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CELL DIVISION PERIODICITY IN 13 SPECIES OF MARINE PHYTOPLANKTON ON A LIGHT:DARK CYCLE[1]

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ABSTRACT

The division rates of 26 clonal cultures representing 13 species of planktonic marine algae (6 diatoms, 2 flagellated chrysophytes, 2 coccolithophores, 1 cryptomonad flagellate, 1 dinoflagellate, 1 green alga) were determined every 2 h for 48 h during exponential growth on a 14:10 LD cycle in nutrient-replete batch culture. Cyclic oscillations in the division rate were detectable in 22 of these clones. Of 14 diatom clones examined, four displayed nearly constant division rates throughout the LD cycle and ten showed strong periodicity favoring division during the light periods. In contrast, all other algae (12 clones) exhibited division rate maxima during periods of darkness, and clearly detectable decreases in cell number for time intervals of 4–8 h during periods of illumination. Intraspecific differences in division periodicity were found among eight clones of the diatom Thalassiosira pseudonana *(Hustedt) Hasle & Heimdal and six clones of the coccolithophore* Emiliania huxleyi *(Lohm.) Hay & Mohler.*

Key index words: cell division; Chaetoceros; Chroomonas; *coccolithophore;* Cyclotella; *diatom; dinoflagellate;* Dunaliella; Emiliania; Hymenomonas; Isochrysis; *light;* Pavlova; Phaeodactylum; *periodicity; phytoplankton;* Scrippsiella; Thalassiosira

Most of the surface waters of the ocean experience cyclic diel fluctuations in light intensity, and many algal processes have been shown to exhibit 24 h periodicity in response to a periodic light:dark (LD) cycle. Periodically oscillating cell division and nutrient uptake rates have been demonstrated both in unialgal cultures under cyclic artificial illumination (e.g., Sweeney and Hastings 1958, Eppley and Coatsworth 1966, Paasche 1967, 1968, Chisholm and Stross 1976a, b, Chisholm et al. 1978) and in natural phytoplankton under ambient solar light conditions (e.g., Eppley et al. 1970, Goering et al. 1973, Azam and Chisholm 1976, Chisholm et al. 1978). In addition, field studies have shown periodic fluctuations in the proportion of dividing or recently divided cells in natural populations of several planktonic algae, implying similar fluctuations in the division rate (e.g., Swift and Durbin 1972, Doyle and Poore 1974, Smayda 1975, Lewin and Rao 1975, Weiler and Chisholm 1976).

The literature on diel periodicity in planktonic algae has been reviewed by Sournia (1974), but generalizations, and even comparisons, based upon data of various investigators are difficult because of differences in parameters measured and experimental procedures employed. As a rule, experiments have been performed on one or two species at a selected light intensity and photoperiod, at either saturating or limiting nutrient concentrations, and have at times resulted in apparently contradictory data. Thus the diatoms *Skeletonema costatum* and *Ditylum brightwellii* grown on LD cycles have been shown to attain maximum division rates during the light period in some studies (Jorgensen 1966, Paasche 1968) and during the dark period in others (Eppley et al. 1971, Richman and Rogers 1969).

Under different photoperiods the division rate maximum has been shown to occur at different points in the LD cycle in the green alga *Dunaliella tertiolecta* (Eppley and Coatsworth 1966), and coccolithophore *Emiliania* (*Coccolithus*) *huxleyi* (Paasche 1967). In these studies there was no simple relationship between the time of maximum cell division and the beginning or end of the light period, but the division rate always reached a maximum at some point during the dark period. These results support the concept that the timing of periodically oscillating algal processes under the LD cycle is flexible and responds to the specific conditions under which the cells are growing, but suggest that there are limits to this flexibility in any given alga.

The present paper reports a study of the diel periodicity of cell division in 26 clonal cultures representing 13 species of planktonic marine algae (6 diatoms, 2 flagellated chrysophytes, 2 coccolithophores, 1 cryptomonad flagellate, 1 dinoflagellate, 1 green alga) growing exponentially in batch culture on a 14:10 LD cycle at 20 C with all nutrients present in excess. Our purpose in conducting these experiments was to eliminate differences in experimental conditions so that the diel phasing of cell division in a moderately large and taxonomically diverse group of algae could be observed under identical light, temperature and nutrient regimes.

MATERIALS AND METHODS

Thirty-eight axenic clonal cultures of planktonic marine algae (Tables 1, 2), were grown in f/2 enriched seawater medium (Guillard and Ryther 1962) with illumination ca. 5×10^{-2} ly·min^{-1} provided on a 14:10 LD cycle using Sylvania cool-white fluores-

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TABLE 1. *Isolation data, inoculation cell densities and mid-experiment dilution factors for the 26 clones yielding periodicity data.*

