

# Characterization of Photosynthetic Rhythms in Marine Dinoflagellates

## I. PIGMENTATION, PHOTOSYNTHETIC CAPACITY AND RESPIRATION<sup>1</sup>

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### ABSTRACT

Circadian rhythms in photosynthesis were defined for the first time in the dinoflagellates *Glenodinium sp.* (M. Bernard strain) and *Ceratium furca* Ehrenberg (B. Meeson strain) and compared with that in *Gonyaulax polyedra* Stein. All three phytoplankton species had photosynthetic rhythms with daily amplitudes ranging from 3 to 5 and maxima displayed about midday. The photosynthetic pigment content and absorption properties of the cells were constant over the circadian cycle. Diurnal periodicities in respiration never accounted for more than 30% of the photosynthetic rhythm and did not persist under constant conditions. There was sufficient similarity between the circadian rhythms of these three dinoflagellates to suggest the mechanism of regulation may be the same for each of them.

Diurnal periodicity in photosynthetic capacity ( $P_{\max}$ )<sup>2</sup> has been documented during the last 20 years for several groups of algae and mixed populations of phytoplankton (6, 12, 13, 21). In these organisms, maximal productivity is restricted to a short time each day, and thus the periodic nature of photosynthesis complicates field measurements of primary production. It appears clear from the extensive data now available that environmental variables significantly affect the magnitude of photosynthesis, but have little or no effect on the timing of the photosynthetic maximum each day. Recently, Stross and his co-workers (2, 22) demonstrated a coincidence between the daily maximum in photosynthesis in *Euglena* and that in the rates of phosphate uptake in phosphorous-sufficient cultures. It appears unlikely that phosphorous availability serves to regulate the timing of the photosynthetic periodicity, since the maximal phosphate uptake rates occurred 6 hr after that in photosynthesis, when cultures were phosphorous-limited (3).

A few marine algae have been examined extensively for endogenous features which might account for their photosynthetic rhythm (4, 8, 9, 14, 18, 19, 21, 25-28). Although several of these studies demonstrate a daily periodicity in various photosynthetic parameters correlated with the cell cycle of synchronized cultures (4, 17, 18, 28), it is not clear that these organisms divide either once a day or in synchrony in natural populations. In the case of the green alga *Acetabularia* (9, 26), the diatom *Phaeodactylum tricornutum* (14), and the dinoflagellate *Gon-*

*yaulax polyedra* (8), the photosynthetic rhythm has been shown to be controlled by a biological clock. In these cases, rhythmicity is an endogenous characteristic of the cell and occurs independently of environmental variables (21, 23). Therefore, photosynthesis not only exhibits rhythmicity when cells are cultured under light-dark cycles (LD), but this rhythmicity persists with a period of about 24 hr when cells are transferred to constant conditions of continuous dim illumination (LL). Unfortunately, few measurements have been made to test whether the diurnal periodicity of photosynthesis observed in natural populations persists under constant conditions.

In the present laboratory study, the circadian natures of the photosynthetic rhythm in three marine dinoflagellates are compared and demonstrated for the first time in two of them. The results indicate that the photosynthetic rhythm of the type previously documented in *G. polyedra* may be widespread among the dinoflagellates and that further work on the regulation of the photosynthesis rhythm in *G. polyedra* should be broadly significant.

### MATERIALS AND METHODS

Algae used in this study and their origins were as follows: *Glenodinium sp.* (L. Provasoli, M. Bernard strain, UCSB code No. 5M29), *G. polyedra* Stein clone 70A (USCB code No. 5M20), and *Ceratium furca* Ehrenberg (B. Meeson strain, UCSB code No. 5M30). All dinoflagellates were grown in unialgal batch cultures in 2.8-liter Fernbach flasks containing 1 to 1.5 liters of half-strength *f* medium (*f*/2) (7). In addition, medium used for culturing *C. furca* contained 65  $\mu\text{M}$  sodium glycerophosphate, in place of 47  $\mu\text{M}$   $\text{NaH}_2\text{PO}_4$ , and 200  $\mu\text{M}$  nitrilotriacetic acid (NTA). Overhead illumination was provided by banks of cool-white fluorescent lamps. Irradiance was measured with a United Detector Technology model 40A light meter. Cells were cultured on an alternating 12-hr light-dark schedule (LD) at 21 C  $\pm$  1 C and 1,000  $\mu\text{W}/\text{cm}^2$  (70 ft-c). In experiments designed to test the circadian rhythmicity of a cell parameter, cultures were transferred at the beginning of the light period (0000 "circadian time" or ct 0) to continuous light (LL) at an irradiance between 400 and 500  $\mu\text{W}/\text{cm}^2$ . Growth was assessed by cell counts made with an AO Bright Line hemocytometer or Hauser Scientific counting chamber.

