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Method for estimating the *in situ* growth rate for a large pelagic diatom of Rhizosoleniacea

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ABSTRACT: A method for estimating the *in situ* growth rate from the nuclear position of the pelagic diatom *Pseudosolenia calcar-avis* is proposed. The algal cells were seen to exhibit characteristic positioning of the nucleus during each cell cycle. Nucleus movement in the first several hours after cell division was consistent in this algal population. The position of the nucleus can be regarded as the quantitative index of duration time after cell division. Applying this index to the method proposed by McDuff & Chisholm (1982; Limnol Oceanogr 27:783–788) has been shown to give an estimation for the daily averaged specific growth rate of the *P. calcar-avis* population. This method could be useful for the estimation of the *in situ* growth rate of other Rhizosoleniacea species in the ocean without the need for bottle incubations.

KEY WORDS: *In situ* growth rate · Large pelagic diatom · Rhizosoleniacea

INTRODUCTION

Large diatoms are generally ubiquitous and present in low numbers in oligotrophic regions of the world's oceans. Why these large species are able to persist in extremely oligotrophic environments and why they sometimes bloom in such regions remain unanswered questions in the field of phytoplankton ecology (Guillard & Kilham 1977).

Recently, Goldman (1988) proposed that the role of these large phototrophs in oceanic primary production may be far more important than previously believed. Large diatoms, blooming episodically, may make a disproportionately high contribution to new production, a possibility that may go unnoticed with conventional sampling strategies.

To further understand the ecology of large diatoms in the pelagic ecosystem, estimation of the *in situ* growth rates of individual populations is necessary. However, there are few studies estimating the growth rate of such diatoms using unialgal culture experiments (Goldman et al. 1992) or data of carbon content and carbon fixation of natural cells (Villareal & Carpenter 1994).

Several methods have been used to estimate species-specific growth rate of phytoplankton in the field. Some make estimations from carbon-specific growth obtained by the measurement of activities of individual algal cells using autoradiography (Knochel & Kalff 1976, Descolas-Gros 1980) or micropipetting labeled single cells from bottle incubations into scintillation vials (Rivkin & Seliger 1981). Others use *in situ* 'cage cultures' of phytoplankton to estimate population growth rate directly (Owens et al. 1977, Sakshaug & Jensen 1978). However, all of these methods are relatively tedious and require incubations which cause additional problems that do not occur under natural conditions (Venrick et al. 1977, Carpenter & Lively 1980).

There are, however, several methods to estimate the *in situ* specific growth rates without incubation by determination of the mitotic index, i.e. that fraction of cells at a particular stage of the cell cycle (Swift et al. 1976, Weiler & Chisholm 1976, Carpenter & Chang 1988, Vaulot 1992). Applying these methods, the aver-

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age growth rate can be calculated according to the equation proposed by McDuff & Chisholm (1982). These methods require an accurate estimate of the duration of a given cell cycle event, which is generally obtained experimentally. Using any long-lasting cell cycle event which can be measured quantitatively is essential for the practical application of growth estimation, particularly for natural populations (Carpenter & Chang 1988).

Rhizosoleniacea are included in the major group of pelagic large diatoms (Guillard & Kilham 1977). This algal group displays a unique positioning pattern of the nucleus during each cell cycle. This feature suggests that the position of the nucleus in each individual cell can be used as a quantitative index of a cell cycle event for this algal group. In this study, we applied this index to estimate the *in situ* growth rate of Rhizosoleniacea in a culture experiment.

MATERIALS AND METHODS

Pseudosolenia calcar-avis (Schultze) Sundström was isolated at a station of the North Pacific central gyre (6° N, 175° E) during a cruise of the RV 'Hakurei- Maru' (NH-91-2) in September 1991. The culture was maintained in modified MET-44 medium (Schöne & Schöne 1982, Villareal 1990) at 20°C under a 14:10 h light:dark cycle (the light was provided by daylight-type fluorescent tubes at ca 50 µmol m^{-2} s^{-1}).

