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MEASUREMENT OF ACRYLIC ACID AND DIMETHYL SULFIDE IN ANTARCTIC COASTAL WATER DURING A SUMMER BLOOM OF *PHAEOCYSTIS POUCHETII*

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Abstract: Water samples were collected during a summer bloom of *Phaeocystis pouchetii* from the beginning of November 1988, in a 15 m water column 10 km off shore of Davis Station, Vestfold Hill, Antarctica. Results of acrylic acid and DMS determined by HPLC and GC show that concentrations of acrylic acid varied from 0.001 to 0.51 μmol L^{-1} and DMS from 0.003 to 0.588 μmol L^{-1}. Both showed an increase after late December 1988 and maximum concentrations in early January 1989 followed by a rapid decrease to low level by middle January to February. The observed changes of both substances showed good agreement with variations in cell numbers of *P. pouchetii*, the correlative coefficients of acrylic acid and DMS against cell numbers of *P. pouchetii* are all 0.998. The observed highest productivities of acrylic acid and DMS were 9.76×10^{-8} μmol $cell^{-1}$ and 13.09×10^{-8} μmol $cell^{-1}$, respectively.

1. Introduction

The prymnesiophyte *Phaeocystis pouchetii* (HARIOT) LAGERHEI is a unicellular alga which is distributed extensively in cool subpolar and polar waters (KASHKIN, 1963). The algae often clog commerial fishing nets due to mucilaginous materials produced (BONEY, 1970). The appearance of *P. pouchetii* bloom is an ill omen to fishermen, aftercalled "the fishermen's sign", "weedy water", or "stinking water". SAVAGE (1930) confirmed fishermen's claims that herring avoid *P. pouchetii* blooms and suggested that the alga was inedible and its blooms were consequently avoided. The widespread observation of low fishing catches associated with *P. pouchetii* blooms implies that the blooms also have the same effect upon other species of fish (CHANG, 1983). Some results of investigations of krill show that there is a negative correlation between krill numbers and phytoplankton cells in the Antarctic sea. It is possible that it responds to chemical effects such as toxic compounds. *P. pouchetii* utilizes amino acids as a source of carbon and nitrogen and releases toxic compounds, acrylic acid and dimethylsulfide (DMS) (FERGUSON and SUNDA, 1984; YANG *et al.*, 1990). These compounds may be a source of chemical deterrent to drive fishes and krill.

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These compounds not only act as deterrent to fish but also affect climate. When DMS is released from water to the atmosphere, it can react with a variety of oxidizing species such as OH, NO_3, and IO radicals to produce dimethysulphoxide, methanesulphonic acid (MSA), sulphur dioxide (SO_2) and non-sea-sulphate (NSS-SO_4^{2-}). MSA and NSS-SO_4^{2-}, are mostly present as aerosol particles acting as a nucleus around which water vapor condeses to form droplets (cloud condesation nuclei-CCN), Hence increasing CCN concentration results in more cloud cover, which in turn increases the overall reflectivity of clouds. Since the global radiation budget is partly dependent on the albedo effect of clouds, a change in the release of DMS by a factor of two may change the earth's temperature by a few degrees. As the earth warms up as a result of the "greenhouse effect", increasing concentratons of DMS could act to counteract the warming by increasing the cloud albedo, hence cooling the earth (MARK and BURTON, 1992; HARVEY, 1992).

So it is important to understand how much acrylic acid and DMS are released to the environment and to obtain the rate of productivity in *P. pouchetii* blooms in Antarctic coastal water. Here we will give the concentrations of acrylic acid and DMS and their natural productivity, estimated during a *P. pouchetii* bloom in Antarctic coastal water, in order to further study the chemical effects of those toxic compounds on biota and the relation between climate and the biology Antarctic sea water.

2. Material and Methods

Sea water samples were collected at 15 m depth, at a coastal site 10 km of north of Davis Station, Vestfold Hill in Antarctica (Fig. 1). Acrylic acid and DMS were determined by High Pressure Liquid Chromatography (HPLC) and Gas Chromatography (GC) at the Davis Station biological laboratory, and *P. pouchetii* were fixed for later enumeration in the Australia Antarctic Division biological laboratory.

