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Cell cycle and growth rate of a natural diatom population in a mesocosm

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Abstract : Diel variations in nuclear DNA concentrations were examined in a mesocosm to study the cell cycle and growth phase of a natural population of a pennate diatom *Nitzschia rectilongae*. Microfluorometry was applied to quantify DAPI-stained DNA molecules. Durations and abundance of cell cycle phases were estimated based on the DNA histograms. S phased cells were consistently present in a considerable amount, and both S and G_2+M phase cells comprised a mojor portion of the pupulation both during the day and night. In contrast, G_1phase cells were less abundant. These observations indicate that cells were directed towards replication shortly after mitosis and that cell division was initiated rather frequently. S and G_2+M phases showed a rhythmic temporal fluctuation. Phase duration of S and G_2+M phases and growth rates were estimated by two different approaches, an application of the model developed for dinoflagellates and the cell cohort analysis. Discrepancy in the results obtained by these two approaches may be ascribed to the characteristic lack of a clear synchrony of cell division in diatoms.

Key words : *diatom, cell cycle, growth rate, mesocosm, microfluorometry*

Introduction

Natural phytoplankton populations are frequently observed to divide at a particular time of the day to vary rhythmically in accordance with the physiological activity over the 24-hour period (SWIFT and DURBIN, 1972 ; SMAYDA, 1975 ; WEILER and CHISHOLM, 1976). The regular light/dark alternation induces varying degrees of synchronization of cell division among species. Although the synchrony or phasing of cell division is common in all major phytoplankton taxa, timing of division during the light/dark cycles varies among different taxa (CHISHOLM, 1981a). In many species so far investigated, division tends to occur during the dark period. In contrast, diatoms do not exhibit a rigid relationship between timing of division and the light/dark cycle, although division can be synchronized by the light/dark cycle (CHISHOLM, 1981b). Hereafter, we use terms "synchronous" and "phased" after CHISHOLM (1981a).

The synchronized cell division allows evaluation of growth rates of individual species in situ (MCDUFF and CHISHOLM, 1982 ; CARPENTER and CHANG, 1988). Using water samples collected at an appropriate time interval, growth rates can be calculated from the temporal changes in maximum frequency of cell division or nuclear DNA content. These approaches provide species-specific growth rates without the problems associated with enclosing natural populations (VENRICK *et al.*, 1977). In the latter approach, temporal variations in the nuclear DNA content are monitored to determine duration of cell cycle phases in a phytoplankton populaation (CARPENTER and CHANG, 1988). The cell division cycle for eukaryotic cells is

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generalized as four discrete sequential intervals, viz., G_1, S, G_2 and M periods between mitosis (M) and the nuclear DNA synthesis (S) referred to as gaps. G_1 occurs just after the cell division, and G_2 just after DNA replication. Duration of these phases is used in the growth rate estimation. However, this method has been applied only to the dinoflagellates in culture (CHANG and CARPENTER, 1988; ANTIA *et al.*, 1990 : YAMAGUCHI, 1992), and has not yet applied for other algal groups or natural phytoplankton populations.

This communication presents the cell cycle and growth rate of a natural diatom population deduced from diel changes in nuclear DNA. A same population was followed during a mesocosm experiment that was conducted in a haline lagoon, Oh-ike (34°17.5′ N, 136°39.2′ E) in Miè Prefecture, Japan. The lagoon is 960 m × 900 m in size ane 12 m in maximum depth, and connected to Gokasho Bay with which one fifth of water in Oh-ike is exchanged daily by tides (WASHIO and YAMAGUCHI, personal communication). The mesocosm was cylindrical, 6 m in diameter and 9 m in depth, and made of flexible polyester sheet with PVC coating. The lowest part was buried into bottom sediment to minimize water exchange between the inside and outside of the mesocosm.

Materials and methods

Water samples from indise the mesocosm were collected from 1-m depth at an interval of 2 hours on July 8 and 9, 1993 using a van Dorn sampler, and immediately fixed with neutralized formalin (2% v/v). Phytoplankton were concentrated by sedimentation, and enumerated under an inverted microscope fitted with a phase contrast device. Samples collected at noon were stored frozen at −20℃ for later analysis of nitrate, nitrite and phosphate (ANONYMOUS, 1987). Chlorophyll *a* was extracted in 90 % acetone from particles collected on Whatman GF/F filters and determined fluorometrically (YENTSCH and MENZEL, 1963). Solar radiation at the surface was recorded using a pyranometer (2770, AANDERAA).

