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Species-Specific Growth Rate of Phytoplankton in the West Pacific Sector of the Southern Ocean. I. *Prorocentrum scuttellum* Schröder

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Phytoplankton samples were collected from the West Pacific Sector of the Southern Ocean to measure the growth rate from November 30 to December 1, 1995. *Prorocentrum scuttellum* was selected for growth rate measurement using the method of cell cycle analysis. During the 24 hr sampling cycle, cells of *P. scuttellum* changed from 2,500 to 5,000 cells/L. The highest abundance was observed at 8:40 AM, December 1, and lowest at 11:40 PM, November 30. Cellular division seemed to occur sometime between 11 : 40 PM, November 30 and 2 : 40 AM, December 1. After cell division, DNA fluorescence shifted slowly towards the right, representing the S phase, and the majority of the cells were in S+G2 phases at 8 : 40 AM, December 1. Between the next six hours, a sharp drop in DNA fluorescence occurred, representing mitosis, and the majority of the cells returned to the G1 phase by 2:40 PM, December 1. We can not determine the duration time of the terminal event from this result. However, the growth rate of *P. scuttellum* was calculated as 0.43 d^{-1} with the help of curve fitting methods. This unexpected result seems to have resulted due to background noise, unsynchronous cell division, unequal sampling, water column unstability, and migrating behavior of *P. scuttellum*.

Keywords: cell cycle, growth rate, phytoplankton, *Prorocentrum scuttellum*

INTRODUCTION

In attempting to construct a scientific model of water ecosystem, it is necessary to calculate the growth rate of phytoplankton, which forms the foundation of the food webs (Chang and Carpenter, 1991). Historically, however, there have existed difficulties in accurate measuring of the growth rate of phytoplankton, i.e., some types of incubation periods have generally been required, and many procedures depended on biochemical markers to indirectly determine growth rates (Chisholm *et al.*, 1984; Carpenter and Chang, 1988; Lin *et al.*, 1994, 1995).

This experiment features a relatively new approach to eliminate these problems, taking advantage of a cell's requirement to propagate. During cellular division, the DNA amount changes through a series of stages, collectively known as the cell cycle (Chisholm *et al.*, 1984; Olson and Chisholm, 1986). Therefore, by gathering samples in a timed series, cells can be obtained from each of the stages. DNA is synthesized during a discrete period of the cell cycle (called S phase), and cells which have not yet replicated their DNA (those in G1 phase) can be readily discriminated from cells which have and are ready to divide (those in G2+M phase) [for review see Chisholm (1981)]. Taking into account previous evidence concerning a strong correlation between cellular division and the natural photoperiod in phytoplankton (Chisholm *et al.*, 1984; Carpenter and Chang, 1988), specifically dinoflagellates, a hypothesis can be proposed as to the synchronous replication of the cells, *i.e.*, each cell entering a specific cell cycle phase within a common time frame (Chisholm *et al.*, 1984; Gerath and Chisholm, 1989). An epifluorescence microscope (EFM) can then be used to quantify DAPI stained DNA volume in the cells, allowing them to be assigned to a specific phase in the cell cycle (Boucher *et al.*, 1991). The growth rate can ultimately be determined using information obtained concerning the duration of a terminal event. The term is defined by the time interval between one or more cycle phases and the conclusion of mitosis (Chang and Carpenter, 1988).

This cell cycle analysis method has been pre-

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viously used in successfully obtaining species-specific growth rates of phytoplankton in Long Island Sound, the Sargasso Sea, and the Equatorial Pacific (Chang and Carpenter, 1994). However, there is no data on Southern Ocean Phytoplankton which experience a unique light and temperature regime (Knox, 1994). Therefore, a calculation of growth rate is needed to understand the structure and function of phytoplankton, which constitute a large portion of the Southern Ocean's population.

The purposes of this study were 1) to determine the growth rate of phytoplankton, 2) to identify the synchronous cell division, and 3) to compare with other ocean's data of growth rate measured by cell cycle analysis. For above purposes, the growth rate of *Prorocentrum scuttellum*, was calculated using the method of cell cycle analysis from the west Pacific Sector of the Southern Ocean.

