

Diel Periodicity of Photosynthesis in Marine Phytoplankton

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Abstract

Short-term changes in photosynthesis were documented for 17 of 24 marine phytoplankton species, representing a range of taxonomic groups. Periodicity in phytoplankton photosynthesis on light-dark cycles (diel periodicity) was widespread but not universal for the species studied. The centric diatoms *Lauderia borealis*, *Ditylum brightwellii*, *Stephanopyxis turris*, *Coscinodiscus rex*, *Chaetoceros gracile*, and *Biddulphia mobiliensis* had strong diel periodicity in photosynthetic capacity (P_{\max}). Amplitudes of the daily variations ranged from 2.9 to >50, with maxima in the morning or near midday, and with minima during the dark period, and these variations were not dependent on changes in cell pigmentation. There was some evidence for sustained photosynthetic periodicity in constant conditions in several diatoms, and an endogenous rhythm may have been present. The photosynthesis-irradiance (P - I) relationship was time-dependent for representative marine diatoms, with both the initial slope (α) and the asymptote (P_{\max}) of P - I curves exhibiting significant synchronous diel oscillations. Moreover, detailed studies of the amplitude and timing of photosynthetic periodicity for the diatoms *L. borealis* and *D. brightwellii* demonstrated large temporal variations in photosynthesis with morning maxima. These P - I oscillations are discussed with reference to models of primary production which use the relationship between photosynthesis and light as a component of predictive equations for phytoplankton growth in the sea.

Introduction

Short-term variations in the photosynthesis of marine phytoplankton under conditions of alternating light and darkness (diel periodicity) have been known for over 20 yr. Early work concentrated on defining when in the course of the day oceanographic measurements of prima-

ry production should be made if photosynthetic rates were not constant. Doty and Oguri (1957), Holmes and Haxo (1958), Shimada (1958), Doty (1959), McAllister (1963), Newhouse *et al.* (1967), Honjo and Hanaoka (1969), Malone (1971), and Harris (1973) have all described diel periodicity in phytoplankton photosynthesis in nature. Some authors suggested that the amplitude and timing characteristics of these daily oscillations could be related to geographic differences in parameters such as species composition, cell size distribution, photoperiod, incident radiation, and nutrition. Other studies by Lorenzen (1963) implicated changes in pigment concentrations as a cause of photosynthetic periodicity. At the same time, laboratory studies with diatoms and dinoflagellates revealed an endogenous component in photosynthetic periodicity; i.e., the daily periodicity persisted under constant conditions for several days (Hastings *et al.*, 1961; Palmer *et al.*, 1964). It was suggested that results from field studies may reflect circadian rhythms in photosynthesis. Recent field studies have further demonstrated the occurrence of significant diel fluctuations in photosynthetic capacity (P_{\max} : Taguchi, 1976; MacCaull and Platt, 1977; Gargas *et al.*, 1979; Prézelin and Ley, 1980), and suggest that the relationship between photosynthesis and light in natural phytoplankton communities is time-dependent, changing over the course of the day. Laboratory studies of diatoms, dinoflagellates, and green algae have also demonstrated diel periodicity in P_{\max} (e.g. Hastings *et al.*, 1961; Eppley and Coatsworth, 1966; Jørgensen, 1966; Eppley *et al.*, 1967; Humphrey, 1979). Research with dinoflagellates has provided the clearest picture to date of diel periodicity of photosynthesis for several species (Prézelin *et al.*, 1977; Prézelin and Sweeney, 1977; Govindjee *et al.*, 1979; Sweeney *et al.*, 1979). These findings show that (1) both light-limited and light-saturated photosynthesis are time-dependent, (2) these diel changes are unrelated to changes in cell pigment content or respiration, (3) the periodicity in photosynthesis persists under constant conditions and is therefore not simply dependent on the light-dark cycle,

Table 1. Results of day-night screening studies for diel periodicity in photosynthetic capacity (P_{\max}) of marine phytoplankton on a per-cell basis and on a chlorophyll *a* basis. Values are means; standard errors in parentheses. CT: circadian time (hours after onset of light period)

Species	P_{\max} cell ⁻¹ ($\mu\text{g C cell}^{-1}\text{h}^{-1}$)		P_{\max} CT 6:CT 18	P_{\max} CT 6-CT 18 ($\mu\text{g C} \times 10^{-1}$ $\text{cell}^{-1}\text{h}^{-1}$)
	CT 6	CT 18		
Diatoms				
<i>Lauderia borealis</i>	12.1 (± 0.609) $\times 10^{-5}$	0.231 (± 0.012) $\times 10^{-5}$	52	119
<i>Ditylum brightwellii</i>	12.0 (± 0.902) $\times 10^{-5}$	0.080 (± 0.015) $\times 10^{-5}$	150	119
<i>Stephanopyxis turris</i>	6.99 (± 0.523) $\times 10^{-5}$	1.46 (± 0.158) $\times 10^{-5}$	4.8	55.3
<i>Coscinodiscus rex</i>	11.0 (± 0.150) $\times 10^{-4}$	1.12 (± 0.310) $\times 10^{-4}$	9.8	988
<i>Chaetoceros gracile</i>	21.2 (± 0.899) $\times 10^{-7}$	7.26 (± 0.785) $\times 10^{-7}$	2.9	1.39
<i>Biddulphia mobiliensis</i>	10.6 (± 0.594) $\times 10^{-5}$	0.934 (± 0.090) $\times 10^{-5}$	11	96.7
<i>Phaeodactylum tricornutum</i>	5.26 (± 0.271) $\times 10^{-6}$	4.99 (± 0.473) $\times 10^{-6}$	1.1	0.270
<i>Nitzschia closterium</i>	2.16 (± 0.054) $\times 10^{-6}$	2.55 (± 0.146) $\times 10^{-6}$	0.85	-0.390
<i>Skeletonema costatum</i>	4.78 (± 0.179) $\times 10^{-7}$	4.96 (± 0.124) $\times 10^{-7}$	0.96	-0.018
<i>Licmophora abbreviata</i>	2.07 (± 0.068) $\times 10^{-5}$	0.124 (± 0.016) $\times 10^{-5}$	17	19.5
<i>Chaetoceros</i> sp.	7.67 (± 1.68) $\times 10^{-7}$	5.90 (± 0.179) $\times 10^{-7}$	1.3	0.177
<i>Chaetoceros</i> sp. (lagoon)	8.00 (± 0.139) $\times 10^{-6}$	2.88 (± 0.073) $\times 10^{-6}$	2.8	5.12
Dinoflagellates				
<i>Gonyaulax polyedra</i>	6.79 (± 0.285) $\times 10^{-5}$	1.36 (± 0.313) $\times 10^{-5}$	5.0	54.3
<i>Glenodinium</i> sp.	2.39 (± 0.507) $\times 10^{-6}$	1.25 (± 0.143) $\times 10^{-6}$	1.9	1.14
<i>Ceratium furca</i>	1.57 (± 0.108) $\times 10^{-4}$	0.647 (± 0.020) $\times 10^{-4}$	2.4	92.3
<i>Peridinium socialis</i>	5.38 (± 0.134) $\times 10^{-6}$	6.14 (± 1.18) $\times 10^{-6}$	0.88	-0.760
<i>Cachonina illdefina</i>	2.72 (± 0.323) $\times 10^{-6}$	0.770 (± 0.080) $\times 10^{-6}$	3.5	1.95
<i>Gyrodinium dorsum</i>	1.80 (± 0.079) $\times 10^{-4}$	1.09 (± 0.062) $\times 10^{-4}$	1.7	71.0
<i>Pyrocystis fusiformis</i>	1.22 (± 0.036) $\times 10^{-3}$	0.165 (± 0.009) $\times 10^{-3}$	7.4	1055
Chlorophyte				
<i>Dunaliella tertiolecta</i>	2.89 (± 0.259) $\times 10^{-6}$	2.50 (± 0.189) $\times 10^{-6}$	1.2	0.390
Chrysophytes				
<i>Isochrysis galbana</i>	1.06 (± 0.060) $\times 10^{-6}$	0.577 (± 0.052) $\times 10^{-6}$	1.8	0.483
<i>Monochrysis lutheri</i>	0.839 (± 0.023) $\times 10^{-6}$	1.28 (± 0.037) $\times 10^{-6}$	0.66	-0.441
<i>Coccolithus huxleyi</i>	5.13 (± 0.117) $\times 10^{-7}$	0.291 (± 0.031) $\times 10^{-7}$	18	0.484
Cyanophyte				
<i>Synechococcus</i> sp.	3.86 (± 0.294) $\times 10^{-8}$	3.10 (± 0.036) $\times 10^{-8}$	1.3	0.008