Among the dominant species, *Nitzschia rectilonga* was selected due to its relatively large cell size. After sedimentation, the cells were washed with methanol to remove algal pigments (YENTSCH, *et al.*, 1983), and rinsed with distilled water. Cells were then resuspended in a Tris buffer containing 20-mM Tris (hydroxymethyl) aminomethane (Sigma), 20-mM 2-mercaptoethylamine hydrochloride (Sigma), 100 mM NaCl (Wako) and 10 mM EDTA 2Na (Wako). pH of the buffer was adjusted to 8.2 (HAMADA and FUJITA, 1983). Nuclear DNA of *N. rectiionga* was stained with 0.5 μg ml^{-1} of 4′, 6′ -diamidino-2-phenylindole (DAPI, Sigma) for 24 hours (MATSUMOTO *et al.*, 1993). Fluorescence intensity of DAPI-stained nuclei was measured using a microfluorometry system composed of an epifluorescence microscope (VFD-TR, Nikon), a high-sensitivity SIT TV camera (CTC-2600, Ikegami), a TV-monitor (PM-123T, Ikegami) and an image analyzer (DVS-3000, Hamamatsu Photonics). Both original images from the TV camera and digitized ones from the image analyzer were recorded by a video recorder (HV-M110, Aiwa) for later reexamination. Excitation light was generated by a mercury lamp (USH-200, Ushio) and passed through an IF 365 filter. The emitted fluorescence was split by a dichroic mirror at 400 nm and passed through barrier filters (a LP 420 and an IF 480 filter). For each sample about 200 cells of *N. rectilonga* were selected randomly and fluorescence was measured to produce the DNA histogram. Fluorescence intensity was standardized using chicken blood cell (CRBC, Sigma, lot No. 40H 4202). CRBC contains uniform amount of DNA at G_1 phase. After rinsing with distilled water the CRBC was stained following procedure same as that for algal cells, and stored in the dark at 5℃ until use. The fluorescence intensity of algal nuclei was normalized to 100 for G_1 cells and 200 for G_2 and M cells (Fig. 1). Since cells at G_2 and M phases could not be separated based on the fluorescence intensity, both phases were combined and are hereafter referred as G_2+M phase. Staining procedure and microfluorometric measurements are detailed in MATSUMOTO *et al.* (1993).

The DNA histogram was analyzed to obtain quantitative estimates of G_1, S and G_2+M phase fractons using a PC-based program (TANAKA and TSUTSUMI, 1988). As exemplified

in Fig. 1, three cell cohorts were extracted by the program at S phase and two cohorts at the G_1 and G_2+M phases. Significance of the cell cohort analysis was examined by χ^2 test, and fitting was repeated to attain maximum χ^2 value.

Population growth rate, μ (h^{-1}), of *N. rectiionga* was estimated using the duration and cell cycle phase fractions following CARPENTER and CHANG (1988) :

$$\mu = \frac{1}{(T_S + T_{G_2+M})n} \sum_{j=1}^{n} \ln\left[1 + f_S(t_j) + f_{G_2+M}(t_j)\right] \quad (1)$$

where T_S and T_{G_2+M} are the duration (h) of S and (G_2+M) phases respectively, n is number of samples, f_s and f_{G_2+M} are fractions of S and G_2+M cells of the population, respectively, and t_j is time when sample j was collected. Daily growth rates were calculated also from in situ phytoplankton abundance:

$$\mu = \ln N_2 - \ln N_1 \quad (2)$$

where N_1 and N_2 are phytoplankton cell number at an interval of 24 hours.

Results and Discussion

Temperature and salinity remained constant during the observation, ranging from 22.5 to 22.6 ℃ and 27.2 to 28.0 respectively. Concentration of nutrient salts and chlorophyll *a* indicated a mesotrophic condition inside the mesocosm (Table 1). Therefore, supply of nutrients did not seem to limit phytoplankton growth. Daily solar radiation was steadily decreasing prior to and during the observation, and the weather was consistently cloudy (Fig. 2). Mean daily radiation that was much lower than the normal value was 139 W m^{-2} and 112 W m^{-2} respectively on July 8 and July 9.

