, is unknown. However, the wide specific range of tolerance to desiccation and light reported (e.g. Castenholz 1963, McIntire & Reimer 1974, McIntire & Moore 1977) and the observed intertidal zonation of species (Castenholz 1963) suggest the existence of such a gradient.

A price to pay for photoadaptation to low irradiance is a weak tolerance to extreme photon flux densities (Bélay & Fogg 1978, Whitney & Darley 1983). The P-I response curve of the community indicated that photoinhibition of the photosynthetic process began at irradiances as faint as 400 $\mu E\ m^{-2}\ s^{-1}$. On the other hand, the frequency distribution of I_m revealed that the microalgae received the optimal illumination for maximum photosynthetic output in only 23 % of the time. Thus, most of the time, the photosynthetic activity of the submerged microalgae was light-limited. We conclude that intertidal epilithic microalgae are stressed both by extreme irradiance when emerged and light limitation when submerged.

Endogenous versus exogenous control of photosynthetic response

The photosynthetic response of the microalgal community exhibited both circadian and circatidal variations. The importance of the 2 signals varied according to the neap-spring cycle in tidal amplitude. In an earlier study, we reported a strong circadian cycle during neap tide in the photosynthetic response of a similar community developing under similar conditions (Lamontagne et al. 1986). The relatively weak diel

periodicity observed here as neap tide ended is consistent with these earlier results. We showed at the time that during neap tide, the community responded more readily to the alternation of daylight and darkness than to the fluctuations in the photon flux density actually reaching the cells (Lamontagne et al. 1986). This suggested that an endogenous clock entrained by the day-night cycle predominated over the actual fluctuations in light availability in the control of the circadian rhythms in the photosynthetic capacity of the algae (P_m). A circadian period found in α further supported the hypothesis of an internal control of the photosynthetic response of the community (Prézelin 1981).

In the present study, an isolation experiment was carried out to test this hypothesis. Circadian variations were detected in the photosynthetic capacity (P_m), the reactivity to light (α), and the resistance to photoinhibition (I_b) of the community isolated under constant conditions. These variations were of comparable amplitude and synchronous to the circadian variations observed in situ. The circadian rhythm in the photosynthetic response of the isolated community lasted for ca 72 h, a duration that compares with the 24 to 72 h reported for phytoplankton isolated under similar conditions (Palmer et al. 1964, Walther & Edmunds 1973, Prézelin & Ley 1980, Harding et al. 1981).

The perpetuation of the weak circadian cycle observed in situ under constant conditions was proof of an endogenous control of the photosynthetic response of the microalgae (Prézelin & Sweeney 1977). Thus, during neap tide, when the stress of semidiurnal exposure is absent, the circadian variations in the photosynthetic response of epilithic microalgae are, at least in part, regulated by endogenous phasing.

With the build up of tidal amplitude during spring tide, the amplitude of the variations in the photosynthetic capacity of the community increased, and a dominant semidiurnal rhythm became superimposed on the circadian rhythm. Photosynthetic capacity was maximum shortly after high tide and dropped drastically at low tide on sunny days. Studies of intertidal macroalgae have suggested that the inhibition of photosynthetic activity during exposure is linked to CO_2, or other nutrient limitation (Thomas & Tregunna 1970, Quadir et al. 1979, Schonbeck & Norton 1979). Because of their high surface/volume ratio, microalgae are generally less susceptible to nutrient limitation than macroalgae (Malone 1971). Yet, based on a simulation model, Ludden et al. (1985) suggested that photosynthesis in exposed microalgal mats could be limited by a reduced supply of CO_2 which is less readily available from the atmosphere than from the water. The hypothesis of CO_2 limitation does not explain however the strong photosynthetic response observed during emersion on Day 8 when clouds shielded the algae from extreme irradiance (Fig. 7).