An HPLC system was used to determine acrylic acid and other volatile fatty acids. The system consists of a Kortec ETP pump, a Kortec mixing chamber, a Rainin microsorb 7125 injector valve with a 100 μL loop, a LDC milton roy variable UV sepectrophotometer and an ion exclusion HPX-87H organic acids column. The operation conditions are as follows: 0.05N H_2SO_4 (pH=1.68), flow rate of 0.6 mL min^{-1}, light absorbance at a wave length of 210 nm and the sensitivity of 0.002 AUFS. The sample injection volume was 100 μL. The peaks were identified by their characteristic retention time and the quantification of concentrations of acryalic acid and other volatile fatty acids was made by measuring area calibrated with standard samples using an integrator (CL-10B).

The method for determining DMS has been described by ANDREAE (1983). The method involves removing DMS from a fixed volume of sea water by sparging helium gas and trapping it on silated glass beads at −95°C. After being released from the cold trapping, the compounds collected are separated by gas chromatograph with a flame ionization detector. The cryogenic enrichment gas

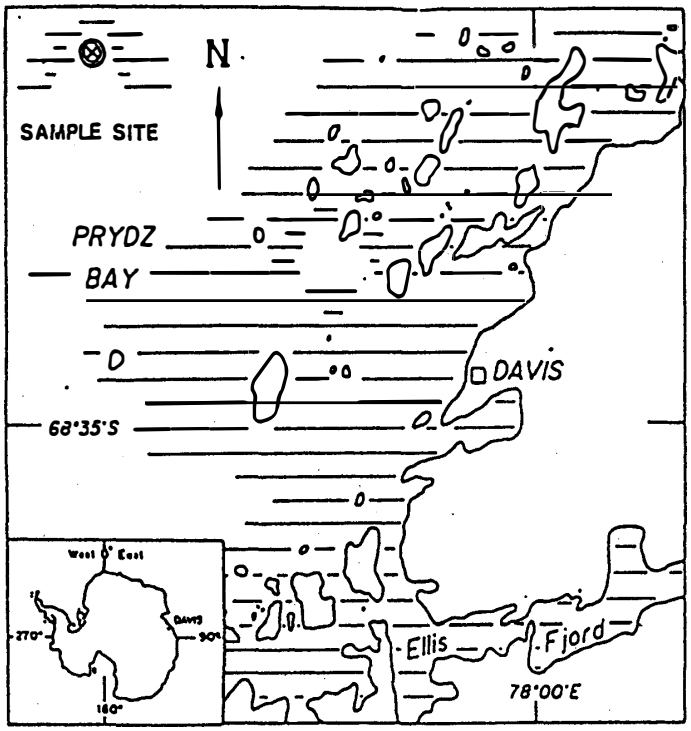

Fig. 1. Sampling site from 10 km northwest of Davis Station.

chromatographic system consists of a gas chromatograph (Varian aerograph series 3700 TCD-FID-FPD), a mass flowmeter, an integrator (LDC milion Ron CL-10B), an immersioncooler (NESLAB cryocool CC100), a digital readout thermometer (NESLAB DR-2), an oxygen trapper (ACTIBON), an isotherm constant temperature bath (KCW), and a six-way stainless steel switching valve (VALCO instrument). The analytical column was a 52.0×3.0 mm ID teflon tube packed with acetone washed porapak QS4.

The rate of change of acrylic acid, DMS and cell numbers from the following formula:

$$r^* = \frac{\log_e N_t - \log_e N_0}{t - t_0},$$

and then the productivity of acrylic acid and DMS are determined from their rates of change, and the number of cells and its rate of change.