Phytoplankton population in the mesocosm was dominated by the diatoms *Chaetoceros distans*, *Cylindrotheca closterium*, *Nitzschia rectilonga* and *Chaetoceros compressum* in the order of abundance. Among them, *N. rectilonga* was used for cell cycle analysis because of its large size and detectability of DAPI fluorescence. The numerical abundance of this species was more or less constant and fluctuated between 33 and 48 cell ml^{-1} (Fig. 3).

Synchrony of DNA synthesis was not clearly seen in the time course of DNA histogram (Fig. 4). S phased cells of the *N. rectilonga* population consistently existed in significant numbers (Fig. 4). Two or three cell cohorts were always extracted significantly at S phase (χ^2-test,

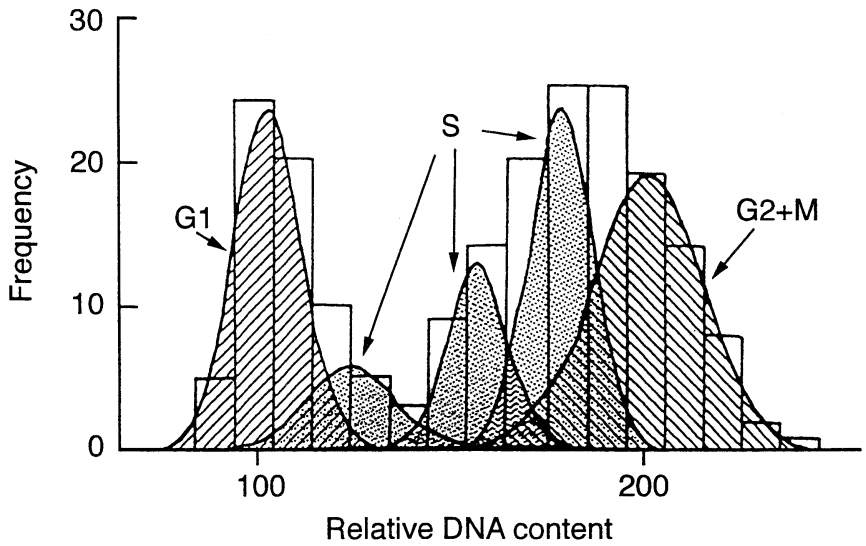

Fig. 1. Fluorescence histogram of *N. rectilonga* representing histogram of relative nuclear DNA content. Cell cohorts obtained by the statistical method are plotted by normal lines.