but driven by a biological clock, and (4) interspecific differences may exist in the amplitude and timing of diel photosynthetic oscillations. Some further studies with natural phytoplankton assemblages, numerically dominated by pennate diatoms, suggest that similar relationships may occur in coastal waters (Prézelin and Ley, 1980).

Despite extensive research on various aspects of photosynthetic periodicity, our knowledge remains somewhat fragmentary and consists chiefly of detailed data for a few species tested over a fairly narrow range of environmental conditions. Dinoflagellates have been the subjects of much research because of their well-described biological rhythms in luminescence (Sweeney and Hastings, 1957), cell division (Sweeney and Hastings, 1958), and photosynthesis (Hastings *et al.*, 1961). However, relatively few data are available on the highly productive marine diatoms or members of other phytoplankton taxa (Harding *et al.*, 1980, and in preparation). Presently, the extent to which diel periodicity of photosynthesis is a common characteristic in marine phytoplankton, with consistent and predictable features, rather than a phenomenon unique to the dinoflagellates, has been inadequately ex-

plored. We report here the results of additional studies on diel periodicity in phytoplankton photosynthesis. We began with a preliminary study of diel oscillations in photosynthetic capacity (P_{\max}) using a simplified day-night criterion for screening phytoplankton species representing various algal taxonomic groups. We then made more detailed studies of (1) diel periodicity of P_{\max} in 6 species of marine centric diatoms which are important net plankton producers in the California Current, (2) diel oscillations in photosynthesis-irradiance ($P-I$) relationship of 4 diatom species, and (3) the amplitude and timing of diel oscillations in P_{\max} for 2 of the diatom species which have large-amplitude photosynthetic oscillations, and which probably contribute importantly to primary production in regions of coastal upwelling.

Materials and Methods

Culture Conditions

Phytoplankton cultures were grown in *f/2* medium (Guillard and Ryther, 1962), with the addition of soil extract.

Table 1 (continued)

P_{\max} chl a^{-1} ($\mu\text{g C } \mu\text{g chl } a^{-1} \text{ h}^{-1}$)		P_{\max} CT 6:CT 18	P_{\max} CT 6-CT 18 ($\mu\text{g C } \mu\text{g chl } a^{-1} \text{ h}^{-1}$)
CT 6	CT 18		
10.2 (± 0.512)	0.174 (± 0.009)	59	10.0
9.88 (± 0.745)	0.065 (± 0.012)	152	9.82
1.19 (± 0.090)	0.412 (± 0.045)	2.9	0.778
1.15 (± 0.016)	0.245 (± 0.068)	4.7	0.905
5.79 (± 0.246)	1.95 (± 0.211)	3.0	3.84
2.96 (± 0.166)	0.587 (± 0.057)	5.0	2.37
12.2 (± 0.630)	11.6 (± 1.10)	1.1	0.600
6.28 (± 0.157)	7.77 (± 0.444)	0.81	-1.49
1.41 (± 0.053)	1.47 (± 0.037)	0.96	-0.060
4.33 (± 0.141)	0.259 (± 0.034)	17	4.07
3.04 (± 0.667)	2.34 (± 0.071)	1.3	0.700
2.39 (± 0.041)	0.859 (± 0.022)	2.8	1.53
0.867 (± 0.036)	0.174 (± 0.040)	5.0	0.693
1.63 (± 0.346)	0.850 (± 0.097)	1.9	0.780
4.66 (± 0.322)	1.92 (± 0.060)	2.4	2.74
0.969 (± 0.024)	1.11 (± 0.214)	0.87	-0.141
2.14 (± 0.254)	0.606 (± 0.063)	3.4	1.53
6.47 (± 0.285)	3.92 (± 0.223)	1.7	2.55
2.87 (± 0.084)	0.388 (± 0.020)	7.4	2.48
2.95 (± 0.265)	2.55 (± 0.193)	1.2	0.400
3.67 (± 0.207)	2.00 (± 0.180)	1.4	1.67
1.92 (± 0.053)	2.94 (± 0.086)	0.65	-1.02
3.33 (± 0.076)	0.189 (± 0.020)	18	3.14
1.73 (± 0.132)	1.39 (± 0.016)	1.2	0.34

The light source consisted of dual banks of General Electric cool-white fluorescent lamps which supplied $1200 \mu\text{W cm}^{-2}$ to the cultures. Irradiance was measured with a United Detector Technology model UDT 40X light meter. Cells were grown on a light-dark cycle of 12 h light–12 h dark (LD 12:12) at $15^\circ\text{C} \pm 1^\circ\text{C}$. Cell numbers were monitored with a Sedgwick-Rafter counting chamber and improved Neubauer haemocytometer, and phytoplankton were collected for experiments when they were in mid- to late-exponential phase. Inocula for the phytoplankters were obtained from the Food Chain Research Group, Scripps Institution of Oceanography, La Jolla, California, and the algal culture collection at the University of California, Santa Barbara. The species studied represent major algal taxa and are listed in Table 1.