Species	WHOI[a] clone designation	Source, date	Isolator	Inoculation cell density ($\times 10^3$ cells·ml^{-1})	Mid-experiment dilution factor	Live or preserved samples counted
DIATOMS						
Thalassiosira pseudonana (Hustedt) Hasle & Heimdal (ex-*Cyclotella nana* Hustedt)	3H	Moriches Bay, Long Island, New York, 1958	R. Guillard	4.5	1:10	Preserved
	C5	Chincoteague Island Lagoon, Virginia, 1964	D. Wilson	4.5	1:5	Preserved
	W	Wumme River estuary, Germany, 1973	E. Paasche	5.0	1:10	Preserved
	SWAN-1	Swan River estuary, Perth, Australia, 1965	R. Davis	4.5	1:10	Preserved
	STX-97	St. Croix, Virgin Islands (Reef), 1969	K. Haines	5.0	1:10	Preserved
	7-15	Continental Slope off Massachusetts, 1958	R. Guillard	4.3	1:5	Preserved
	13-1	Sargasso Sea, 1958	R. Guillard	3.6	1:10	Preserved
	FCRG-66	Central gyre of North Pacific	J. Jordan	5.1	1:5	Preserved
Thalassiosira weissflogii (Grunow) Fryxell & Hasle (ex-*T. fluviatilis*)	Actin	Gardners Island Pond, Long Island, New York, 1958	R. Guillard	5.5	1:5	Preserved
	SA	Portugal, 1973	M. A. Sampayo	1.5	1:5	Preserved
Thalassiosira sp.	289-1	Peru upwelling region, 1976	D. Nelson	0.5	1:5	Preserved
Cyclotella cryptica Reimann, Lewin & Guillard	WT-1-8	West Tisbury Pond, Massachusetts, 1969	A. White	4.3	1:5	Preserved
Chaetoceros simplex Ostenfeld	Blom	Sargasso Sea, 1960	R. Guillard	5.3	1:10	Preserved
Phaeodactylum tricornutum Bohlin	Pet Pd	Aquaculture pond, Dennis, Massachusetts	E. Petrovitch	14.5	1:5	Live
COCCOLITHOPHORES						
Emiliania huxleyi (Lohm.) Hay & Mohler (ex-*Coccolithus huxleyi*)	BT6	32°10′N, 64°30′W, 1960	R. Guillard	4.2	None	Live
	MCH	Off Bermuda, 1967	W. Maddux	4.4	None	Live
	451B	Oslo fjord, strain "F", 1958	E. Paasche via W. Blankley	5.2	None	Live
	G4	Oslo fjord, 1968	? via D. Klaveness	5.8	None	Live
	92A		M. Parke	3.7	None	Live
	WHA	Off Woods Hole, Massachusetts, 1963	D. Klaveness (?)	6.1	None	Live
Hymenomonas carterae (Braarud & Fagerl.) Braarud	Cocco	Woods Hole, Massachusetts, 1958	I. Pintner	1.7	None	Live
OTHER TAXA						
Scrippsiella trochoidea (Stein) Loeblich III (Dinophyceae)	Peri			20.0	None	Live
Chroomonas salina (Wislouch) Butcher (Cryptophyceae)	3C	Milford Harbor, Connecticut, 1957	R. Guillard	4.6	1:5	Preserved
				5.2	1:5	Live
Dunaliella tertiolecta Butcher (Chlorophyceae)	Dun			3.3	1:5	Live
Pavlova lutheri (Droop) Green (ex-*Monochrysis lutheri*) (Chrysophyceae)	Mono		M. Droop	9.1	1:5	Live
Isochrysis galbana Parke (Chrysophyceae)	Iso		M. Parke	8.3	None	Live

[a] WHOI = Woods Hole Oceanographic Institution collection.

cent lights.[3] All were grown at 20 C, and additional cultures of *Thalassiosira pseudonana* clones 3H, C5 and W were grown at 29 C. Exponential growth of each clone under these conditions was maintained for 5–8 days prior to the experiment by 1–3 transfers into fresh f/2 medium. During this conditioning period all cultures were maintained at <0.1 the maximum cell density attainable in f/2 medium, thus preventing nutrient limitation and possible nutrient-starvation synchrony (Lewin 1962, Darley and Volcani 1971).

Experimental culture vessels (500 ml borosilicate erlenmeyer flasks containing 200 ml sterile f/2 medium) were inoculated with 1–50 ml of exponentially growing culture at approximately the mid-point of the 14 h light period preceding the time interval over which division was to be monitored. The cultures were then sampled, beginning at the start of the next light period (i.e. ca. 17 h after inoculation) every 2 h for 48 h. Sampling required ca. 15 min, but the cultures were sampled in the same sequence each time, so that the sampling interval for each clone was 2 h ± 2 min. During dark periods samples were drawn by the light of a flashlight directed away from the culture flasks, so that the light intensity incident on the cultures was $<1 \times 10^{-4}$ ly·min^{-1} during the 15 min sampling periods. To keep the cultures in exponential growth throughout the 48 h experiment, clones known to have maximum division rates >2 divisions·day^{-1} were diluted either 1:5 or 1:10 with fresh f/2 medium 24 h after sampling was initiated. The undiluted cultures were sampled 6 and 10 h after sampling was shifted to the diluted cultures, and the diluted cultures sampled 6 and 10 h after termination of the experiment, to verify that exponential growth continued for at least 10 h after the period for which division rates were determined, and that dilution did not affect the observed division rates.

Samples, consisting of 10 ml of culture fixed at the time of sampling with 2 drops of 37% formaldehyde solution, were counted on a Coulter Counter with a 70 μm aperture and a 16-channel particle-size analyzer. Internal precision of counts in the cell-density range used in these experiments was ±5%.