Pigments were extracted after the methods of Jeffrey (10). Details of extraction, TLC, and extinction coefficients have been described previously (15).

For photosynthetic measurements, freshly harvested cells (3 min, 1,300 rpm, room temperature) were suspended in a small volume of fresh *f*/2 medium with the addition of 5 to 10 mM  $\text{NaHCO}_3$  (pH 8). Dilute suspensions used for  $\text{O}_2$  production measurements contained between 2.5 and 5  $\mu\text{g}$  Chl *a*/5-ml sample. Prior to photosynthetic measurements,  $\text{O}_2$  levels in the

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<sup>2</sup> Abbreviations:  $P_{\max}$ : photosynthetic capacity; LD: light-dark cycles; LL: continuous illumination; ct: circadian time; UCSB: University of California, Santa Barbara algal culture collection.

sample were reduced by passing  $N_2$  gas over the surface of the cell suspension. The sample was stoppered and kept at 20 C in the dark for 15 to 30 min. Photosynthetic  $O_2$  evolution was measured polarographically with a Delieu and Walker (5) arrangement of the Clark-type  $O_2$  electrode by the experimental procedure previously described (15). Temperature was controlled in the reaction vessel at 20 C by a constant temperature water bath (Forma Scientific Inc.). Collimated light was supplied by a cooled 500 w tungsten bulb from a Viewlex model V-25 slide projector and was filtered through 5 cm of 1% cupric sulfate solution. The irradiance of the incident beam was varied by placing wire mesh screens between the lamp and the reaction chamber.

## RESULTS

Circadian rhythms of photosynthesis were defined for the first time in the dinoflagellates *Glenodinium sp.* and *C. furca* and compared with the well defined system in *G. polyedra*. Diurnal periodicity of  $P_{max}$  was established in LD cultures (Figs. 1a, 2a, 3a) with measurements made at 6-hr intervals, corrected for dark respiration, and expressed both on a cellular and Chl *a* basis. Each dinoflagellate displayed maximum photosynthetic capability at midday, followed by a 2- to 6-fold decline in the rates of  $O_2$  evolution to a minimum during the subsequent dark period. Increased rates of  $O_2$  evolution were reinitiated shortly prior to the onset of the next light period. The circadian nature of these fluctuations in  $P_{max}$  was established in the same LD cultures by transferring them to LL. Rates of  $O_2$  evolution continued to demonstrate a rhythmicity with a period of approximately 24 hr and were maintained for several days under continuous dim illumination (Figs. 1a, 2a, 3a). During this time and