Screening Studies of Diel Periodicity in Photosynthetic Capacity (P_{\max})

The 24 phytoplankton species listed in Table 1 were tested for day-night differences in photosynthetic capacity (P_{\max}) to elucidate which algae show photosynthetic periodicity and to estimate the amplitude of diel oscil-

lations for those species exhibiting this periodicity. Aliquots of 10 ml were withdrawn from stock cultures in triplicate at CT 6 and CT 18 (CT = circadian time, the time corresponding to hours after the onset of the light period). Samples were placed in test tubes and approximately $0.25 \mu\text{Ci } ^{14}\text{C}$ -sodium bicarbonate was added. The specific activity was determined at each sampling time. The tubes were stoppered with disposable foam plugs, agitated to mix, and incubated for 1 h at an irradiance of $6000 \mu\text{W cm}^{-2}$ supplied by high-output Power Groove fluorescent lamps, at 15°C . This irradiance was shown in preliminary experiments to be saturating for photosynthesis for cells grown under the conditions described. A fourth sample was taken for measurement of non-photosynthetic ^{14}C -assimilation in darkness. Each "light" ^{14}C -assimilation value was corrected by subtracting the value for "dark" uptake. Following incubation, samples were gently filtered under low vacuum pressure (150 to 200 mm Hg) onto HA Millipore filters ($0.45 \mu\text{m}$ pore size, 25 mm diameter). The filters were fumed over HCl, and placed in glass scintillation vials to which 10 ml of scintillation cocktail (ACS, Amersham Co., USA) was added. The samples were counted with a Beckman Model LS 100 C liquid scintillation counter. Total CO_2 ($t\text{CO}_2$) was measured by the method of Culbertson *et al.* (1970). Cell numbers were routinely determined as described above. Pigment concentrations (chlorophylls *a* and *c*) were measured by the method of Jeffrey (1974). Photosynthetic rates were calculated according to the equations in Strickland and Parsons (1968), and are expressed as $\mu\text{gC cell}^{-1} \text{ h}^{-1}$ and $\mu\text{gC } \mu\text{g chlorophyll } a^{-1} \text{ h}^{-1}$.

Diel Oscillations in Photosynthetic Capacity (P_{\max})

Cultures of the marine centric diatoms *Lauderia borealis*, *Ditylum brightwellii*, *Stephanopyxis turris*, *Coscinodiscus rex*, *Chaetoceros gracile*, and *Biddulphia mobiliensis* were grown in 1.5 l f/2 medium in Fernbach flasks under the conditions described above. P_{\max} measurements were made at 6-h intervals for 72 h by the methods described above. Routine measurements of $t\text{CO}_2$, cell numbers, and pigmentation were made throughout these experiments. At the beginning of the light period on the fourth day (CT 0, 72 h), cells were transferred to constant dim light (LL) at an irradiance of $180 \mu\text{W cm}^{-2}$. A 24 h delay was imposed before measurements of P_{\max} were resumed, to allow the pigment composition to readapt to the reduced irradiance. Following the break, P_{\max} measurements were made for an additional 48 h to check for the persistence of diel photosynthetic oscillations in constant conditions as an indication of circadian rhythmicity.

Diel Periodicity in Photosynthesis-Irradiance ($P-I$) Relationship

Culture of *Lauderia borealis*, *Ditylum brightwellii*, *Stephanopyxis turris*, and *Coscinodiscus rex* were grown