The virtually unsilicified diatom *Phaeodactylum tricornutum* and all non-diatoms except the cryptomonad *Chroomonas salina* yielded erratic cell counts due to fragmentation of cells in formaldehyde-preserved samples. For these clones, counts were per-

[3] The response of division rate to light intensity has not been determined for most of the clones examined in this study. A light intensity of 5×10^{-2} ly·min^{-1} is either in or near the region of light-saturation for most planktonic algae, and is that at which these cultures have been growing for periods of 1–20 yr (R. R. L. Guillard, pers. comm.). Subsequent experiments (Nelson, Woodward and Guillard, unpubl.) have shown that division in several diatom clones examined becomes light-saturated in the range of $1–2.5 \times 10^{-2}$ ly·min^{-1}.

formed on living cultures immediately after sampling. Counting of each of the 18 clones required ca. 1 min, but again the cultures were counted in the same sequence each time so that the time interval between counts for a given clone was 2 h ± 2 min. Samples collected during light periods were kept in clear plastic cuvettes until counted (5–25 min) and those collected during dark periods were kept in plastic cuvettes darkened with black electrical tape.

The counted cell density at each sampling time (N_t) and those at the immediately preceding and immediately following sampling times (N_{t-2h} and N_{t+2h}, respectively) were used to compute the instantaneous specific cell division rate (μ: $\equiv 1/N \cdot dN/dt$) of each clone as a function of time. A finite-difference approximation of μ was calculated for each clone in 2 h time steps using the following equation:

$$\mu_t = \frac{1}{N_t} \cdot \frac{N_{t+2h} - N_{t-2h}}{4h} \quad (1)$$

Equation 1 considers the data for each clone in a series of 23 overlapping 4 h time intervals. An alternative method would be to compute μ_t over 24 consecutive 2 h time intervals using the equation:

$$\mu_t = \frac{2}{N_{t+1h} + N_{t-1h}} \cdot \frac{N_{t+1h} + N_{t+1h}}{2h} \quad (2)$$

However, the random counting error of ±5% imposes an uncertainty of $\pm 0.025 h^{-1}$ in μ_t when computed using Equation 2. Some time-resolution is lost by using Equation 1, but the uncertainty in μ_t is reduced to $\pm 0.0125\ h^{-1}$.

Division periodicity data on 26 clones were obtained in these experiments. Isolation data and experimental parameters for these clones are presented in Table 1. An additional 12 clones failed to produce data relevant to the question of the existence or timing of cell-division periodicity, and these are listed in Table 2 with the reasons for failure.

RESULTS

As an example of the cell count data and division rate estimates derived from them using Equation 1, N_t and μ_t for *Thalassiosira pseudonana* clone 3H at 20 C are plotted vs. time in Fig. 1. As discussed, the ±5% uncertainty in N_t subjects any individual estimate of μ_t to a random error of $\pm 0.0125\ h^{-1}$, and

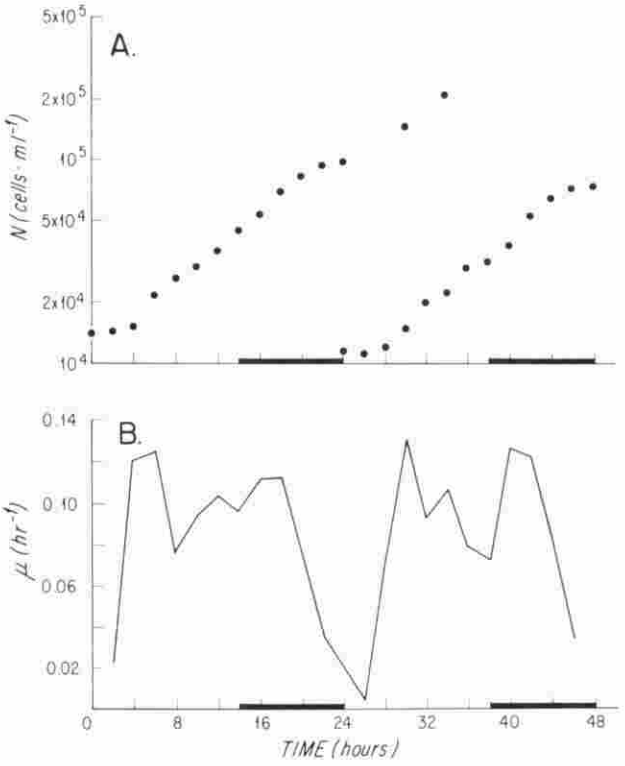

FIG. 1. Cell count and division rate of *Thalassiosira pseudonana* (3H) on 14:10 LD cycle, 20 C: culture diluted 1:10 at 24 h, and both diluted and undiluted cultures counted at 24, 30, 34 h. FIG. 1A, cell count (N) vs. time: FIG. 1B, specific division rate (μ) vs. time, computed from data in FIG. 1A according to Equation 1.

Fig. 1 shows that the computed value of μ_t is reasonably sensitive to changes in N_t that appear to be simple random scatter in the cell count data. Nevertheless, a strong periodicity in cell division is apparent: μ_t increased from ca. 0.01–0.02 h^{-1} at the be-