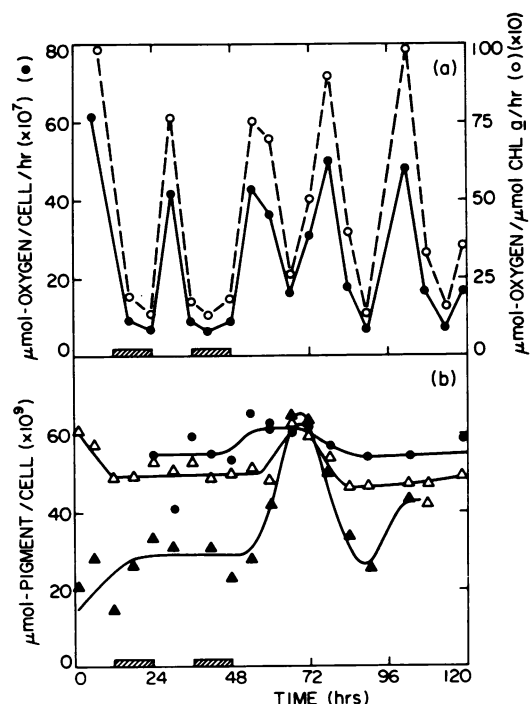


FIG. 1. a: Rhythms of photosynthetic capacity in *Glenodinium sp.*, expressed as  $\mu\text{mol } O_2/\text{cell} \cdot \text{hr}$  (●—●) or as  $\mu\text{mol } O_2/\mu\text{mol Chl } a \cdot \text{hr}$  (○—○). Light-dark cycle was 12:12 hr; irradiance of light phase was  $1,000 \mu\text{w}/\text{cm}^2$ . Dark periods are indicated by hatched bars on abscissa. Cultures were transferred to continuous dim illumination ( $500 \mu\text{w}/\text{cm}^2$ ) at circadian hr 00. Cell division occurred at beginning of light period of light-dark cycle and ceased upon transfer to constant dim light. Cells were in midexponential phase of growth. b: Whole cell pigmentation of *Glenodinium sp.* over circadian time. Concentrations of peridinin (●), Chl *a* (Δ), and Chl *c*<sub>2</sub> (▲) are expressed as  $\mu\text{mol} \times 10^{-10}/\text{cell}$ .

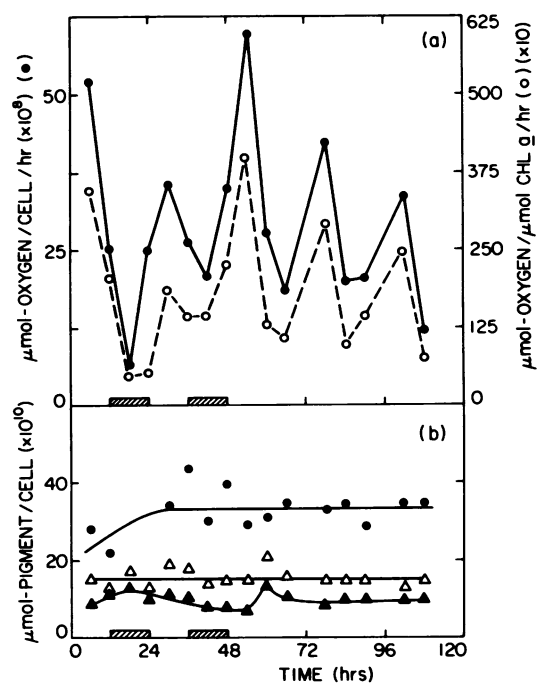


FIG. 2. a: Rhythms of photosynthetic capacity in *G. polyedra*, expressed as  $\mu\text{mol } O_2/\text{cell} \cdot \text{hr}$  (●—●) or as  $\mu\text{mol } O_2/\mu\text{mol Chl } a \cdot \text{hr}$  (○—○). Light-dark cycle was 12:12 hr; irradiance of light phase was  $800 \mu\text{w}/\text{cm}^2$ . Dark periods are indicated by hatched bars on abscissa. Cultures were transferred to continuous dim illumination ( $500 \mu\text{w}/\text{cm}^2$ ) at circadian hr 00. Cell division occurred at beginning of light period and ceased upon transfer to constant dim light. Cells were in late exponential phase of growth. b: Whole cell pigmentation of *G. polyedra* over circadian time. Concentrations of peridinin (●), Chl *a* (Δ), and Chl *c*<sub>2</sub> (▲) are expressed as  $\mu\text{mol} \times 10^{-10}/\text{cell}$ .

under the conditions of continuous dim illumination, cell division ceased. Although cells were viable and photosynthetically active, the photosynthetic rhythm of these dinoflagellates is not closely linked to their cell cycle. Unlike  $O_2$  evolution, dark respiration rates displayed diurnal periodicity in LD cultures which appeared to damp out upon transfer of the cultures to constant illumination (Fig. 3a).

Although a difference of almost two orders of magnitude was noted in the cellular photosynthetic capacities of the three species of dinoflagellates examined, the amplitudes of the rhythms were approximately the same for *Glenodinium sp.*, *C. furca*, and *G. polyedra* cultured under similar growth conditions averaging at about 3.4, 3, and 5.2, respectively. Apparently unrelated to circadian control, the differences in  $P_{max}$  rates between the dinoflagellate species were correlated with differences in such cell parameters as cell size, cell volume, photosynthetic pigment content, and perhaps vacuolar space (inversely related to cell density) (Table I).