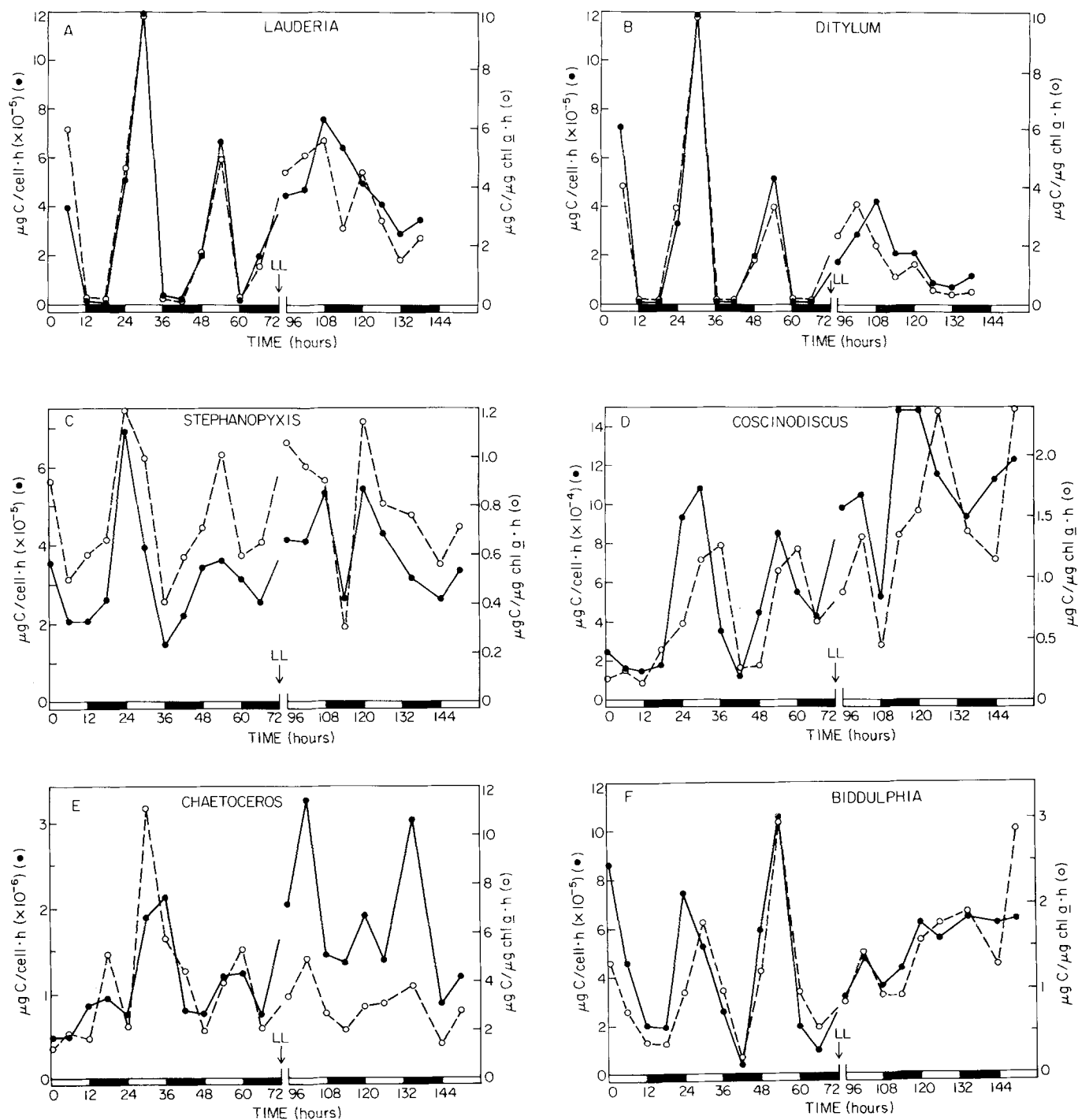


Fig. 1. Diel periodicity in photosynthetic capacity (P_{\max}) for 6 species of marine centric diatoms: (A) *Lauderia borealis*; (B) *Ditylum brightwellii*; (C) *Stephanopyxis turris*; (D) *Coscinodiscus rex*; (E) *Chaetoceros gracile*; (F) *Biddulphia mobiliensis*. Data are expressed as P_{\max} cell⁻¹ and P_{\max} chl *a*⁻¹. Cultures were grown on LD 12:12 at 1200 $\mu\text{W cm}^{-2}$ and 15 °C. Photosynthetic measurements were made at 6-h intervals. At 72 h, cultures were transferred to constant dim light (LL at 180 $\mu\text{W cm}^{-2}$), a 24-h delay was imposed, and photosynthesis measurements were then resumed for an additional 48 h (see text for further details). Black bars indicate dark periods, or subjective dark when in constant dim light

in 1.5 l f/2 under the conditions described above. Aliquots of 10 ml each were withdrawn at CT 3, CT 6, CT 12, and CT 18. Photosynthesis was measured (at a range of 15 to 20 irradiances from 30 to 6000 $\mu\text{W cm}^{-2}$, as supplied by Power Groove fluorescent lamps in com-

bination with layers of neutral density screens) with the ¹⁴C-sodium bicarbonate assimilation methods described above. Data on pigmentation, cell numbers, and rCO_2 were collected by the methods described above.

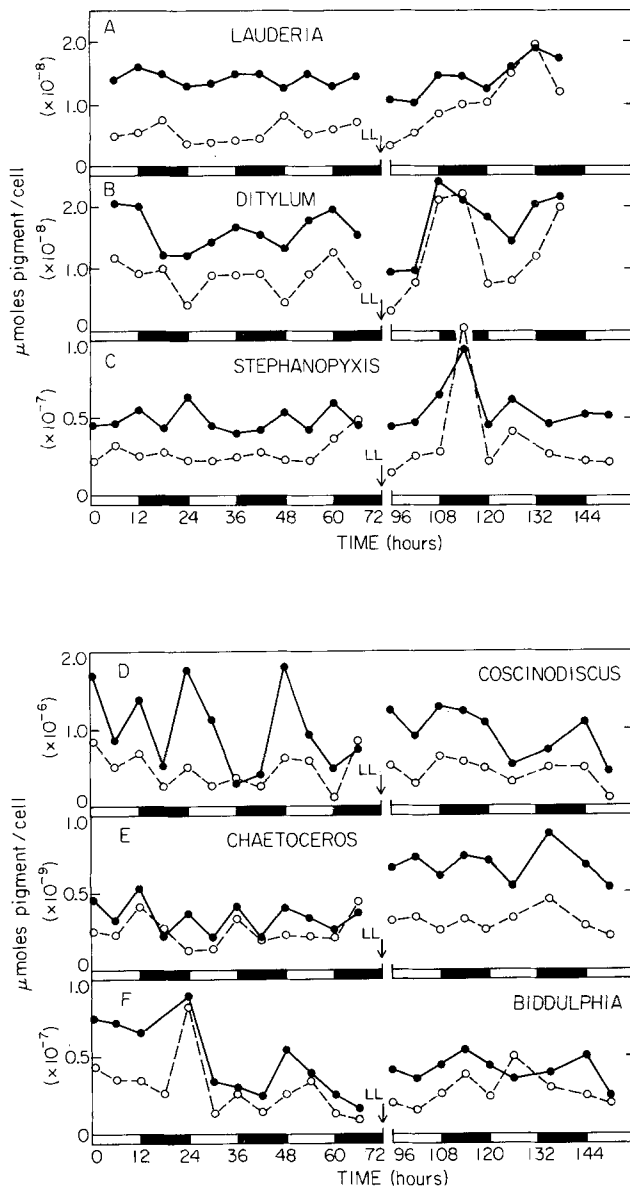


Fig. 2. Pigment concentrations during studies on diel periodicity in photosynthetic capacity (P_{\max}) for diatom species of Fig. 1. Both chl *a* (filled circles) and chl *c* (open circles) concentrations are presented

Amplitude and Timing of Diel Oscillations in Photosynthetic Capacity (P_{\max})

Lauderia borealis and *Ditylum brightwellii* were cultured as described above and used for experiments on the amplitude and timing of diel oscillations in P_{\max} . Measurements of P_{\max} were made by techniques already given, and the sampling schedule was compressed in time to provide for intensive sampling in the daylight hours. This method provided the temporal resolution necessary to define the position of the daily maximum in P_{\max} with respect to time of day. Pigmentation, cell numbers, and other needed data were collected by the methods already outlined.