TABLE 2. *Clones for which experiments described failed to produce data.*

Reason unsuccessful	Species	WHOI[a] clone designation
1. Formalin-preserved samples yielded erratic data on Coulter Counter due to fragmentation of cells, and cells were either too large or too small to be counted live on Coulter Counter using a 70 μm aperture.		
	Ditylum brightwellii (T. West) Grunow (Bacillariophyceae)	DB
	Gonyaulax polyedra Stein (Dinophyceae)	GP60c
	Gymnodinium sp. (Dinophyceae)	Gymno
	Prorocentrum sp. (Dinophyceae)	Salp
	Monallantus salina Bourrelly (Eustigmatophyceae)	GSB Sticho
2. Chain-forming diatoms could not be counted accurately on Coulter Counter as intact chains. We were unable to duplicate the finding of Falkowski and Stone (1975) that sonication yielded intact, individual cells that could be counted accurately.		
	Skeletonema costatum (Greville) Cleve (Bacillariophyceae)	Skel 200-1
3. Division rate was too low to be partitioned into 4 h intervals.		
	Thalassiosira nordenskioeldii Cleve (Bacillariophyceae)	T nord
	Coscinodiscus sp. (Bacillariophyceae)	Cos 4 TN 2
	Rhizosolenia setigera Brightwell (Bacillariophyceae)	Rhizo
	Prorocentrum sp. (Dinophyceae)	Exuv

[a] WHOI = see Table 1.

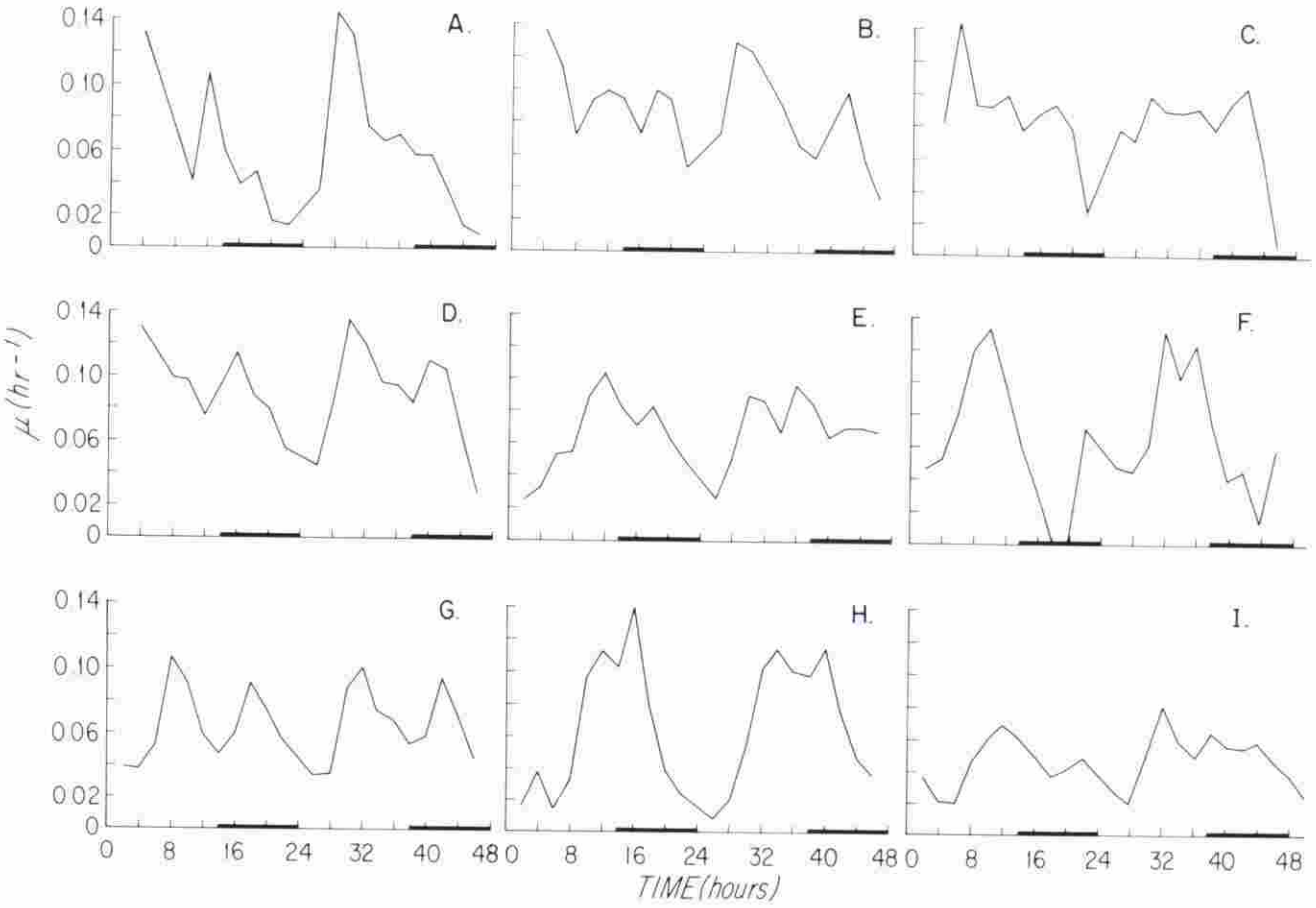

FIG. 2. Division rate vs. time in diatom clones exhibiting LD periodicity in cell division: A–D, *Thalassiosira pseudonana*, A = clone C5, B = clone W, C = clone Swan-1, D = clone STX-97; E, *T. weissflogii* clone SA; F, *T.* sp. clone 289-1; G, *Cyclotella cryptica* clone WT-1-8; H, *Chaetoceros simplex* clone Bbsm; I, *Phaeodactylum tricornutum* clone PetPd.

ginning of each light period to a maximum of >0.12 h^{-1} after 6 h of illumination. Cell division then proceeded at high (although perhaps somewhat reduced) rates through the remainder of the light period and ca. 4 h into the ensuing dark period, after which μ_t declined sharply to the minimum observed in the early morning hours.