$P_{max}$  within a single species of dinoflagellate was dependent on irradiances during growth. While photosynthetic rhythms persisted with roughly the same amplitude, the magnitude of the photosynthetic capacity was lower in cultures of *G. polyedra* grown at irradiances below  $1,000 \mu\text{w}/\text{cm}^2$ . Light-saturated photosynthetic rates were reduced by a factor of 5, from  $200$  to  $40 \times 10^{-7} \mu\text{mol } O_2/\text{cell} \cdot \text{hr}$ , when cells were cultured at  $800 \mu\text{w}/\text{cm}^2$  rather than at  $1,200 \mu\text{w}/\text{cm}^2$  at ct 6 on the same day (Table I). The photosynthetic stress response of *G. polyedra* to lowered irradiance has been further defined and will be described elsewhere (B. Prézélin and B. Sweeney, in preparation).

At the same time that photosynthetic capacity was being measured, the photosynthetic pigment content of an aliquot of the same culture was analyzed by acetone-extraction and TLC.

Although some alterations with time were observed, the cellular concentrations of Chl *a*, Chl *c*<sub>2</sub>, and peridinin did not fluctuate with a period of 24 hr (Figs. 1b, 2b, 3b). The only significant fluctuations in pigment content occurred as an irradiance-induced response when cultures were transferred to constant dim illumination. Since incident irradiance of LL was about half that of LD, the total irradiance per day was approximately equivalent under both LD and LL conditions. Cellular pigment content of *G. polyedra* and *C. furca* was most sensitive to the transfer and began to increase markedly 6 to 12 hr after being placed in LL. Pigment concentrations reached a maximum after 12 to 24 hr, and returned to values comparable to those measured in the LD cultures after 24 to 48 hr in LL. Additional experimental measurements on similarly cultured dinoflagellates indicated that the pigment content of all three genera was stable and nonperiodic

in LD cultures and in LL cultures which had been in constant illumination for a day or 2.

## DISCUSSION

The presence of circadian rhythms in photosynthesis has been established for three genera of marine dinoflagellates, *G. polyedra* and now *Glenodinium sp.* and *Ceratium furca*. There is sufficient similarity between the rhythms of these three dinoflagellates to suggest that the mechanisms of circadian regulation of photosynthesis may be the same for each of them. The maximal rates of photosynthetic O<sub>2</sub> evolution occurs about midday, *C. furca* displaying a maximum during the late morning (ct 5) and a few hr before the occurrence of the maxima of *Glenodinium sp.* (ct 7) and *G. polyedra* (ct 7) (B. Meeson, unpublished). Although the magnitudes of the rhythms differ in relation to cell size and culture condition, each of the dinoflagellates examined shows a 3- to 5-fold change in magnitude over the day and a free running periodicity of about 24 hr. In no species does rhythmicity depend on fluctuations in cell absorption (16) or photosynthetic pigment content of the cells (Figs. 1b, 2b, 3b), these cell parameters being found to be constant in photoadapted log phase cultures. The photosynthetic rhythms are detected when photosynthetic O<sub>2</sub> evolution is calculated either on a cellular or Chl basis (Figs. 1a, 2a, 3a). These characteristics are consistent with the circadian rhythmicity of photosynthesis described for *Phaeodactylum tricoratum* (14) and *Acetabularia crenulata* (9, 26) and the diurnal fluctuations in photosynthesis observed in *Chlorella pyrenoidosa* (20) and *Scenedesmus obliquus* (17-19).

In addition, the three dinoflagellates show diurnal periodicities in respiration which can never account for more than 30% of the photosynthetic rhythm and which did not persist under constant conditions. It is clear that variations in the rate of respiration cannot account for the characteristic rhythm observed in photosynthesis. Like *G. polyedra* (24), *C. furca* also displays a circadian rhythm in cell division, the maximum number of early division pairs appearing just prior to the beginning of the light period (B. Meeson, unpublished). The greatest number of dividing pairs of *Glenodinium sp.* also occurs at the beginning of the light period in cultures entrained to a 12:12 hr LD cycle, but the circadian rhythmicity of its cell division has not been tested.