Results

Screening Studies

The results of screening studies for diel photosynthetic periodicity in 24 species of marine phytoplankton are presented in Table 1. Photosynthetic capacity (P_{\max}) is expressed on both a per-cell and chlorophyll (chl) *a* basis for CT 6 (local noon on LD 12:12) and CT 18 (midnight). The daily changes in P_{\max} cell⁻¹ and P_{\max} chl *a*⁻¹ are expressed as the ratio of CT 6 to CT 18 values, and by the difference in P_{\max} cell⁻¹ and P_{\max} chl *a*⁻¹ between CT 6 and CT 18 for each species. Diel periodicity in P_{\max} was documented in representatives of 3 phytoplankton groups. The marine diatoms, including *Lauderia borealis*, *Ditylum brightwellii*, *Stephanopyxis turris*, *Coscinodiscus rex*, *Chaetoceros gracile*, *Biddulphia mobilensis*, *Licmophora abbreviata*, and *Chaetoceros* sp. (lagoon), and the dinoflagellates *Gonyaulax polyedra*, *Glenodinium* sp., *Ceratium furca*, *Cachonina ildefina*, *Gyrodinium dorsum*, and *Pyrocystis fusiformis* showed particularly strong day-night changes in P_{\max} cell⁻¹ and P_{\max} chl *a*⁻¹, as did the chrysophyte *Coccolithus huxleyi*. Several other species showed little or no fluctuations when values at these two times of day were compared. The photosynthesis of some of the rapidly dividing centric and pennate diatoms was the same at CT 6 and CT 18. Moreover, some plants in other taxa failed to show diel changes as indicated by the simplified day-night criterion. Thus, photosynthetic periodicity is widespread but not necessarily universal in marine phytoplankton. However, it is possible that our simplified screening method is sufficient only to detect strong rhythms, with peak activity centered near midday, and that we could miss photosynthetic oscillations which reached maxima at times other than noon or were damped by other environmental considerations. This technique is valid, therefore, principally to indicate in a preliminary test the occurrence of photosynthetic diel variations, and the possibility remains that periodicity occurs even in species that showed no difference in P_{\max} at CT 6 as compared to CT 18.

Diel Oscillations in Photosynthetic Capacity (P_{\max})

Diel oscillations in photosynthetic capacity (P_{\max}) were studied further in 6 species of marine centric diatoms (Fig. 1), all showing significant periodicity in P_{\max} (either P_{\max} cell⁻¹ or P_{\max} chl *a*⁻¹) when cells were grown and measured on a light-dark cycle. Daily maxima in P_{\max} occurred in the morning or midday in all 6 species, and the values for light-saturated photosynthesis declined to minima in the dark period. The amplitudes of the diel oscillations in P_{\max} ranged from 4.8 for *Stephanopyxis turris*, to >50 for *Lauderia borealis* and *Ditylum brightwellii*. The photosynthetic rates for the latter two species declined to very low levels in the dark periods. These large-amplitude variations, as measured by both daily ratios of maximum to minimum P_{\max} values (max:min)

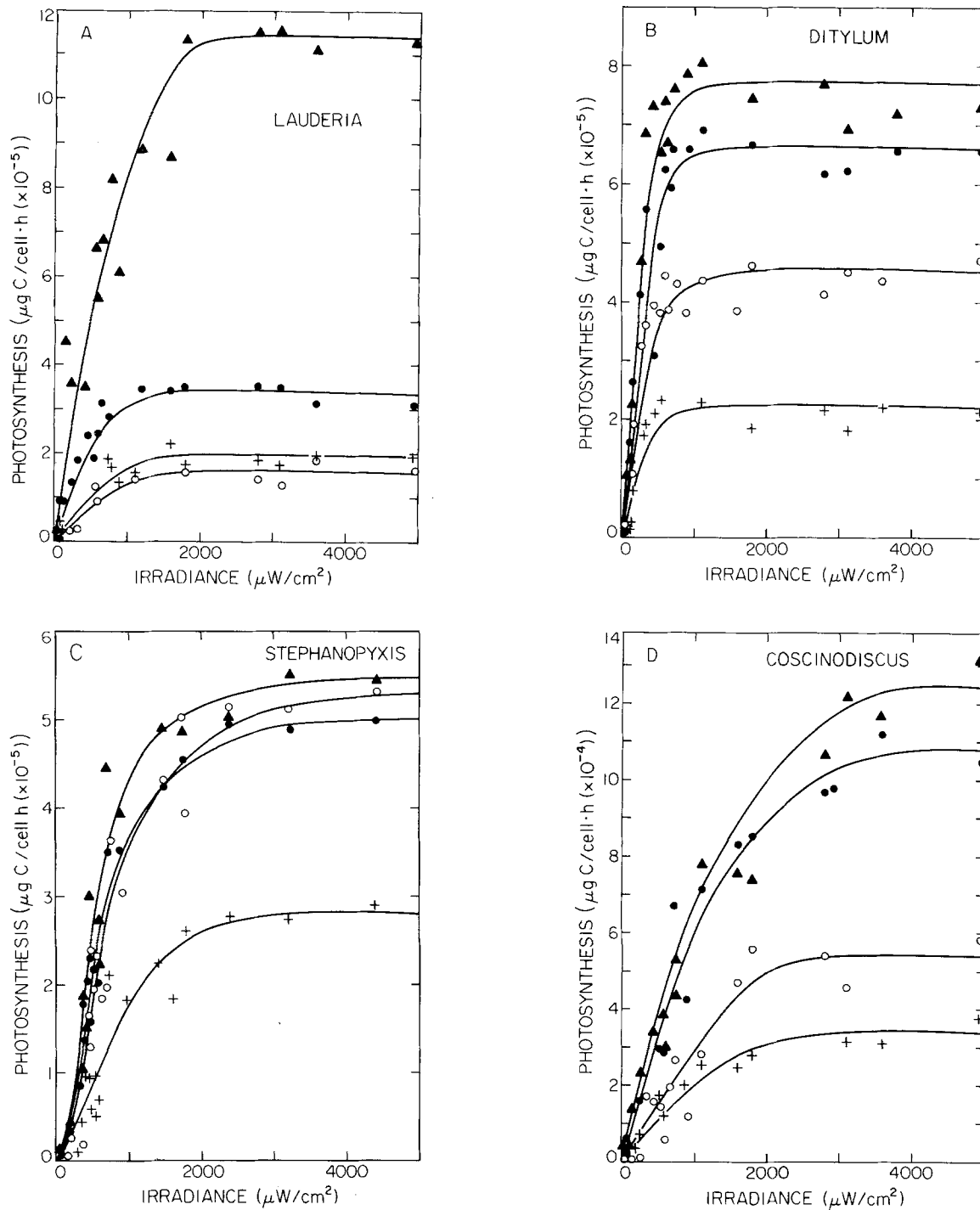


Fig. 3. Diel periodicity of photosynthesis-irradiance ($P-I$) relationship for 4 species of marine centric diatoms: (A) *Lauderia borealis*; (B) *Ditylum brightwellii*; (C) *Stephanopyxis turris*; (D) *Coscinodiscus rex*. $P-I$ measurements were made at CT 3 (filled circles), CT 6 (triangles), CT 12 (open circles), and CT 18 (crosses), where CT 0 is the onset of the light period. Curves represent best fit to data points by eye. Calculated α and P_{max} values are presented in Table 2

and the difference between daily maximum and minimum P_{max} values (max-min) (see Table 1), were comparable to those of the other large diatoms (*Coscinodiscus rex* and *Biddulphia mobiliensis*). Since the minimum values for P_{max} cell⁻¹ and P_{max} chl *a*⁻¹ were nearly zero at mid-

night (CT 18) in *L. borealis* and *D. brightwellii*, amplitude estimates for these species may be somewhat inflated. Strong diel oscillations in photosynthesis at least as pronounced as those previously described for marine dinoflagellates were observed in the 6 diatom species