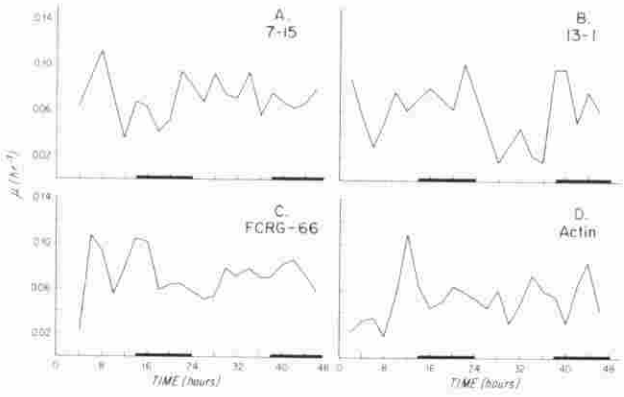

FIG. 3. Division rate vs. time in *Thalassiosira* clones with division rates not detectably periodic: A–C, *T. pseudonana*, A = clone 7–15, B = clone 13–1, C = clone FCRG-66; D, *T. weissflogii* clone Actin.

We propose that true periodicity in cell division can be distinguished from random fluctuations in the μ_t vs. time data by the following criteria, which take into account both the magnitude and the repeatability of the observed changes: cell division in a given clone was considered detectably periodic if μ_t exhibited both a maximum and a minimum, each of which was repeated at an interval of 24 ± 2 h; and, if the mean of the two periodic maxima exceeded the mean of the two periodic minima by at least a factor of 2. A secondary periodic division rate maximum was taken to have been present if it followed the primary maximum by the same number of hours each day, and if on each day it exceeded the intervening division-rate minimum by at least a factor of 1.5.

By these criteria, detectable LD periodicity in cell division was present in 22 clones and absent in 4. A secondary division-rate maximum was evident only in *Cyclotella cryptica*, although the data suggest the possibility of secondary maxima in *Thalassiosira pseudonana* clones 3H, W, STX-97.

Figure 2 presents μ_t as a function of time for the nine diatom clones in addition to *T. pseudonana* clone 3H that exhibited periodic cell division at 20 C.