The present studies establish *G. polyedra* as a test organism representative of those dinoflagellates which display a daily rhythmicity in their photosynthetic capacity. Extended studies on *G. polyedra* are being conducted to examine more fully how the regulation of photosynthetic capacity is mediated by a biological clock. Some of those results are presented in a following study (16). Finally, increasing documentation of *in situ* diurnal periodicity in photosynthesis and Chl fluorescence (11) requires a closer examination of natural populations under constant conditions, to define what role biological clocks may play in regulating primary production.

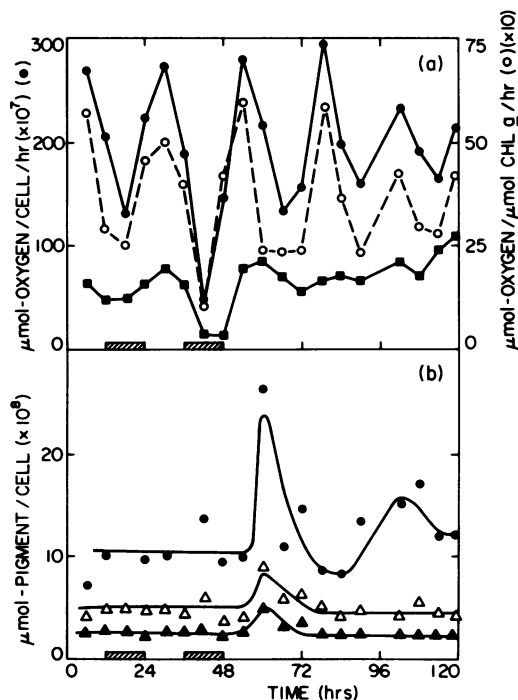


FIG. 3. a: Rhythms of photosynthetic capacity in *C. furca*, expressed as  $\mu\text{mol O}_2/\text{cell}\cdot\text{hr}$  (●—●) or as  $\mu\text{mol O}_2/\mu\text{mol Chl } a/\text{hr}$  (○—○). Respiration is expressed as  $\mu\text{mol O}_2/\text{cell}\cdot\text{hr}$  (■—■). Light-dark cycle was 12:12 hr; irradiance of light phase was  $1,000 \mu\text{w}/\text{cm}^2$ . Dark periods are indicated by hatched bars on abscissa. Cultures were transferred to continuous dim illumination ( $500 \mu\text{w}/\text{cm}^2$ ) at circadian hr 00. Cell division occurred at beginning of light period of light-dark cycle and ceased upon transfer to constant dim light. Cells were in midexponential phase of growth. b: Whole cell pigmentation of *C. furca* over circadian time. Concentrations of peridinin (●), Chl *a* (△), and Chl *c*<sub>2</sub> (▲) are expressed as  $\mu\text{mol} \times 10^{-8}/\text{cell}$ .

Table I. Comparison of cell parameters and photosynthetic rates of three marine dinoflagellates.

Species Growth Irradiance	Cell Size	Cell Volume (cm <sup>3</sup> /cell X 10 <sup>10</sup> )	Cell Density (gm/cell X 10 <sup>8</sup> )	chl <i>a</i> /cell (μmol X 10 <sup>10</sup> )	P <sub>max</sub> /cell <sup>1</sup> (X107)	P <sub>max</sub> /cm <sup>3</sup>	P <sub>max</sub> /chl <i>a</i>
<i>Glenodinium sp.</i> 1200 μw/cm <sup>2</sup>	16μ X 11μ	60	5.0 - 8.3	15	3.5	58	23
<i>G. polyedra</i> 1200 μw/cm <sup>2</sup>	60μ X 40μ	540	2.22	760	175 - 225	32 - 42	23 - 30
<i>G. polyedra</i> 800 μw/cm <sup>2</sup>		410		500	40	10	8
<i>C. furca</i> 1200 μw/cm <sup>2</sup>	130μ X 135μ	800	1.37	500	300	38	60

<sup>1</sup>P<sub>max</sub> is measured as μmoles oxygen/hr at 5000 μw/cm<sup>2</sup> and is corrected for dark respiration.

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