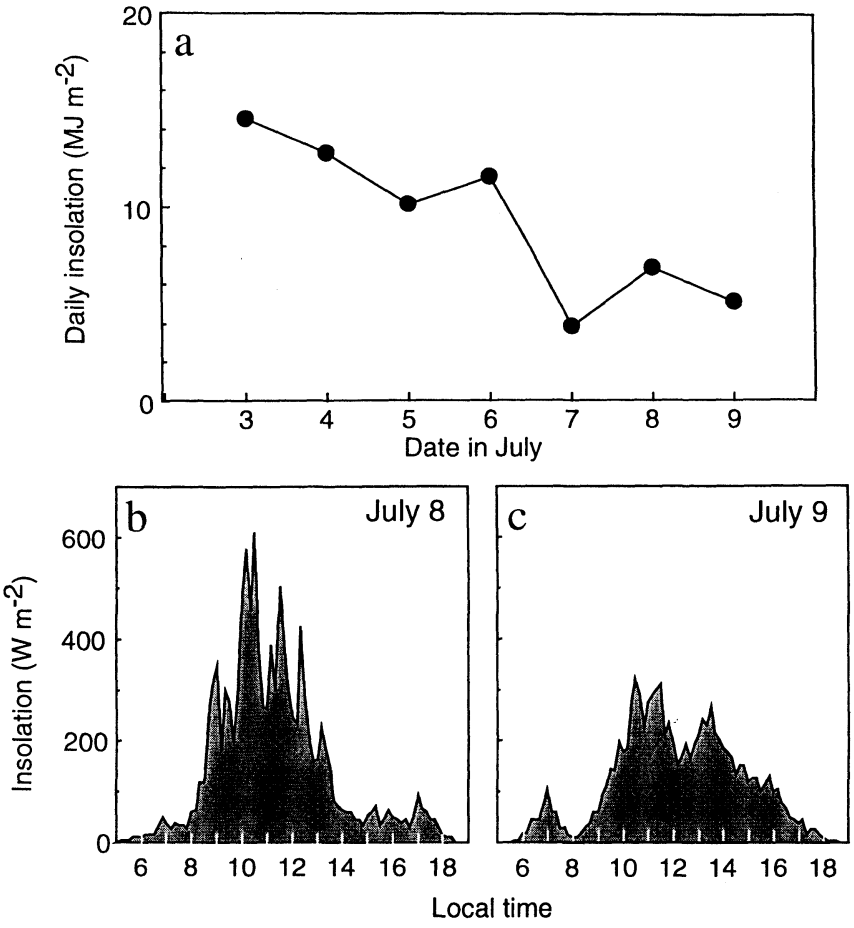

Fig. 2. Temporal changes in solar radiation during the peroid of observation (a) between July 2 and 9, (b) July 8 and (c) July 9.

Table 1. Nutrient and chlorophyll *a* concentration observed at the surface of the mesocosm.

Date	Nitrate (μM)	Nitrite (μM)	Ammonia (μM)	Phosphate (μM)	Chlorophyll *a* ($\mu g l^{-1}$)
July 8	2.1	0.2	3.4	0.5	2.6
July 9	3.1	0.5	7.9	0.3	3.4

$p<0.05$). Cell cohorts represented by normal curves were superimposed on the DNA histograms (Fig. 4). Mode of fluorescence intensity of extracted G_1 cell cohort was 100.7 ± 1.91, and that of G_2+M was 200.1 ± 2.10. Mean fluorescence ratio of G_2+M cell cohorts to G_1 cell cohorts was 2.0 ± 0.03. Coefficient of variation (CV) of the area under G_1 normal curves ranges from 4.9 to 10.8% of the estimated area, and the CV of G_2+M areas ranged from 4.6 to 8.6%. Based on the cell cohort analysis, temporal changes in cell cycle phase fractions were obtained. Three peaks of G_2+M phase cells were observed at 10 : 00 hr on July 8, 02 : 00 hr and 14 : 00 hr on July 9 (Fig. 5). S phase cells also showed three peaks at 20 : 00 hr on July 8, 06 : 00 hr and 20 : 00 hr on July 9. G_1 phase cells were substantially low throughout the sampling, and fluctuated less intensively than other phases.

The constant occurrence of S and G_2+M phase cells, and minor abundance of G_1 phase cells indicate that cells did not stay long at G_1 phase and were directed toward replication shortly after the mitosis. In other words, cell division was initiated rather frequently. This agrees with the general feature of diatom that replication of cells is not fixed at a certain period of the day as observed in other algal taxa (CHISHOLM, 1981a). Although synchrony was not obvious from the DNA histograms abundance of S and G_2+M phase fractions exhibited some rhythms suggesting the cell cycle to be weakly synchronized, or at least phased.

Peaks of S phase cells appeared to shift to those of G_2+M phase cells (Fig. 5). The peak of S phase at 20 : 00 hr on July 8 seemingly moved to that of G_2+M phase at 02 : 00 hr on July 9. Another peak of S Phase at 06 : 00 hr on July 9 likely shifted to that of G_2+M phase at 14 : 00 hr on the same day. Time interval of these possible transits of the population from S phase to G_2+M phase was 6 to 8 hours. CARPENTER and CHANG (1988) proposed a model which provides duration time of S and G_2+M phase. The model assumes clock-based regulation of cell cycle. Although this assumption itself is hardly applicable to diatoms (CHISHOLM, 1981b), the observed rhythms in the phase duration of *N. rectilonga* allows the model to be used to data shown in Fig. 5. Adopting the peaks at 6 : 00 on July 9 and 14 : 00 on July 9 for time of