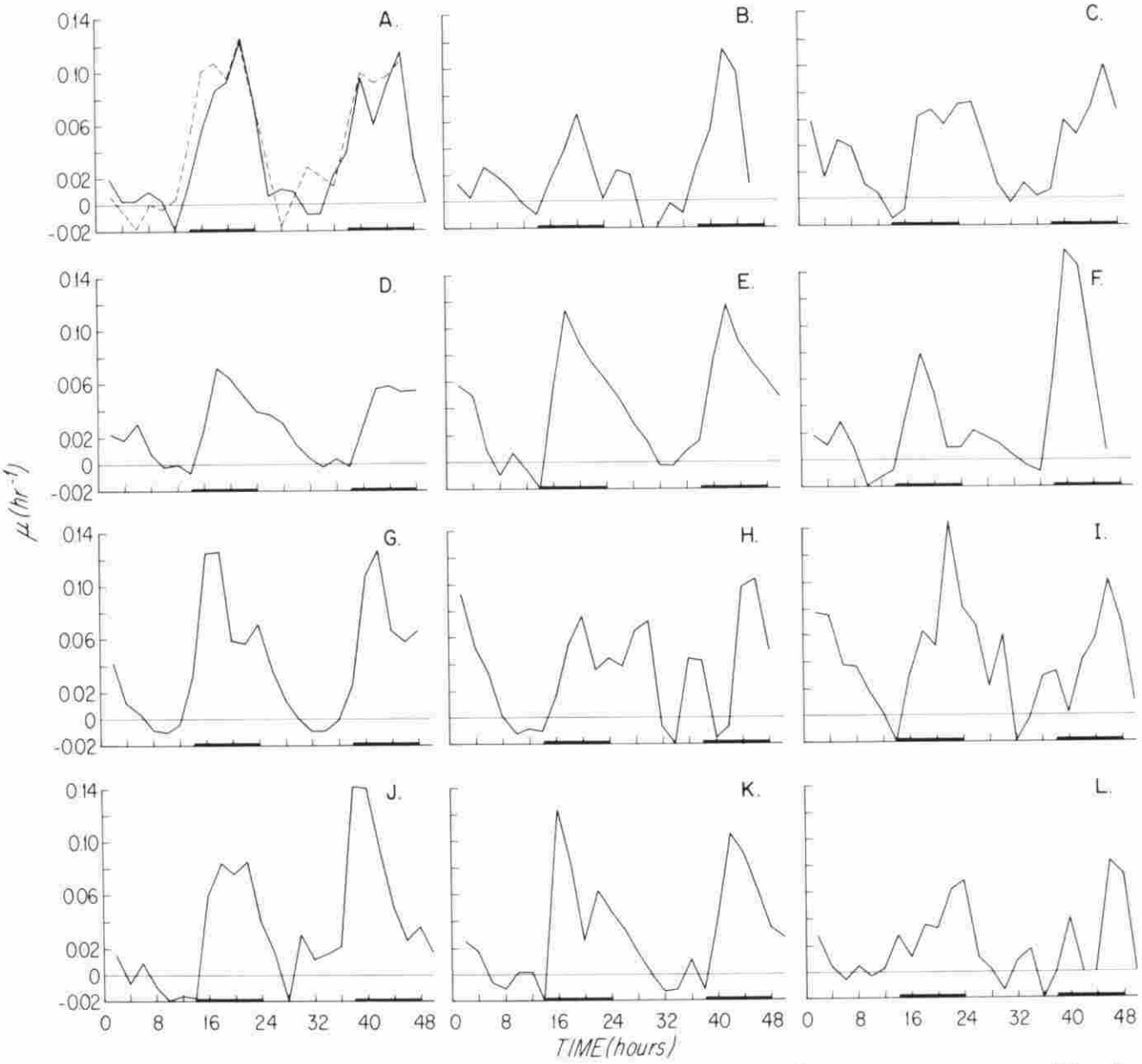

FIG. 4. Division rate vs. time in non-diatom clones: A, *Chroomonas salina* clone 3C (●——● = live counts; ○·····○ = counts of formalin-preserved samples); B–G, *Emiliania huxleyi*, B = clone BT6, C = clone MCH, D = clone 451B, E = clone G4, F = clone 92A, G = clone WHA; H, *Hymenomonas carterae* clone Cocco; I, *Dunaliella tertiolecta* clone Dun; J, *Pavlova lutheri* clone Mono; K, *Isochrysis galbana* clone Iso; L, *Scrippsiella trochoidea* clone Peri.

Comparable data for the four clones, all diatoms, in which division was not detectably periodic are presented in Fig. 3, whereas Fig. 4 shows periodicity data for the 12 non-diatom clones.

Two striking differences between the diatoms and all other algae investigated are apparent from these data. First, a negative value of μ_t (i.e., a decrease in N_t over a 4 h time interval) was never observed for any diatom, but every non-diatom exhibited at least two 4 h time intervals within the 48 h experiment during which the cell count was decreasing with time. Secondly, every non-diatom exhibited a division rate maximum during the dark period, whereas diatoms exhibited either a nearly constant division rate throughout the LD cycle or periodicity favoring division during hours of illumination.

Division rate data at 20 C and 29 C for *T. pseudonana* clones 3H, W, C5 are compared in Fig. 5. Division was strongly periodic in all three at both temperatures, and a 9 C increase in temperature had no detectable effect on either the maximum or the minimum division rate obtained. However, the elevated temperature produced a distinct phase shift in clones 3H and W (Figs. 5A, B) with all

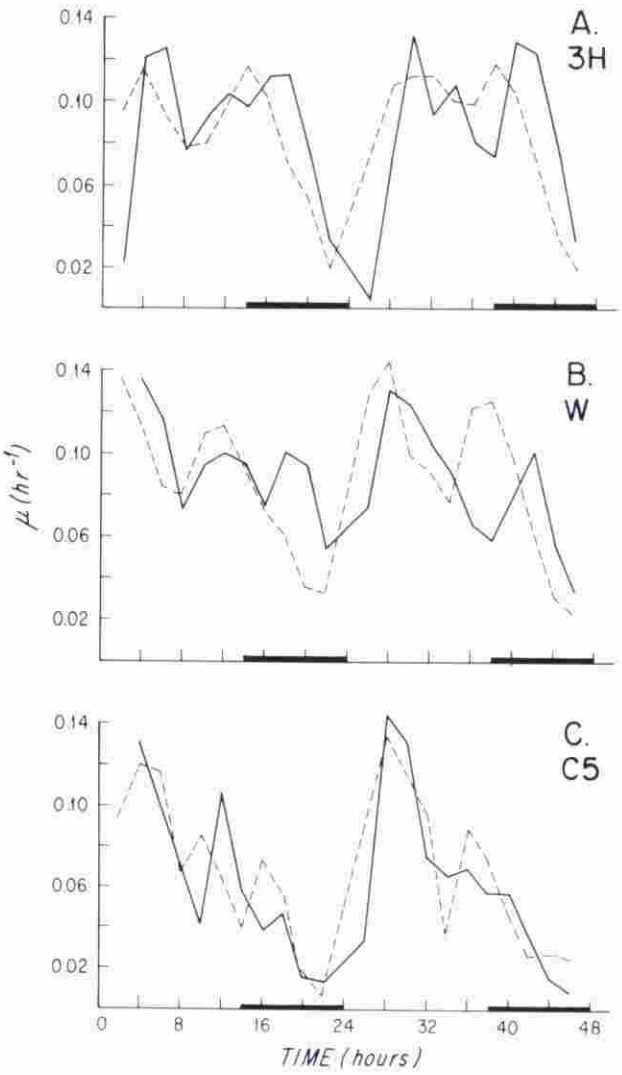

FIG. 5. Division rate vs. time at 20 C (●——●) and 29 C (○· · · · ·○) in three estuarine clones of *Thalassiosira pseudonana* (clone numbers on figure).

changes in μ_t occurring 2–4 h earlier in the LD cycle at 29 C than at 20 C. No such phase shift was evident in clone C5 (Fig. 5C); μ_t vs. time curves for this clone at 20 C and 29 C are analytically indistinguishable from one another.

DISCUSSION

The three most notable features of cell-division periodicity observed in these experiments are the periodic occurrence of "negative division rates" in every non-diatom clone, the distinct periodicity difference between the diatoms and the other major taxa, and the variability of division periodicity within single species, especially the diatom *Thalassiosira pseudonana*.