MATERIALS AND METHODS

Water samples were collected every 3 hr interval over the course of a 24 hour period from 8:40 PM, November 30 to 8:40 PM, December 1, 1995 from the Southern Ocean at 50 °S and 145.26 °E using 10 l Niskin bottles attached to a CTD rosette. Water samples were concentrated immediately by centrifugation at 2000×g for 5 min, and all supernatants were removed but 0.5 mL of pellet. The phytoplankton pellet was suspended in 10 mL of chilled (0°C) absolute methanol to fix the cells and remove chlorophyll (Olson *et al.*, 1983). Methanol fixed samples were stored at 4°C for more than 2 days to assure complete extracting of chlorophyll. Upon reaching the laboratory, samples in methanol were spun at 2000×g for 5 min, and the pellet of each tube was washed with 1.5 mL of a phosphate-buffered saline (PBS, pH = 7.4), and then resuspended in 180 μL of a PBS (pH = 7.4). 20 μL of 4'6-diamindino-2-phenylindole (DAPI, concentration of 10 μg/mL) was added for DNA staining (Chang and Carpenter, 1988). Following the DNA staining, an epifluorescence microscope (EFM) was used to identify and determine each portion of the cell cycle phases (G1, S, G 2, and M). Using a camera and imaging system in conjunction with the microscope, DAPI stained photographs of *P. scuttellum* were taken under ultraviolet light illumination. The relative fluorescence and the area of the DAPI stained nucleus were measured from the frozen image, and the process was repeated until 150 cells were counted for each sample.

DNA histograms were made, plotting the relative fluorescence of the cells against the number of cells in each of those fluorescence bins. Using the information obtained concerning the length of the terminal event, the growth rate of *P. scuttellum* was calculated using the formula of McDuff and Chisholm (1982):

$$\mu = 1/(nTx)\ \Sigma\ln[1 + fx(ti)]$$

where, μ = the growth rate per day, n = the number of samples, Tx = the duration of the terminal phase, and *fx* = the fraction of cells in the terminal phase (McDuff and Chisholm, 1982; Chang and Carpenter, 1991).

RESULTS AND DISCUSSION

The abundance of phytoplankton cells ranged from 2.8×10^4 cells/L (11:40 PM, November 30) to 3.6×10^4 cells/L (8:40 AM, December 1) during the sampling cycle from 50°S and 145.26°E of the Southern Ocean (Fig. 1). The total cell number showed slightly bimodal diurnal distribution. The major peak of phytoplankton cell abundance was observed after sun rise (8:40 AM), and minor peak after sun set (8:40 PM). Low abundances of phytoplankton cells were observed at 11:40 PM, November 30 and 5:40 PM, December 1.

The most abundant phytoplankton was *Cylindrotheca closterium*. More than 1.0×10^4 cells/L [average of $1.15(\pm0.5)\times10^3$ cells/L and 30% of total phytoplankton] were observed during the sampling cycle. Among dinoflagellates, the *Gymnodinium* species

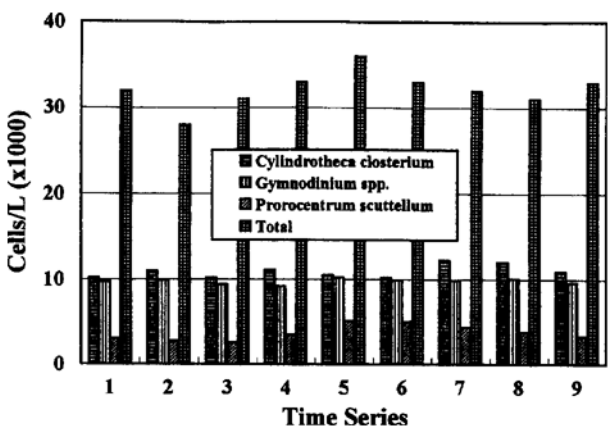

Fig. 1. The abundances of major phytoplankton and *P. scuttellum* in the Southern Ocean from 8:40 PM, November 30, to 8:40 PM, December 1, 1995. Time series 1; 8:40 PM, Nov. 30, 2; 11:40 PM, Nov. 30, 3; 2:40 AM, Dec. 1, 4; 5:40 AM, Dec. 1, 5; 8:40 AM, Dec. 1, 6; 11:40 AM, Dec. 1, 7; 2:40 PM, Dec. 1, 8; 5:40 PM, Dec. 1, 9: 8:40 PM, Dec. 1.

complex was observed around 9.7×10^3 cells/L. Though they appeared more frequently than other phytoplankton, small and unclear DAPI stained nuclei (*Cylindrothecaclosterium*) and bad identification (*Gymnodinium* species complex) prevented them from being used to measure growth rate by cell cycle analysis. *P. scuttellum*, cells which occurred an average of 3,700 cells/L, were stained clearly by DAPI and identified easily under EFM. Therefore, we used *P. scuttellum* for this study based on its easiness to identify, well stained nuclei, and high relative abundance.