Experiments were carried out in the modified MET-44 medium under the same temperature and light conditions as mentioned above. To examine the positioning of the nucleus of *Pseudosolenia calcar-avis* during the cell cycle, several cells of this alga were inoculated in each of 24 holes of a multiwell culture dish (Corning Cell Wells 25820) and incubated for 24 h. The position of the nucleus, x, and the whole cell length, l, of each cell of this population (see Fig. 1a) were determined every 2 h during incubation. In this study, x was defined as a distance from the center of the nucleus to the near end of the valve. The length-dependent effect on the nuclear position was analyzed by nonparametric analysis of variance, i.e. Kruskal-Wallis test (Sokal & Rohlf 1995). The time taken for nucleus positioning in each cell was determined from tracing the nucleus position. To determine the specific growth rate of this alga, a batch culture experiment was conducted using Erlenmeyer flasks with light and culture conditions as mentioned above. The initial volume of the culture was 300 ml. Over a 2 wk period, 10 ml of sample was removed every day for cell counting Each sample was fixed with glutaraldehyde (2.5%, v/v) and settled in chambers before counting with an inverted microscope (Utermöhl 1958).

RESULTS

Positioning pattern of nucleus during the cell cycle

The cell growth patterns of *Pseudosolenia calcar-avis* during a cell cycle were examined throughout a 24 h period using a cultured population. Fig. 1 shows a typical cell growth pattern. This algal cell elongated from 420 µm at the beginning of the experiment to a maximum of 550 µm at Hour 12 and then divided into 2 daughter cells which were 200 and 350 µm long, respectively (Fig. 1b). Each daughter cell began to elongate immediately after cell division. At the beginning of the experiment, the nucleus of the algal cell was located 70 µm from the near end of the cell, and then was seen to move to the center of the cell during elongation (Fig. 1c). The nucleus was seen to divide 220 µm from the near end of the cell prior to mother cell separation. It was then found to be located 30 µm from the near end of each separated daughter cell. Each nucleus then moved to the center of each daughter cell, where the positions were almost the same up to 4 h after cell division. After this time, the positioning of the nuclei in the 2 daughter cells was seen to differ. Relative positions of nuclei in the cells were obtained by dividing nucleus position by the whole cell length. These relative positions were seen to differ after cell division occurred, since the cell lengths of the daughter cells were different (Fig. 1d).

These results indicated that *Pseudosolenia calcar-avis* exhibited a characteristic positioning pattern of nuclei during a cell cycle (Fig. 1a). Nuclei of these algal cells were located near the dividing end of the cell immediately after division. Following this, the nucleus shifted towards the center of the cell as the cell lengthened. The nucleus was divided near the center of the mother cell prior to central mother divisions, and was seen to be located at the end of each separated daughter cell. Each nucleus exhibited a similar position in each daughter cell for several hours, despite the cell lengths of each daughter cell being different.

The distribution of nuclei positions within the culture population broadened after cell division (Fig. 2a). The mean position of the nucleus increased almost linearly from 30 µm at 2 h to 73 µm at 10 h after cell division. The average speed of the moving nucleus was obtained from the slope of Fig. 2a and found to be 5.4 µm h^{-1}. The variance of nucleus position within this algal population also increased with time, as did the standard deviation of the distributions, which was found to increase from 5 µm at 2 h to 22 µm at 10 h after cell division. These results indicated that each cell of the population exhibited a similar positioning rate of the nucleus for the first few hours (<4) after cell division.