Table 2. Values for photosynthesis-irradiance (*P-I*) relationship parameters α and P_{\max} for 4 diatom species

Species	Time (CT)	α (\pm SE)				P_{\max} (\pm SE)			
		$\mu\text{g C} \times 10^{-8}$ $\text{cell}^{-1}\text{h}^{-1}$ $\mu\text{W}^{-1}\text{cm}^2$	n	r	% Daily maximum	$\mu\text{g C} \times 10^{-5}$ $\text{cell}^{-1}\text{h}^{-1}$	n	% Daily maximum	
<i>Lauderia borealis</i>	3	1.68 (±0.363)	14	0.800	33.7	3.41 (±0.096)	7	29.9	
	6	4.98 (±0.700)	14	0.899	100	11.4 (±0.110)	5	100	
	12	0.624 (±0.355)	10	0.539	12.5	1.49 (±0.081)	6	13.1	
	18	0.495 (±0.336)	14	0.391	9.94	1.45 (±0.174)	6	12.7	
<i>Ditylum brightwellii</i>	3	7.71 (±0.731)	15	0.946	100	17.5 (±0.384)	3	100	
	6	6.40 (±0.818)	15	0.908	83.0	15.8 (±0.519)	4	90.3	
	12	2.92 (±0.308)	12	0.943	38.7	6.77 (±0.290)	4	38.7	
	18	0.126 (±0.057)	14	0.536	1.63	0.745 (±0.065)	5	4.26	
<i>Stephanopyxis turris</i>	3	3.17 (±0.404)	13	0.921	90.6	4.67 (±0.127)	6	93.4	
	6	3.50 (±0.544)	13	0.889	100	5.00 (±0.201)	6	100	
	12	3.06 (±0.453)	13	0.894	87.4	4.81 (±0.237)	6	96.2	
	18	1.73 (±0.368)	13	0.817	49.4	2.37 (±0.194)	6	47.4	
<i>Coscinodiscus rex</i>	3	30.6 (±3.76)	14	0.920	83.8	102 (±3.74)	4	91.9	
	6	36.5 (±3.41)	13	0.955	100	111 (±8.86)	4	100	
	12	18.2 (±2.77)	16	0.869	49.9	52.6 (±3.24)	4	47.4	
	18	8.99 (±2.24)	13	0.771	24.6	28.6 (±3.17)	5	25.8	

studied. The diel periodicity in P_{\max} is probably not attributable to cyclic changes in cell pigmentation, since no temporal pattern in chl *a* or *c* concentrations was detected which could completely account for the photosynthetic periodicity. Fig. 2 depicts pigment concentrations that correspond to the photosynthesis data for the 6 diatom species presented in Fig. 1. Some diel trends in chl *a* concentrations could be seen in *D. brightwellii* and *C. rex* which might reflect periodicity in pigment synthesis. *C. rex* showed relatively large diel fluctuations in chl *a* content per cell, with maxima in the morning. There was some indication in Fig. 2D that this pattern continues in constant light. The other 5 species varied less in cellular pigment concentrations, probably because of scatter. The fluctuations in chl *a* and *c* contents in no case accounted for the periodicity described for P_{\max} , however, and cyclic changes in pigmentation may represent an independent variation in chlorophyll synthesis. Following the transfer of the phytoplankton cultures to constant light, P_{\max} measurements were continued to test for an endogenous component in the photosynthetic periodicity. The diel oscillations in P_{\max} persisted in constant light to a varying extent for the 6 species, but were rapidly damped out and complicated by photoadaptive changes in cell pigmentation. Even so, Fig. 1 suggests that periodicity persists in constant light for several species. Moreover, during the light-dark regime, several diatoms achieve relatively high photosynthetic rates prior to the onset of the light period. The data are insufficient, however, to support a conclusion that diel periodicity in phytoplankton photosynthesis uniformly constitutes a circadian rhythm.

Diel Periodicity in Photosynthesis-Irradiance (*P-I*) Relationship

Families of photosynthesis-irradiance (*P-I*) curves for the diatoms *Lauderia borealis*, *Ditylum brightwellii*, *Stephanopyxis turris*, and *Coscinodiscus rex* provided evidence for significant diel oscillations both in the initial slope (α) and asymptote (P_{\max}) of the *P-I* relationship during the day (Fig. 3). Table 2 gives the pertinent data on the *P-I* parameters. α was determined by least-squares linear regression of photosynthesis values in the light-limited region of each *P-I* curve. P_{\max} was calculated as the mean of photosynthesis values in the asymptotic, light-saturated region of the curve. The results of statistical analyses of slopes (α) and asymptotes (P_{\max}) are presented in Tables 3 and 4, respectively. Analyses of covariance were used to test for the significance of temporal differences in α (Snedecor and Cochran, 1967). The significance of diel oscillations in P_{\max} was determined using Student's *t*-test for differences between means (Sokal and Rohlf, 1969).

Both light-limited and light-saturated photosynthesis were clearly time-dependent. These *P-I* curves, constructed from measurements made at CT 3, CT 6, CT 12, and CT 18, showed the same general pattern as seen in studies of P_{\max} oscillations for these same species (cf. Figs. 1 and 3): a daily maximum in the morning or near midday for both α and P_{\max} , and a decline to low levels in the dark period. This pattern was consistent for the 4 diatoms, although the amplitudes of the diel oscillations clearly differed from one species to the next.