During the 24 hr sampling cycle, cells of *P. scuttellum* changed from 2,500 to 5,000 cells/L. Like the result of total cells, the highest abundance of *P. scuttellum* was observed at 8:40 AM, December 1, and the lowest abundauce at 11:40 PM, November 30.

The DAPI stained nuclei of *P. scuttellum* were round or oval shaped, and the CV (coefficients of variation) of the G1 phase DNA was around 15%.

A clear shifting trend of the relative DNA fluorescence peak was observed from DAPI stained *P. scuttellum* samples (Fig. 2). The relative DNA fluorescence peak of *P. scuttellum* shifted from 130 units at 8:40 PM, November 30 to 140 units at 11:40 PM, November 30. 140 units of DNA fluorescence peak was the highest peak from this study. Therefore, 140 units of DNA fluorescence would represent G2 phase, and the maximum portion of *P. scuttellum* cells seemed to be in G2 phase at 11:40 PM, November, 30. After midnight, the peak reduced to 120 units at 2:40 AM, December 1. Over the next six hours, the DNA fluorescence peak increased slowly until 8:40 AM, December 1, when the peak of DNA fluorescence reached to 135 units. After 8:40 AM, December 1, sharp decline was witnessed again down to 120 units at 11:40 AM, December 1. The increase began once again at this point, reaching 135 units by 8:40 PM, December 1. This data leads to the hypothesis that the cells of *P. scuttellum* synthesized DNA (representing S phase) from 8:40 PM, November 30 and reached the G2 phase at 11:40 PM, November 30, and cellular division occurred sometime between 11:40 PM, November 30 and 2:40 AM, December 1, leaving the majority of the cells in G1 at the end of that time. Decreasing of *P. scuttellum* concentration was observed during this period of six hours, and a consistent increase of concentration appeared following that time frame.

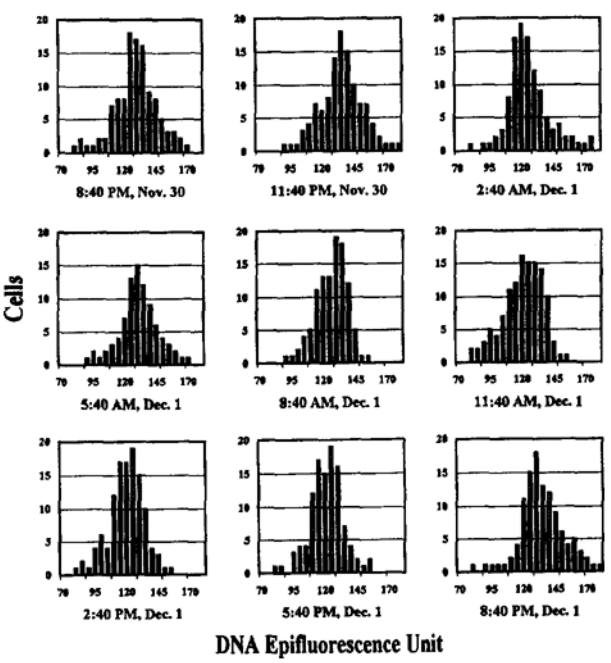

Fig. 2. Distributions of DNA epifluorescence unit of *P. scuttellum* in the Southern Ocean from 8:40 PM, November 30 to 8:40 PM, December 1, 1995.

The cell cycle pattern on the DNA histograms in the six hour period from 2:40 AM, December 1 to 8:40 AM, December 1 is confusing. Initially, it appears that most of the DNA has a relatively low fluorescence, peaking around 125 units. This would correspond to a majority of the cells being in G1 phase. As time progresses, a shift in the DNA fluorescence slowly occurred towards the right, representing the S phase. By 8:40 AM, Demceber 1, the majority of the cells yielded a fluorescence of 125 units, representing the S phase or G2 phase. Between the next three hours, a sharp drop in the DNA fluorescence occurred again, representing mitosis, and the majority of the cells returned to the G1 phase, i.e., the peak of DNA fluorescence of 120 units.