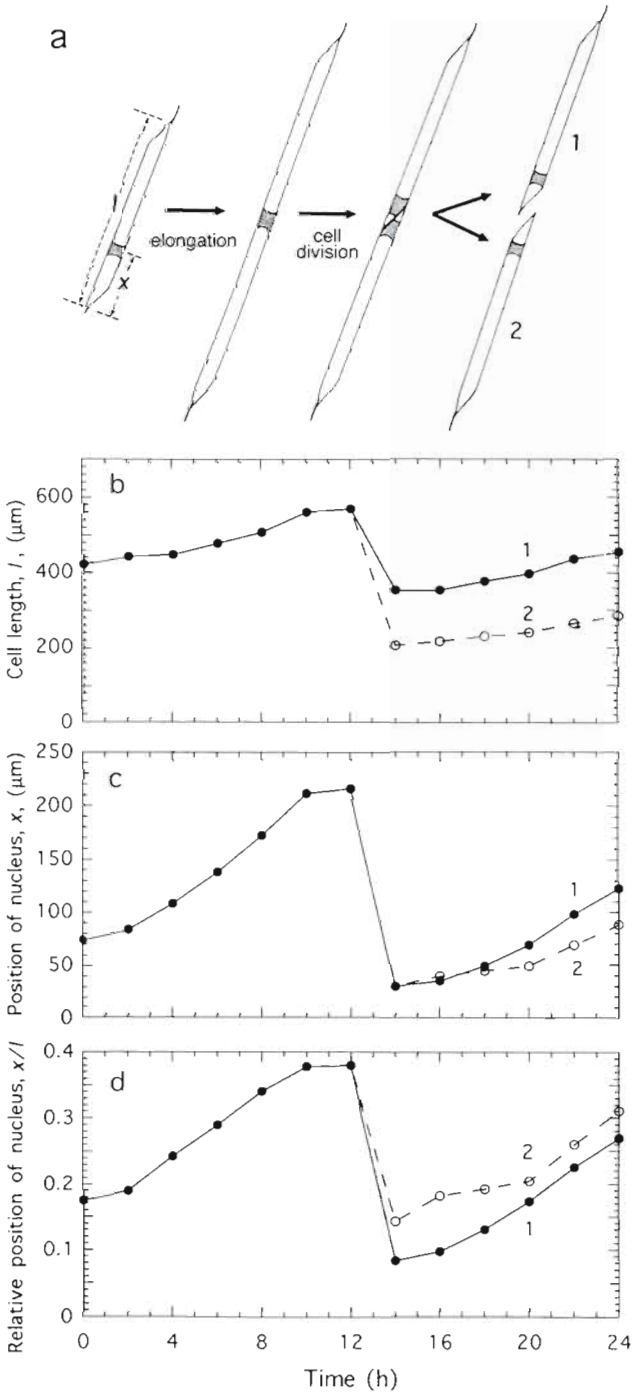

Fig. 1 *Pseudosolenia calcar-avis*. (a) Schematic diagram of positioning of nucleus (hatched area); and changes in (b) cell length, l; (c) position of nucleus, x; and (d) relative position of nucleus of a individual cell, x/l during 1 cell cycle throughout 1 d. Numbers 1 and 2 represent 2 daughter cells

Nucleus position was independent of cell size within 4 h after cell division (Kruskal-Wallis test, $p = 0.957$) (Table 1). Thus, the early position of the nucleus can be used as an index of a cell cycle event.

Estimation of growth rate from positioning of nuclei

To estimate the *in situ* average population growth rate of individual phytoplankton species, an accurate estimate of the duration of 1 cell cycle stage is needed. On estimating the *in situ* growth rate using this index, only information as to the position of the nucleus in each cell within the natural population was available. The duration of each individual cell with each cell nucleus position was determined in a cultured population of *Pseudosolenia calcar-avis*. As the position of the nucleus moved towards the center of the cell, the average duration increased almost linearly from 3.1 h at 30 μm to 7.6 h at 60 μm (Fig. 2b). The standard error of the average duration for each position at the early stage after cell division was around 0.5 h. The minimum value was 0.42 h for <40 μm and the maximum was 0.64 h for <60 μm.

Using these values of duration obtained from the culture experiment, the daily averaged growth rate of a cultured population of this alga can be estimated by modifying the method proposed by McDuff & Chisholm (1982).

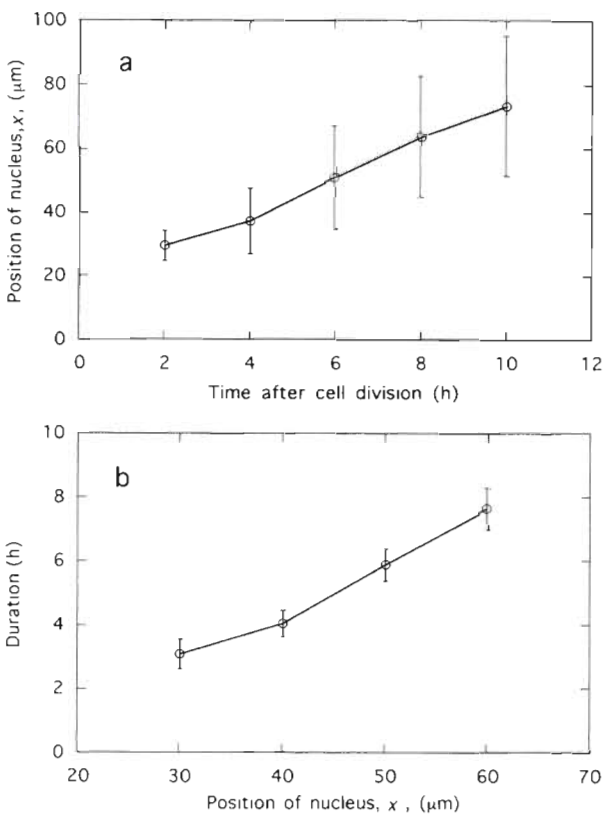

Fig. 2. *Pseudosolenia calcar-avis*. (a) Changes in the mean position of the nucleus, x, of a cultured population after cell division. Each bar represents standard deviation. (b) Changes in the mean duration time along with the moving of the nucleus, x, of a cultured population. Each bar represents standard error

