Growth Rate of *Oxytoxum* cf. *variable* Schiller in the Southern Ocean

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1. Introduction

The Southern Ocean has unique physical and chemical characteristics including the circumpolarity, variability of light regime, and high concentrations of inorganic nutrients (Knox, 1994). These characters influence the distribution and productivity of marine organisms (El-Sayed and Fryxell, 1993; Knox, 1994). The Antarctic continental side of the Southern Ocean is known to be predominated by krill, and their preference of large diatoms make dinoflagellates dominance over diatoms (Kopczynska, 1992). We found a small dinoflagellate, *Oxytoxum* cf. *variable*, which dominated in cell number with *Gymnodinium* spp. from west Pacific Sector of the Southern Ocean in November 1995.

To understand the structure and function of phytoplankton, which constitute a large portion of the Southern Ocean's population, a calculation of their growth rate is indispensable. We calculated the growth rate of a dinoflagellate, *Oxytoxum* cf. *variable*, using cell cycle analysis from the Southern Ocean to measure the growth rate of phytoplankton.

2. Materials and Methods

Water samples (800 ml) were collected over the course of a 24 hour period (2:40 PM, Nov. 30 - 2:40 PM, Nov. 30, 1995). The sampling site was located at 50 °S and 145. 26 °E. Samples were concentrated immediately by centrifugation, and the phytoplankton pellet was resuspended in 10 *ml* of chilled methanol. Upon reaching laboratory, samples in methanol were spun and the pellet of each tube was first washed with 1.5 *ml* of a

phosphate-buffered saline (PBS, pH=7.4), and then resuspended in 180 μl of a PBS. 20 μl of 4'6-diamindino-2-phenylindole (DAPI, concentration of 10 $\mu g/ml$) was added for DNA staining (Chang and Carpenter, 1988), and an epifluorescence microscope (EFM) is used to determine each portion of the cell cycle phases. Using a camera and imaging system in conjuction with the microscope, photographs of *Oxytoxum* cf. *variable* were taken under ultraviolet light. The relative fluorescence and the area of the nucleus was obtained from the frozen image, and the process was repeated until 150 cells were counted for each sample.

Relative DNA proportion was plotted against the time series, and using the information obtained concerning the length of the terminal event, a calculation of the growth rate (μ) of the phytoplankton was measured using McDuff and Chisholm (1982):

$$\mu = 1/(nTx) \sum_{i=1}^{n} \ln[1 + fx(ti)]$$

where n=the number of samples, Tx=the duration of the terminal phase, $fx(ti)$=the fraction of cells in the terminal phase in ith sample.

3. Results

The most abundant dinoflagellate was *Gymnodinium* spp. from this studied area. They accounted more than 70 % of total dinoflagellates througout the sampled period.

Oxytoxum cf. *variable* was the second dominant species, and they occupied around 10 % of total dinoflagellates. Maximum cell number of *Oxytoxum* cf. *variable* was observed 22 h after initial sampling. As the cells of *Oxytoxum* cf. *variable* were nano- to netplankton size (14 to 25 μm length and 5 to 10 μm width), DAPI stained DNA was carefully countered.

A clear trend was observed in relative DNA proportion. G0-G1 phase was maximum (aroud 100 %) from 0 to 6 h after initial sampling. Over next three hours, a sharp decrease of G0-G1 phase was seen, and S phase increased reciprocally. High proportion of S phase lasted for 8 h and decreased 16 h after initial sampling. The division phase, G2+M phase, increased 18 h after initial sampling. 21 h after initial sampling, around 80 % of DNA were G2+M phase. This data lead to the hypothesis that the majority of the cells were in the G0-G1 phase when first sampling was made and division phase was begun after 21 h of first sampling. Therefore,

celllular division occurred sometime between 18-24 h after initial sampling. It coinsides well with the cell number data.

The growth rate of *Oxytoxum* cf. *variable* was calculated 0.38 d^{-1} from above data. It is similar with mean growth rate of phytoplankton from this area measured by incubation method.

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