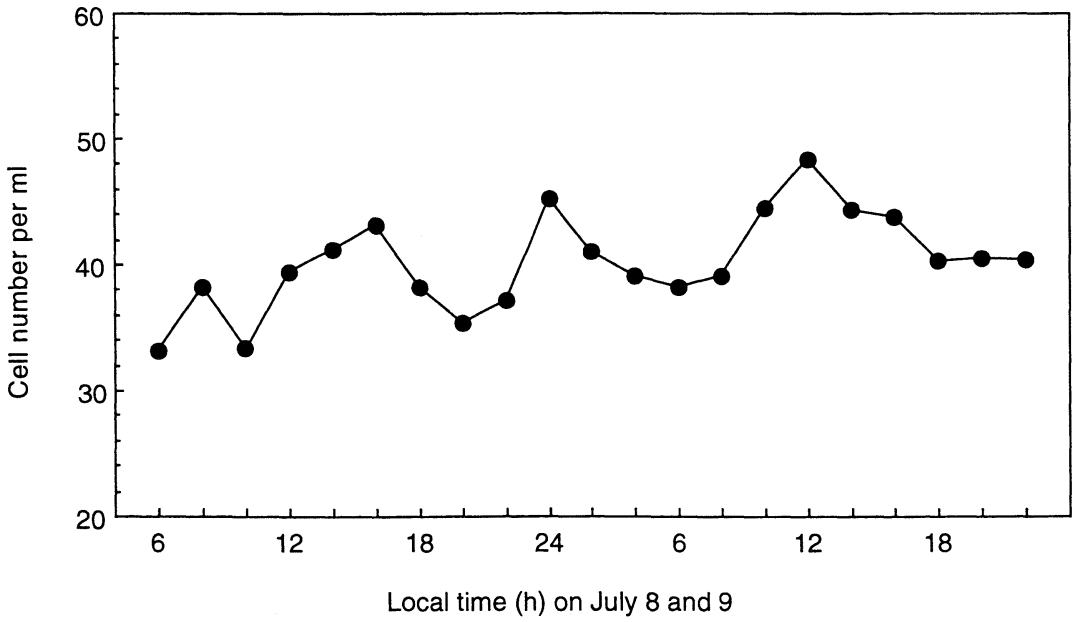

Fig. 3. Variations in the numerical abundance of *N. rectilonga* at 1-m depth inside the mesocosm.