Table 1. *Pseudosolenia calcar-avis*. Size dependency of the position of the nucleus 4 h after cell division

	Cell length (µm)			
	150–200	200–250	250–300	>300
Position of nucleus relative to end of cell (µm)				
Mean ± SD	35.1 ± 3.7	37.2 ± 11.2	37.6 ± 11.5	39.2 ± 10.6
Maximum	39.2	63.7	49.0	53.9
Minimum	31.9	24.5	24.5	29.4
No. of samples	3	17	6	4

Of the algal population, whole cell numbers and numbers of cells already divided during time t_d (the duration after cell division) which are distinguished by having a nucleus position less than a certain distance x at time t are $N(t)$ and $P(t)$, respectively. The total number of cells at time $t - t_d$ can be expressed as:

$$N(t - t_d) = N(t) - P(t)/2 \quad (1)$$

Assuming the logarithmic growth of the population from $t - t_d$ to t, total cell numbers of the population at time t can be expressed with the following equation:

$$N(t) = N(t - t_d)e^{\bar{\mu}(t-t_d,t)} \quad (2)$$

where $\bar{\mu}(t - t_d, t)$ is the mean value of the growth rate over the interval from $t - t_d$ to d.

Thus,

$$\bar{\mu}(t - t_d, t) = \frac{1}{t_d} \ln \frac{N(t)}{N(t - t_d)} \quad (3a)$$

$$= \frac{1}{t_d} \ln \frac{N(t)}{N(t) - P(t)/2} \quad (3b)$$

The fraction of dividing cells in the population, f, at time $t - t_d$ can be expressed with the following equation:

$$f(t - t_d) = \frac{P(t)/2}{N(t) - P(t)/2} \quad (4)$$

Then,

$$\bar{\mu}(t - t_d, t) = \frac{1}{t_d} \ln[1 + f(t - t_d)] \quad (5)$$

To apply Eq. (5) when $\bar{\mu}$ is not constant, we can calculate the daily averaged population growth rate from sequential observations of f according to the same equation proposed by McDuff & Chisholm (1982):

$$\mu = \frac{1}{n\, t_d} \sum_{i=1}^{n} \ln(1 + f_i) \quad (6)$$

where μ is the daily averaged population growth rate (d^{-1}), f_i is the fraction of dividing cells in the ith sample; and n is number of samples in a 24 h measuring cycle.

The daily averaged growth rate of a cultured population of this alga was estimated using Eq. (6) of this method (Table 2). Estimated growth rates using present nuclei position analysis varied from 0.36 to 0.63 d^{-1}, and the rate was seen to increase slightly with a change of nuclei position (Table 2). The average population growth rate of the same population determined by the direct cell count in the multiwell dish gave a value of 0.49 d^{-1} (Table 2), which was close to the value of 0.54 d^{-1} obtained by bottle culture experiment of the same strain under the same culture condition (Table 2). Considering the 4 different positions of a nucleus as indexes of cells immediately after cell division, a position of <40 µm gave the best estimate, 0.53 d^{-1}, of the average rate of population growth of the present species.

DISCUSSION

A number of species of Rhizosoleniacea, including *Pseudosolenia calcar-avis*, are common large diatom species in the pelagic ocean (Sundström 1986). Pelagic large diatoms typically exist in very small population densities (Guillard & Kilham 1977). This scarcity makes it difficult to determine their *in situ* growth rate by ordinary bottle incubations.

McDuff & Chisholm's (1982) method for the estimation of the *in situ* growth rate has the advantage of avoiding bottle incubation. This method requires a cell cycle event which exhibits a longer duration than a sampling interval; also, precise determination of this period results in the accurate estimation of the *in situ* growth rate. The mitosis stage, with paired nuclei in the cell, has been commonly used as a cell cycle event

Table 2. *Pseudosolenia calcar-avis*. Daily averaged population growth rates estimated from the nucleus position analysis and cell counts

Method of estimation	Growth rate (d^{-1})
Nucleus position analysis	
Position of nucleus relative to the end of the cell:	
<30 µm	0.36
<40 µm	0.53
<50 µm	0.61
<60 µm	0.63
Direct cell count	
Single cell culture in a multiwell dish, the same population for nucleus analysis	0.49
Bottle culture	0.54

for such estimations (e.g Swift et al. 1976, Weiler & Chisholm 1976, Braunwarth & Sommer 1985). However, a disadvantage of this technique is that the interval of cell division can be relatively short, and the microscopic observation of cytokinesis can be subjective (Carpenter & Chang 1988). In fact, it was hard to distinguish cells in the mitosis stage from those in the other stages of the cell cycle population even when observing nuclei of *Pseudosolenia calcar-avis* population stained with 4',6-diamidino-3-phenylindole (DAPI) under a fluoromicroscope. The duration of mitosis of this algal cell was also short, i.e. less than 2 h.