Table 3. Results of pairwise comparisons of α values for 4 diatom species, determined at 4 times during day. Analysis of covariance was used, and results are expressed as F (DF). Levels of significance are *, $P < 0.05$; **, $P < 0.01$; ns, not significant

Species		CT 3	CT 6	CT 12	CT 18
<i>Lauderia borealis</i>	CT 3		17.6 (1,24)**	3.99 (1,20) ^{ns}	5.70 (1,24)*
	CT 6			17.6 (1,24)**	33.4 (1,24)**
	CT 12				0.067 (1,20) ^{ns}
	CT 18				
<i>Ditylum brightwellii</i>	CT 3		1.31 (1,26) ^{ns}	14.8 (1,22)**	151 (1,25)**
	CT 6			3.97 (1,22) ^{ns}	44.2 (1,25)**
	CT 12				64.4 (1,21)**
	CT 18				
<i>Stephanopyxis turris</i>	CT 3		0.242 (1,22) ^{ns}	0.030 (1,22) ^{ns}	6.96 (1,22)*
	CT 6			0.380 (1,22) ^{ns}	7.31 (1,22)*
	CT 12				5.11 (1,22)*
	CT 18				
<i>Coscinodiscus rex</i>	CT 3		1.13 (1,23) ^{ns}	6.78 (1,26)*	18.1 (1,23)**
	CT 6			17.5 (1,25)**	45.4 (1,22)**
	CT 12				5.96 (1,25)*
	CT 18				

Table 4. Results of pairwise comparisons of P_{\max} values from $P-I$ curves for 4 diatom species, determined at 4 times during day. Student's t -test was used to test significance of differences between means. Values are expressed as t (DF). Levels of significance are *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant

Species		CT 3	CT 6	CT 12	CT 18
<i>Lauderia borealis</i>	CT 3		54.4 (10)***	15.0 (10)***	10.3 (11)***
	CT 6			73.8 (9)***	45.9 (9)***
	CT 12				0.230 (10) ^{ns}
	CT 18				
<i>Ditylum brightwellii</i>	CT 3		2.44 (5) ^{ns}	22.8 (5)***	56.9 (6)***
	CT 6			15.2 (6)***	32.6 (7)***
	CT 12				22.8 (7)***
	CT 18				
<i>Stephanopyxis turris</i>	CT 3		1.42 (10) ^{ns}	0.560 (10) ^{ns}	9.93 (10)***
	CT 6			0.600 (10) ^{ns}	9.45 (10)***
	CT 12				8.00 (10)***
	CT 18				
<i>Coscinodiscus rex</i>	CT 3		0.920 (6) ^{ns}	10.1 (6)***	15.1 (7)***
	CT 6			6.21 (6)***	9.63 (7)***
	CT 12				5.23 (7)**
	CT 18				

Amplitude and Timing of Diel Oscillations in Photosynthetic Capacity (P_{\max})

When grown in nutrient conditions which were saturating for growth, photosynthesis of *Lauderia borealis* reached a peak early in the light period around CT 4 (10.00 hrs on LD 12:12), and *Ditylum brightwellii*, around CT 3 (Fig. 4). The amplitudes of the diel oscillations in P_{\max} cell⁻¹ and P_{\max} chl *a*⁻¹ were very large, even when only the differences during the 12-h light period are considered. These two diatom species showed maximum to minimum ratios of 3.10 and 3.26 for *L. borealis*, and 6.26 and 9.25 for *D. brightwellii*, on a per-cell and chl *a*

basis, respectively. These data support the contention that large-amplitude variations in photosynthesis occur within the light period, and that the changes in photosynthesis we report do not merely reflect decreased photosynthetic capacity during darkness.

Discussion

It is clear from our results that photosynthesis may fluctuate greatly over the day in some phytoplankton species but not in others. The results of our screening of 24 phytoplankton species for photosynthetic periodicity

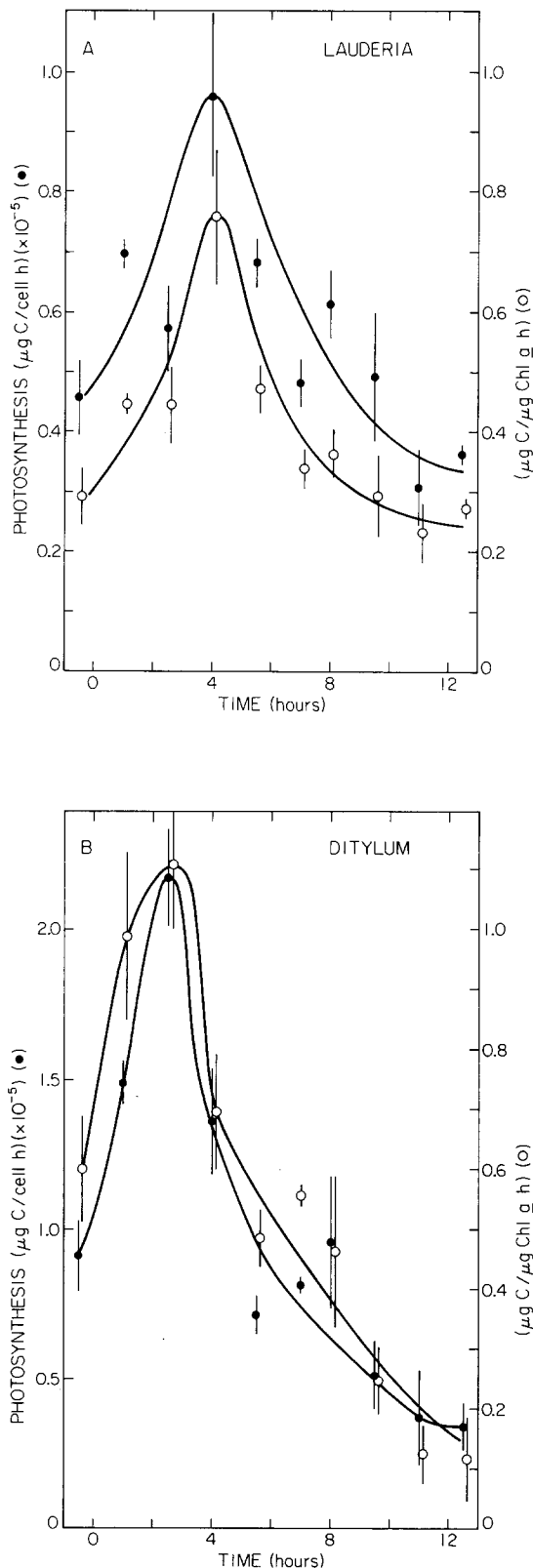


Fig. 4. Photosynthetic capacity (P_{\max}) versus time of day for: (A) *Lauderia borealis*; (B) *Ditylum brightwellii*. P_{\max} cell⁻¹ and P_{\max} chl *a*⁻¹ values are presented, a vertical bar indicating the standard error of triplicate determinations for each data point

demonstrate an interesting relationship between cell size, growth rate, and the occurrence of these diel oscillations in P_{\max} (Table 1) that may account for this difference. Most of the species that exhibited no significant day-night differences in P_{\max} are small, rapidly dividing phytoplankters, while the larger, slower growing cells almost uniformly showed marked changes. Curiously, a few species previously reported to show photosynthetic periodicity were among those which showed no differences in P_{\max} at CT 6 compared to CT 18 (Palmer *et al.*, 1964; Eppley *et al.*, 1967). *Phaeodactylum tricornutum*, *Skeletonema costatum*, and *Dunaliella tertiolecta* all divided up to 3 times per day under the growth conditions used in our studies without exhibiting day-night differences in P_{\max} .