Not being protected by teguments, microalgae are probably more vulnerable to strong irradiance and desiccation than macroalgae. In this study, a detailed examination of the conditions prevailing at low tide indicated that a combination of desiccation or high temperature and extreme irradiance was necessary to depress the photosynthetic response of the microalgae (Fig. 7). These observations are consistent with the conclusions of Satoh (1970) who showed that photoinactivation of chloroplasts increased with temperature over a range of temperature (2 to 25 °C) comparable to the range experienced by the microalgae in this study (4 to 18 °C). We conclude that the sharp decline in photosynthetic capacity at low tide on sunny days resulted from a photoinactivation of the chloroplasts at high temperature.

The photoinactivation of the photosynthetic response was reversible (Fig. 7). On the first day of exposure to desiccation and extreme irradiance, 2 to 3 h were needed after resubmergence for the algae to fully recover. The relative shortness of this delay indicates that little bleaching or photo-oxidation of the pigments occurred (Satoh 1970). On the following days, the impact of exposure on the photosynthetic response of the algae declined and time to full recovery decreased, the community showing a progressive acclimatization to 'sunburns'.

While the endogenous nature of the circadian cycle in photosynthetic capacity during neap tide was confirmed by the isolation experiment, no evidence for a circatidal clock was found. The semidiurnal rhythm during spring tide clearly resulted from the external agencies of extreme irradiance and temperature. These and earlier observations (Lamontagne et al. 1986) lead us to the general conclusion that rhythms in the photosynthetic activity of intertidal epilithic microalgae are controlled primarily by endogenous phasing in neap tide and by tidal forcing in spring tide.

Production in situ

Whether on soft or hard substrates, the direct measurement of benthic microalgal production in situ is technically difficult. The approach used in the present study was developed by students of phytoplankton dynamics and consists in measuring in vitro the Photosynthesis-Irradiance response of algae collected in the field. Based on ambient irradiance, the production rate taking place in situ is then extrapolated (e.g. Platt & Jasby 1976).

A central assumption of the approach is that temperature and nutrient conditions in the incubator reflect those prevailing in the field. In this study, nutrient conditions in the incubator were those prevailing in the field since the algae were incubated in the water they were collected from. The incubation temperature was maintained at

12 ± 1 °C, the water temperature that prevailed in the field for most of the sampling period. However, during the last 40 h a cooling trend from 12 to 8 °C was observed in the field (Fig. 1b). According to the relation linking P/B to temperature, this drop in temperature from 12 to 8 °C reduced production in situ by a factor of 10 to 20 % (Eppley 1972). It is thus likely that we overestimated production in situ by 10 to 20 % during these last 40 h. Keeping in mind this potential bias, the approach nevertheless enabled us to reconstruct the time course of the production of the community in situ and to quantify the effects of exposure on this production.

Our results indicate that emersion at low tide on sunny days induced a physiological stress sufficient to completely inhibit the photosynthetic response of epilithic microalgae especially on the first days of exposure (Fig. 9). The microalgae quickly acclimatized to this stress and, in the following days, production began to peak after exposure as well as before exposure. The greater light availability immediately before and after emersion combined with the higher photosynthetic capacity of the algae to produce higher peaks of production than in neap tide. These higher peaks somewhat compensated for the loss of production during exposure, and total production in neap and spring tide were comparable.

The rates of production measured in situ (0 to 300 mg C m^{-2} h^{-1}) compared well with the range (0 to 800 mg C m^{-2} h^{-1}) reported by Colijn & de Jonge (1984) in their exhaustive review of microalgal production for intertidal and shallow coastal sediments, or the rates measured by Revsbech et al. (1983) using microelectrodes for the profiling of oxygen production in microbial mats. Thus, epilithic microalgae, which cannot use adaptations such as vertical migrations to avoid resuspension or photoinhibition and optimize their light regime (Harper 1977, Baillie & Welsh 1980, Riaux 1983, Admiraal 1984, Heckman 1985), nevertheless appear as productive as soft-sediment diatoms. In epilithic microalgae, a first adaptation to resist the mechanical stress of tidal scouring and wave pounding is the development of special modes of attachments including mucus pads and mucilaginous stalks. Our results further show that photoadaptation to the prevailing low irradiances associated with immersion and a potential to quickly recover from the stress of emersion are part of the strategy developed by epilithic microalgae to exploit highly energetic intertidal environments.

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