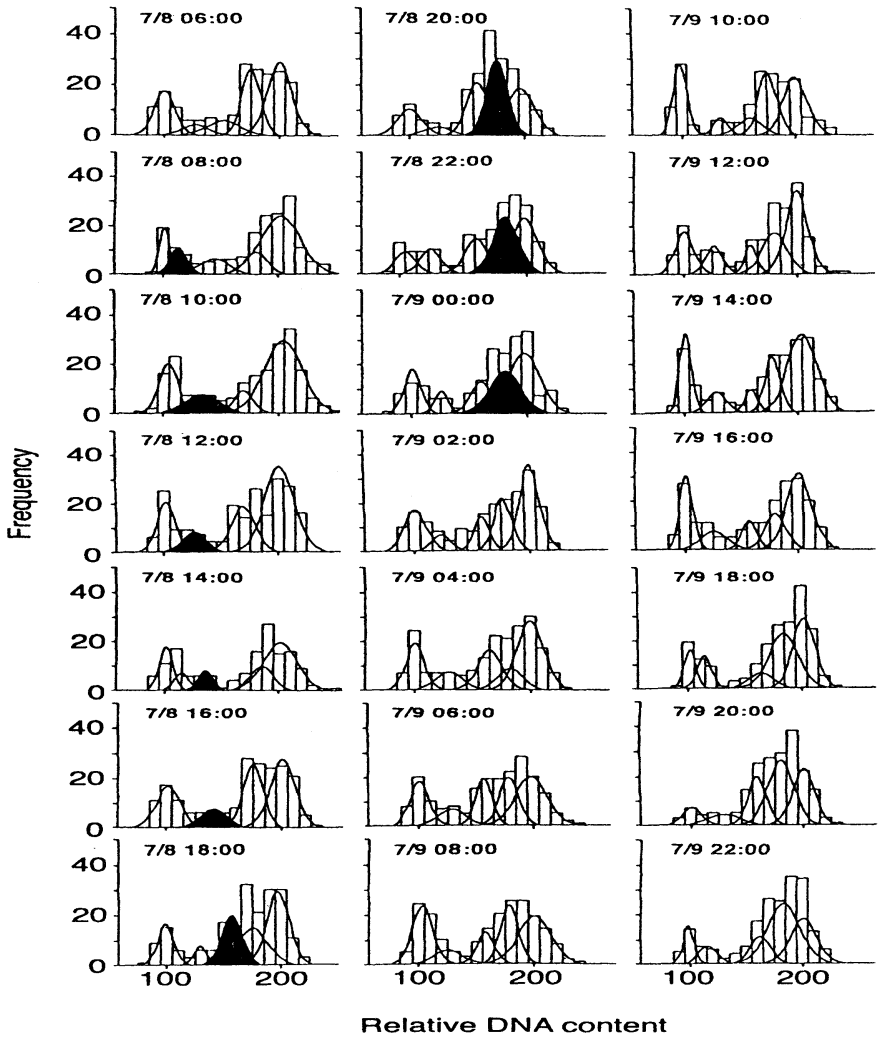

Fig. 4. Time courses of DNA histogram of *N. rectilonga*. Cell cohorts obtained by statistical analysis are superimposed. Shaded areas depict time course of a cohort at S phase.

maximum occurrence of S and G_2+M phases respectively, the duration of S and G_2+M phases was estimated to be 7.2 and 8.8 hours respectively. Applying these estimates to equation (1), daily population growth rate μ was calculated to be 0.91. By using a different set of peaks of S and G_2+M phases e.g., peak of S phase at 20 : 00 on July 8 and that of G_2+M at 02 : 00 on July 9, we obtained the daily population growth rate as 1.21. A striking contrast was observed between these growth rates and in situ numerical abundance of *N. rectilonga*. Net population growth within the mesocosm was small, and the low instantaneous specific growth rates calculated by equation (2) ranged between <0 and 0.20 (d^{-1}). The difference in growth rates based on the two methods was probably attributable to loss processes, such as grazing and sinking.

Phase duration can be also examined by tracing the extracted cell cohorts (Fig. 4). Modes of these cohorts are plotted in Fig. 6. Possible sequence of S phase cohorts are shown by lines, assuming that they do not split or combine

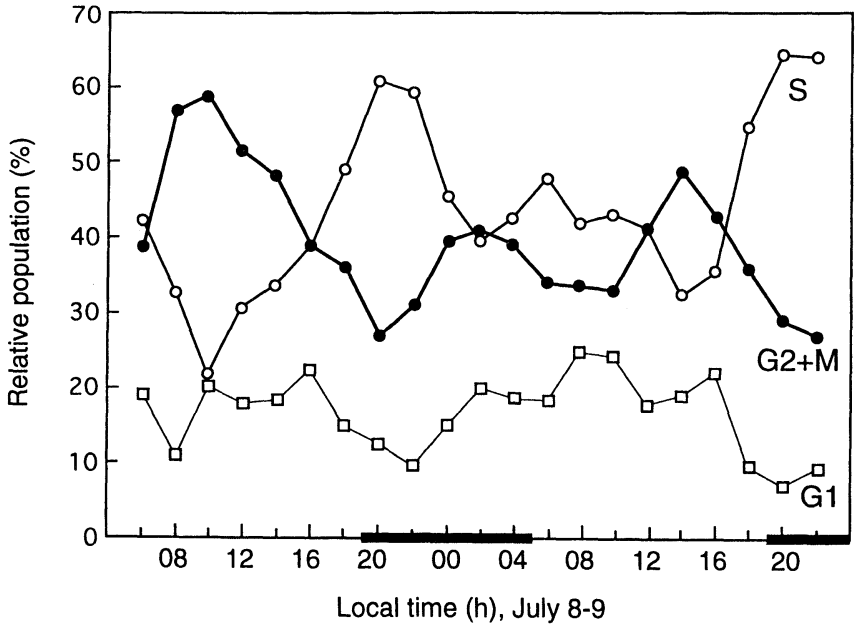

Fig. 5. Temporal variations in the cell cycle fractions of *N. rectilonga*. Dark bars on the X axis indicate night time.