This method has been modified in order to estimate the *in situ* growth rate of a planktonic algal population without the need for a culture experiment by determining the proportion of the subpopulation entering the G2 and M stages in the cell cycle which lasted a relatively long period (Carpenter & Chang 1988, Chang & Carpenter 1988). However, this method can only be applied when using the microfluorometry technique to determine cellular DNA contents.

In this study, it was revealed that *Pseudosolenia calcar-avis* exhibits a characteristic positioning of the nucleus during 1 cell cycle (Fig 1). It was also revealed that the intracellular movement of the nucleus for the first 4 h after cell division is consistent within the *P. calcar-avis* population (Fig 2a, Table 1). The position of the nucleus in this species can be used as a quantitative index of duration after cell division for McDuff & Chisholm's (1982) method (Fig 2b). Estimations of specific growth rates using these indices were consistent with those obtained using the direct cell counting method, to within 30% (Table 2). Using a duration corresponding to a nucleus movement of less than 40 µm from the near end of the cell produced the most accurate estimation of the specific growth rate, i.e. within 10% of estimations using direct cell counting (Table 2).

To estimate the *in situ* growth rate using the method of McDuff & Chisholm (1982), 1 cell cycle event of greater duration than the sampling interval is preferred (McDuff & Chisholm 1982). Due to the very small population density of this algal population in pelagic water, large volumes of water are needed which may require long time periods for collection. The time taken for the nucleus to shift to less than 40 µm from the near end of the cell is about 4 h, which is long enough to enable adequate field sampling (Fig 2b).

In applying this method to obtain an estimation of the *in situ* growth rate, it should be noted that small changes in the duration, t_d, could make a large difference in the calculated growth rate using Eq. (6). The accuracy of this method depends on the maintenance of a constant duration of nucleus movement under varying conditions that might occur in natural water. For example, natural temperature variation can effect the duration time of this event, as observed by Weiler & Epply (1979) in the duration of mitosis of dinoflagellates. Furthermore, the early positioning of the nucleus after cell division appears to be involved in the G1 phase, although correlations between nucleus position and cell cycle stages were unclear in this study. For example, it has been reported that a marine diatom *Thalassiosira weissflogii* was arrested in G1 phase under light and nutrient limitation (Olson et al. 1986, Vaulot et al. 1987). This phenomena might occur in *Pseudosolenia calcar-avis* and affect the duration of early nucleus movement. The duration of *P. calcar-avis* nucleus movement may also vary depending on conditions such as temperature, light and nutrients. However, this study was carried out under only one regime, at 20°C with a 14 h light (ca 50 µmol m^{-2} s^{-1}) : 10 h dark cycle and sufficient nutrients. If an accurate estimation of the *in situ* growth rate is required, estimations of duration of nucleus movement under varying conditions might be necessary.

In conclusion, although this method using the duration of nucleus movement after cell division might have limited accuracy, it could be available for the estimation of the *in situ* growth rate of this algae in the oligotrophic ocean. This method is convenient for field study since it needs neither bottle incubation nor special equipment for determination of cellular DNA content.

Since the nucleus being surrounded by cytoplasm and between large vacuoles within a solenoid shaped cell is a common feature of Rhizosoleniacea cells, characteristic intracellular movement of the nucleus during each cell cycle as observed in *Pseudosolenia calcar-avis* (Fig. 1) can be expected to be a general feature of Rhizosoleniacea species. Therefore, this method could be applicable to other species of Rhizosoleniacea than *P. calcar-avis*, although culture experiments to determine the duration of moving nucleus after cell division of these species are necessary for accurate estimation. When the growth rate of a species of Rhizosoleniacea in the field is required and no unialgal culture is available, the data from *P. calcar-avis* could be useful for the estimation of the *in situ* growth rate of such other species, even though this would be an approximation.

Recently, it was revealed that some Rhizosoleniacea species might play an important role in the oligotrophic pelagic ecosystem (Villareal et al. 1993). However, no estimation of the *in situ* growth rate of Rhizosoleniacea has yet been attempted. This method could be very useful for gaining an understanding of this potentially important algal group in the ecosystem of the oligotrophic ocean.

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