According to a cell cycle analysis done on a dinoflagellate, *Ceratium teres* (Chang and Carpenter, 1994), G1 cells appeared to exist most abundantly during daytime. However, *P. scuttellum* cells showed low DNA fluorescence between 11:40 AM, December 1 and 5:40 PM, December 1 from this study. Just after midnight, DNA synthesis began in the *C. teres* experiment, but the S phase began after 2:40 AM from the Southern Ocean data collection. *C. teres* cells entered G2 phase synchronously a few hours later, and the onset of cell division occured near sunrise (Chang and Carpenter, 1994). In this study, however, the onset of cell division occurred

at 11:40 PM, i.e., at midnight. The discrepancy of this result with the cell cycle of *C. teres* is uncertain. The most conspicuous difference of this study with *C. teres* experiment is the study site. Our sampling site has a unique light and temperature regime (Knox, 1994) which influences the growth and behavior of phytoplankton (Olson *et al.*, 1986; Armbrust *et al.*, 1990; El-Sayed and Fryxell, 1993). It is well known that cell division in unicellular algae can be entrained to periodic supplies of light (Chisholm, 1981), and temperature can change the duration of cell cycle phases (Olson *et al.*, 1986). Therefore, the above discrepancy seems to result from the difference of environmental factors such as light and temperature.

According to the cell cycle theory, DNA in the G2 phase should exactly twice the amount of DNA in the G1 phase (Chisholm *et al.*, 1984; Chang and Carpenter, 1990). Therefore, the relative fluorescence should correspondingly be double in G2 cells. In this study, however, only a 20-30 percent increase in DNA fluorescence occurred in the *P. scuttellum* cells from G1 to G2. A reason for this (and for the unexpected results in the relative area vs. fluorescence plots) could be a flaw in the image analysis. Indeed, background noise was impossible to eliminate completely from this study, and contributions to the fluorescence measurements from this source of error may have caused quantification of not only DNA fluorescence but also background fluorescence. Since the background fluorescence varied from cell to cell (greatly influenced by light intensity of UV and DAPI concentration), different amounts of background noise might have contributed to each reading. As a result, the scattergram would not show a clear separation between G1 and G2 cells. Therefore, though histograms showed a strong trend, specific time frames for each phase were difficult to determine. Due to this fact, a calculation of the growth rate of *P. scuttellum* was not possible. However, with the help of curve fitting methods, the duration of terminal phase was calculated as 6 h. As a result, the estimated growth rate of *P. scuttellum* would be 0.43 d^{-1}. This value is very high compared to the growth rate of *Leptocylindrus danicus* (0.12 d^{-1}), but similar with the growth rates of *Prorocentrum triestimum* (same genus as our sample) calculated using polynomial curve fitting (0.44 d^{-1}) and periodic curve fitting (0.43 d^{-1}) from the sample of Long Island Sound, USA (Chang and Carpenter, 1991).

There are hypotheses as to why the DNA histograms did not provide the information needed to calculate a duration for a terminal phase. The cells may not have been growing synchronously to the light cycle (Olson and Chisholm, 1986). If this were true, no clear majority of cells would exist in one cell cycle phase at a specific time. However, dinoflagellates appear to have been highly synchronized in previous experiments, including a *Prorocentrum* species, *P. triestinum* (Chang and Carpenter, 1991). Another possibility would be the fact that a single water mass was not continuously sampled. Since sampling for this study was performed from easterly flowing open ocean, a single population might not have been sampled. Continuous flow could transport a certain synchronous population out of collection range during specific time periods. Finally, since a motile species is being dealt with, there exists the possibility that cellular populations moved up or down the water column during the sampling time with the change in light intensity.

Although the growth rate of the *P. scuttellum* was unable to be calculated accurately, there was a clear trend in the DNA fluorescence shift with time, corresponding to a progression through the cell cycle. With this information, the cell cycle method for measuring phytoplankton growth rates appears to work well, although some improvement should be made when repeating the experiment. A single water mass must be followed, and the motility of phytoplankton should be considered. However, there is ample evidence showing that dinoflagellate species migrate little through a water column over the course of two days (Chang and Carpenter, 1994). In addition, measurements taken every hour, instead of every three hours, may provided more clear understanding of a certain species' cell cycle. For example, additional samplings between the time period of 11:40 PM, November 30 and 2:40 AM, December 1 for the Southern Ocean may have shown even sharper decrease in G2 cells (with a sharp increase in G1). Finally, improvement in image analysis would allow more accurate data for calculation of growtn rate.

CONCLUSIONS

1) There were no major difficulties in applying the method of cell cycle analysis to natural population of *P. scuttellum.*

2) Cells stained clearly and the DNA content of each cell was easily quantified by microfluorometry.

3) Both DNA synthesis and cell division of the *P. scuttellum* occurred at distinct periods during a 24 h

cycle.

4) The growth rate of phytoplankton can be measured more accurately by reducing background noise and improving the sampling methods.

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