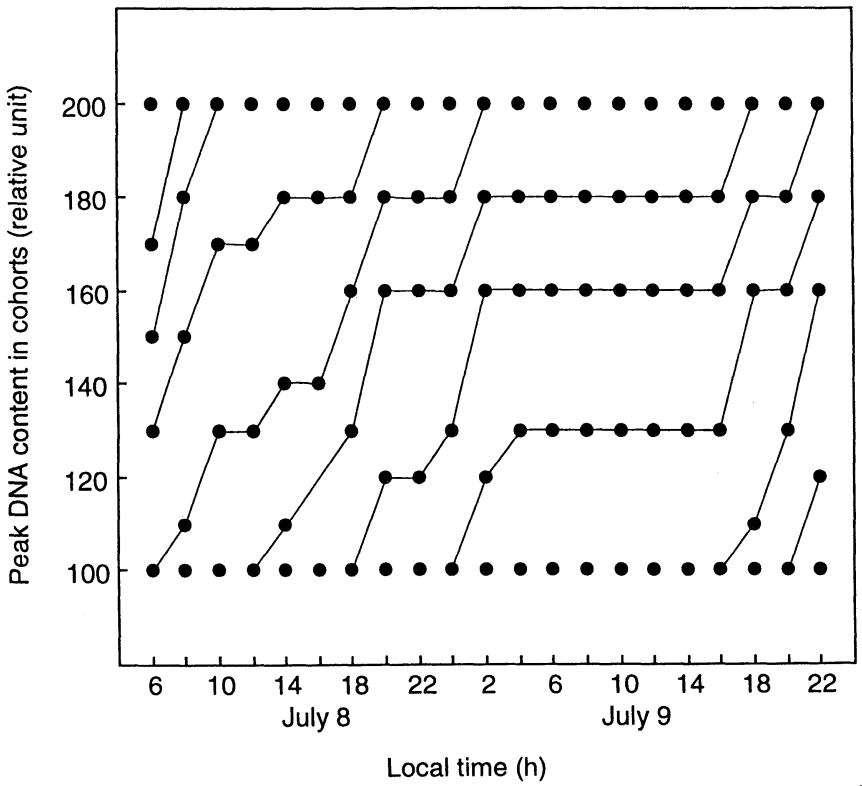

Fig. 6. Temporal distributions of extracted cohorts as shown by closed circles. Possible time courses of each cohort are connected. For details see text.

during DNA synthesis following G_1 phase and until reaching G_2+M phase. Three cell cohorts were generated on both the days. Additionally, three cohorts were in progress at the beginning of the observation. Duration of S phase starting at 06 : 00 hr on July 8 was 14 hours. After this the duration of two subsequent cohorts became much longer; 30 and 28 hours. Since the connection of the cohorts lacked in rigorous criteria, it should be regarded as arbitrary and there could be another sets of connection. However, in all the sets attempted, a common feature noticed was the lower synthesis of DNA synthesis on July 9 compared to that on July 8. This could be ascribed, in part, to the lower light intensity on July 9. Solar radiation on July 7, 8 and 9 was much lower than normal value, and phytoplankton growth was light-limited during the daytime on both days as revealed by the P-E experiments (HARADA, unpublished). *In vitro* oxygen evolution was lower on July 9 than July 8 (HARADA, unpublished), confirming the lowered phytoplankton productivity.

Duration of S phase was estimated to be 3–4 times longer in Fig. 6 than that obtained from Fig. 4. There is no adequate explanation for the discrepancy. Nevertheless the disagreement seemed to be originated from the difference in mode of division cycle between diatoms and other algal groups. Diatoms exhibit only a weak relationship between timing of division and the light/dark cycle, and they have several "preferred" division times in a day (CHISHOLM, 1981b). On the other hand cell division is synchronized and the timing of division is phase restricted in clock-controlled groups such as dinoflagellates (CHISHOLM, 1981a). In the present study S phase was regularly observed and several cell cohorts were extracted at the S phase. These features made it difficult to apply the model originally developed for dinoflagellates, and uncertainties remained in estimation of phase duration and growth rates. The rhythm observed in phase fractions should be considered as forced oscillations rather than entrained by the light/dark cycle (CHISHOLM, 1981a).

In conclusion, the natural population of a diatom *N. rectilonga* did not show a clear synchrony in cell division. This caused difficulty in estimating population growth rate from the cell cycle analysis. Since it does not require bottle incubation and is not influenced by grazing, advection and other loss processes, the cell cycle analysis is of great potential for growth rate of natural populations. Better understandings of diatom cell division and consequent improvements of model analysis are needed.

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