Negative μ_t values were not observed in any of the 14 diatom clones listed in Table 1, but were consistently observed over at least two 4 h time intervals in each of the 12 non-diatom clones studied. In several of the latter (e.g., *Emiliania huxleyi* clones 451B, WHA) no individual estimate of μ_t was more negative than -0.0125 h^{-1}, and thus the decrease in N_t over any single 4 h time interval was less than the 5% counting error. However, in these clones the low negative μ_t values persisted for 6–8 h, and the decline in N_t over this longer time interval was significant analytically. Thus, periods of genuine decrease in cell density were observed in all 12 non-diatom clones for which periodicity data were obtained.

In these closed-system, batch culture experiments a negative μ_t implies not only death but also disappearance of cells from the medium by fragmentation or lysis. Present data do not allow us to determine whether this disappearance was confined to periods of measured negative μ_t or was occurring at all times and masked by cell division during periods of positive μ_t. The absence of negative μ_t values among the diatoms may indicate that they did not undergo lysis or fragmentation, or it may simply reflect the fact that they have siliceous shells, which are preserved in the medium (and thus counted) regardless of the physical condition of the cells.

The other consistent difference between the diatoms and all other classes studied was in the timing of cell division. All non-diatom clones that yielded periodicity data exhibited distinct division rate maxima at some time during the 10 h dark period. In contrast, no diatom exhibited a periodicity favoring division during hours of darkness; ten clones showed division rate maxima during light periods while four divided at apparently constant rates throughout the LD cycle. The 38 clones used in this study were selected with little prior knowledge of their division periodicities under the conditions used in these experiments, and the 12 that failed to yield data on periodicity were rejected for solely analytical reasons (see Table 2.). Thus, although we have no reason to believe that the 14 diatom clones we selected for experimentation encompass the entire spectrum of division periodicities present in planktonic marine diatoms, or that the 12 members of other algal classes similarly represent all other taxa present in marine phytoplankton, there was nothing in our selection process that biased our sample in the direction of a distinct periodicity difference between major taxonomic groups. The probability of a sample of 14 diatom and 12 non-diatom clones showing the observed periodicity difference by chance, if there were no systematic difference in cell-division periodicity between diatoms and other planktonic marine algae, is less than 1 in 4×10^6. We therefore interpret the data in Figs. 1–4 as indicating a general tendency toward daytime cell division or non-periodic cell division among the diatoms and nocturnal cell division among other planktonic marine algae.

Division periodicities contrary to the general trend described here have been reported for the diatoms *Phaeodactylum tricornutum* (Palmer et al.

1964), *Ditylum brightwellii* (Richman and Rogers 1969) and *Skeletonema costatum* (Eppley et al. 1971), which all exhibited maximum division rates during dark periods. These findings are in apparent contradiction to other studies on the same species reporting division rate maxima in the light for *S. costatum* (Jorgensen 1966), *D. brightwellii* (Paasche 1968) and *P. tricornutum* (this paper). Periodicity favoring division in the light in algae other than diatoms has been observed in the dinoflagellates *Prorocentrum micans* Ehr., *Gymnodinium splendens* Lebour (Sweeney 1959) and *Scrippsiella sweeneyae* (Sweeney and Hastings 1964). Clearly, the observed division periodicity of a planktonic alga is determined to some degree by the particular conditions of the experiment (e.g. Eppley and Coatsworth 1966, Paasche 1967). Additionally, the data presented here for *Thalassiosira pseudonana* (Figs. 1–3) and *Emiliania huxleyi* (Fig. 4) indicate detectable differences among different isolates of a single species, even when experimental conditions are identical. Thus, apparent contradictions concerning the division periodicity of any species may have either environmental or genetic causes.

Differential diel periodicity in nutrient assimilaton has been proposed (e.g., Williams 1971, Stross et al. 1973, Doyle and Poore 1974, Chisholm and Nobbs 1976) as a possible factor serving to resolve Hutchinson's (1961) "paradox of the plankton", in which numerous species apparently coexist while in competition for the same limiting resources. Differential cell division periodicities may serve to separate the nutrient demands of different algae temporally in the 24 h day, but the only potentially limiting nutrient in the ocean whose assimilation has been shown to be tightly in phase with the cell division cycle is silicic acid (Lewin 1962, Lewin et al. 1966, Darley et al. 1976, Sullivan 1977; Chisholm et al. 1978). Other nutrients can be stored intracellularly in amounts sufficient to support one to several cell divisions (e.g., Caperon and Meyer 1972, Droop 1973, Rhee 1974). Thus, differences among phytoplankton species in the timing of cell division need not imply similar differences in the timing of nutrient assimilation.

We have no completely satisfying explanation for the striking difference observed between the division periodicities of diatoms and other marine phytoplankters. The following speculative explanation of our data rests upon the untested assumption that temporal separation of metabolic processes imparts a selective advantage to a unicellular alga.

All cells appear to partition the synthesis of metabolic structures, division structures and nucleic acids so that these syntheses occur at different times in the division cycle (Yost 1972). Such partitioning may avoid disruptive and wasteful interaction such as competition for common energy sources or metabolic precursors. Furthermore it has been shown that overall metabolic rates are lowered during mitosis and cell division (DuPraw 1968), and diatoms have shown substantial decreases in cellular carbon and carbohydrate content concurrent with cell wall formation (Coombs et al. 1967). Thus in planktonic algae it may be advantageous, or even necessary, for photosynthesis and mitotic events such as chloroplast division, cytokinesis and cell wall formation to be separated temporally. If so, then natural selection acting upon the phytoplankton would favor genotypes that divide at night when photosynthesis cannot occur. It is conceivable that most planktonic algae developed such a strategy, and data presented here suggest that the majority of algae other than diatoms carry it through to the present.