Perhaps our screening procedure prevented detection of periodicity in these species. If their daily maxima in P_{\max} did not coincide with midday, tests at only CT 6 and CT 18 could conceivably miss any oscillations at other times of day. Another possible explanation for the failure of our comparisons of photosynthesis at CT 6 and CT 18 to detect periodicity is that no diel oscillations in photosynthesis occurred under the conditions of our experiments. We have recently completed studies with the diatom *Ditylum brightwellii* which indicated that periodicity may or may not be manifested, depending upon the physiological state of the cells (Harding *et al.*, unpublished data). Should a similar relationship hold for *Phaeodactylum tricornutum*, *Skeletonema costatum*, and *Dunaliella tertiolecta*, the lack of significant difference in P_{\max} at CT 6 versus CT 18 in our screening studies could reflect differences in conditions between our experiments and those of the previous studies. Caution must be exercised before species which did not show large photosynthetic changes are classified as not having diel oscillations.

The diatoms and dinoflagellates have many representatives with marked photosynthetic periodicity, the amplitude of which is highly variable. Perhaps the presence or absence and the amplitude of photosynthetic periodicity are both species-specific and tied to environmental conditions. Further work is needed to elucidate cause and effect in that relationship, since our data reveal little about the coupling of diel periodicity in photosynthesis with other aspects of phytoplankton physiology.

The 6 diatoms we studied more extensively were selected for three reasons. First, these species had large CT 6 versus CT 18 ratios in P_{\max} cell⁻¹ and P_{\max} chl *a*⁻¹, which suggested the occurrence of diel periodicity in photosynthesis. Second, they are important net plankton primary producers, often typical of dense blooms in rich coastal upwelling regions. Third, the photosynthetic periodicity of these diatoms in nature could be a very significant modifying factor in accurate calculations of integral daily production. Clearly, the diel oscillations in P_{\max} of the type reported here for *Lauderia borealis*, *Ditylum brightwellii*, *Stephanopyxis turris*, *Coscinodiscus rex*, *Chaetoceros gracile*, and *Biddulphia mobiliensis* were sufficiently large to influence production in the sea. We have described short-term daily changes in photo-

synthesis which should make the validity of production data collected at a single time during the day the object of substantial concern.

Whether the circadian rhythms in photosynthesis for the diatom species studied are endogenous remains uncertain. Several continued to show oscillations in constant conditions, which damped out very quickly. This observation is not unlike that for *Euglena gracilis* (Walther and Edmunds, 1973; Laval-Martin *et al.*, 1979; Lonergan and Sargent, 1979) and *Acetabularia crenulata* (Terborgh and McLeod, 1967), where an endogenous rhythm has been suggested to control photosynthesis. The existence of an endogenous component in the diel periodicity of photosynthesis is indicated for the diatoms we studied, since photosynthesis generally declined prior to the end of the light period, and similarly increased before the start of the light period. The possibility that the rhythms we observed are endogenous is strengthened by the findings of Prézelin and Ley (1980) that natural assemblages of diatoms showed circadian regulation of photosynthesis.

It has been pointed out previously that detecting photosynthetic rhythmicity may be complicated by physiological changes in the cells induced by the shift to continuous low light. Palmer *et al.* (1964) reported a rhythm in photosynthesis for *Phaeodactylum tricornutum* and found that the persistence of oscillations in constant light depended on the light intensity used. Getting conditions exactly right is clearly very important in analyzing for circadian rhythms, and perhaps an inappropriate light intensity for the constant light regime is partly responsible for the inconclusive results in constant conditions.

Fig. 3 shows diel oscillations in the photosynthesis-irradiance (*P-I*) relationship for 4 diatom species. Significant diel changes occurred in both the slope (α) and the asymptote (P_{\max}) of the *P-I* curves of all 4 species (see Tables 2 and 3); a similar pattern was described for the dinoflagellate *Gonyaulax polyedra* (Prézelin and Sweeney, 1977). The significance of such oscillations in α and P_{\max} to biological oceanography depends on the role assigned to them in predictive models of phytoplankton photosynthesis and growth in the sea. All major models of phytoplankton production use the relationship between photosynthesis and light as their basis. Generally, models combine data on incident radiation, light penetration, photosynthetic pigments, and the *P-I* relationship in estimating daily production. A common feature of all models proposed to date is their use of the *P-I* relationship (Steele, 1962; Vollenweider, 1965; Fee, 1969, 1975; Bannister, 1974; Jassby and Platt, 1976; Platt *et al.*, 1977), although diel oscillations in the parameters of this relationship are often inadequately considered. Most models have assumed constancy over the day in the parameters used in the estimation of integral daily production (e.g. Steele, 1962; Vollenweider, 1965; Bannister, 1974), despite suspicion for over 20 yr that variations in amplitude and timing of diel photosynthetic oscillations make single daily *P-I* measurements unrepresentative (see Sournia, 1974).

Both the amplitude and timing of diel oscillations in photosynthesis (e.g. Fig. 4) are so complex as to make any assumptions of constancy a serious oversimplification. In the two diatom species *Lauderia borealis* and *Ditylum brightwellii*, changes in photosynthesis occurring over the day were offset temporally by up to several hours from a midday maximum. These characteristics certainly argue against using constants for α and P_{\max} in production equations, since the peak in photosynthetic activity may be quite sharp, as in *L. borealis* and *D. brightwellii*, and the oscillations are not necessarily symmetrical about midday. By neglecting possible changes in α and P_{\max} which occur during the day, calculations based on a single *P-I* curve will generally tend to overestimate integral daily production, assuming that the curve is determined at or near the daily peak of photosynthetic activity. Temporal patterns in photosynthesis, such as occur in the two diatom species we have studied in detail, may profoundly affect production calculations if similar patterns occur in natural phytoplankton communities. Taguchi (1976), MacCaull and Platt (1977), Gargas *et al.* (1979), Prézelin and Ley (1980), and Platt *et al.* (in press) indicate that diel periodicity may be a common feature in the phytoplankton.

We are currently testing the hypothesis that diel oscillations in the *P-I* relationship, resembling those described in this paper for monospecific cultures, occur in coastal phytoplankton assemblages in the California Current. If significant short-term changes in photosynthesis in the *P-I* relationships of natural algal communities are detected, we must conclude that methods presently used to determine integral daily production are inadequate. The results of these quantitative studies will then provide a framework in which to incorporate diel oscillations in photosynthesis into models of phytoplankton production in the sea.

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