The first siliceous diatom frustules appear relatively late in the fossil record—in the middle Cretaceous (Burckle 1978). Presumably frustules provide the diatoms with some selective advantage, such as resistance to predation or parasitism, enhanced sinking ability or a mechanism for attaining greater cell size while retaining a high surface-to-volume ratio by draping the cytoplasm on the frustule. While imparting such advantages, formation of a silica frustule may preclude a reproductive strategy favoring nocturnal cell division. Silicic acid is not stored intracellularly by diatoms in significant amounts (Mehard et al. 1973, Azam et al. 1974) and uptake is almost entirely confined to a specific period within the division cycle, following cytokinesis and preceding separation of the two daughter cells (Lewin 1962, Lewin et al. 1966, Sullivan 1977). Thus silicic acid uptake and cell division are tightly coupled temporally. If silicic acid uptake and frustule formation require substantial amounts of energy (Lewin 1955, Coombs et al. 1967) and the cell cannot store sufficient energy to maintain uptake and deposition in the absence of photosynthesis, then the cell would be forced to divide during the daytime. The observed range of periodicities in the diatoms, in which ten showed division rate maxima during the light period (and continued dividing for varying lengths of time in the dark) and four divided at nearly constant rates throughout the LD cycle may thus reflect the capacities of the different diatoms to store energy for later use in silicic acid uptake and frustule formation.

We consider the discussion presented here to be only a working hypothesis, inasmuch as present data are clearly insufficient to test this, or any, general theory of division periodicity in marine phytoplankton. Further research on temporal separation of metabolic processes, on the energy requirements of silicic acid uptake and silica deposition, and on the energy storage capabilities of cells would help greatly in any effort to explain division periodicity data.

Division periodicity differences among isolates of a single species are detectable in *Emiliania huxleyi*, and very pronounced in *Thalassiosira pseudonana*. The five *T. pseudonana* clones isolated from estuarine or coastal waters (3H, W, C5, Swan 1, STX-97)

all demonstrated strong periodicity, with the division rate maxima occurring 4–6 h after the beginning of the light period. In contrast, the isolate from the western Atlantic slope water (7–15) and the two open ocean clones (FCRG-66, 13-1) showed no detectable division periodicity. Numerous physiological differences between estuarine and open ocean clones of *T. pseudonana* have been reported (e.g., Guillard and Ryther 1962, Carpenter and Guillard 1971, Guillard et al. 1973, Nelson et al. 1976) and have generally been interpreted as genetic adaptations to variable, high-nutrient and stable, low-nutrient environments, respectively. The periodicity data are consistent with this hypothesis, and suggest that the lower instantaneous rates of nutrient uptake and cell division imposed on oceanic *T. pseudonana* isolates by their low-nutrient environments are compensated for somewhat by the ability to continue these low rates throughout the 24 h day.

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OSCILLATORIA (TRICHODESMIUM) THIEBAUTII (CYANOPHYTA) IN THE CENTRAL NORTH ATLANTIC OCEAN[1,2]

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ABSTRACT

Physiological rate measurements were made with Oscillatoria thiebautii *(Gom.) Geitler in the subtropical north Atlantic Ocean between Spain and Bermuda during May and June of 1975. The near surface C:N fixation ratios averaged 6.5, and the cellular composition ratio was 6.2, suggesting that N_2 fixation is the major path of nitrogenous nutrition for this alga. Compared to other oceanic phytoplankters, it has a low affinity for orthophosphate at oceanic concentrations ($k_s = 9.0$); however, it has a high potential for utilizing phosphomonoesters (170–300 ng atoms $P \cdot \mu g$ chl $a^{-1} \cdot h^{-1}$). Maximal photosynthesis occurred at 450–700 μ Einstein $\cdot m^{-2} \cdot s^{-1}$, and was inhibited by full sunlight. Calculated cell division rates (ca. 180 days) suggest that relative to other phytoplankters in this oceanic region,* O. thiebautii *must be subjected to negligible grazing pressure. No major differences in C, N, chl* a *or ATP were observed between the tuft (fusiform) and puff (spherical) colonies. ATP concentrations relative to other cellular constituents varied greatly between colonies, suggesting a general inter-colony physiological variability in the open Atlantic. With increasing depth in the euphotic zone, there was no evidence for chromatic adaption. The observations that* O. thiebautii *represents only a small fraction of total phytoplankton biomass and that its growth rate is 10–100 times slower than that of the other indigenous phytoplankton, strongly suggest that N_2 fixation by this alga is a virtually insignificant component of the nitrogenous nutrition for the phytoplankton of the North Atlantic central gyre in late Spring.*

Key index words: Atlantic Ocean, North; ATP; carbon; chlorophyll a*; Cyanophyta; ecology, phytoplankton; nitrogen; nitrogen fixation; nutrition;* Oscillatoria*; photosynthesis; phosphorus; phytoplankton, ecology;* Trichodesmium

There are numerous reports on the distribution, production and nitrogen fixing capacity of marine *Oscillatoria* (*Trichodesmium*); however, relatively little is known of its physiology. The first workers to study

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