3. Results and Discussion

An increase of *P. pouchetii* population at a depth of 15 m was observed from November 23, 1988 at the sampling site in Prydz Bay, Antarctica (Fig. 2 or 3). The maximum cell number was approximately 6.01×10^7 cell L^{-1} on January 3, 1989. Total cell numbers of *P. pouchetii* increased 226 times within six days between December 14 and 20, 1988, and started to decline after January 3, 1989 until reaching a low of 1390 cell L^{-1} on February 14, 1989.

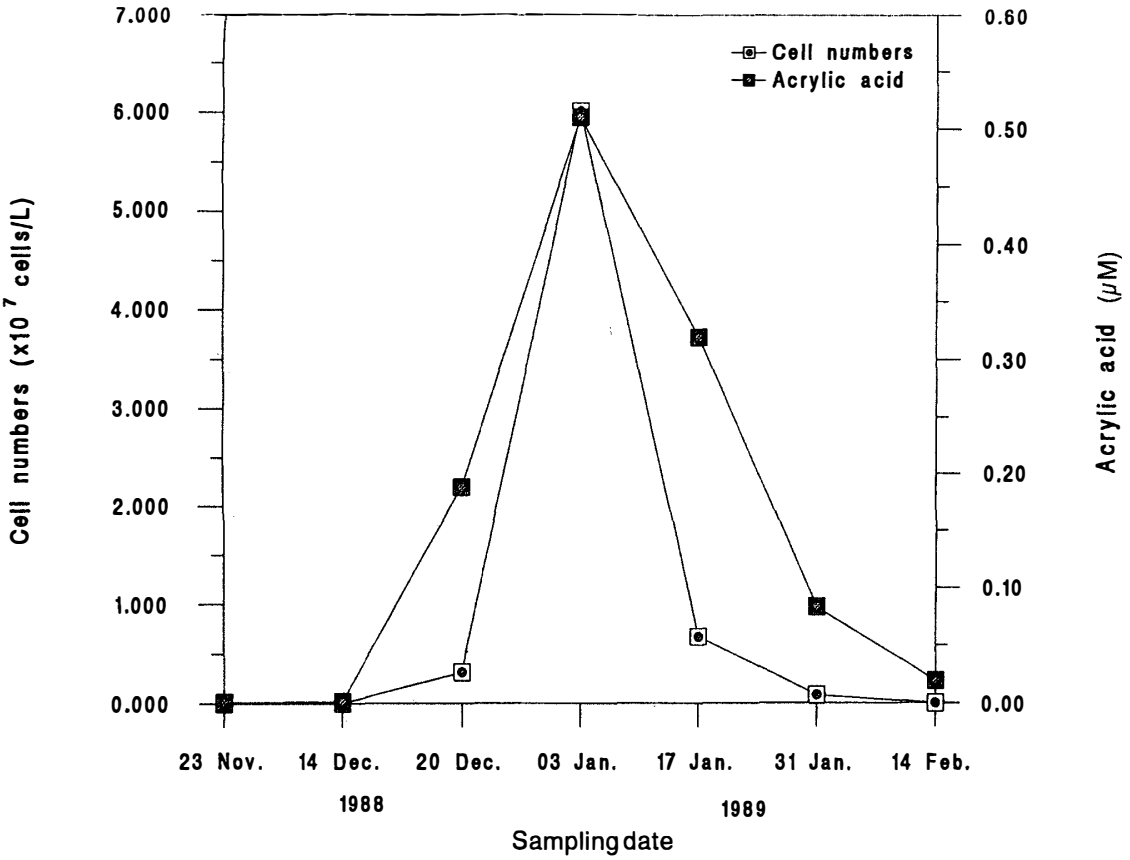

Fig. 2. Cell numbers of P. pouchetii *and acrylic acid concentrations at 15 m depth in coastal Antarctic sea water.*

Concentrations of acrylic acid and DMS were detected and paralleled the changes of the population of *P. pouchetii* (Figs. 2 and 3). Their concentrations remained at low levels on December 14, 1988 followed by rapid increase with the bloom of *P. pouchetii*. The concentrations of acrylic acid and DMS increased 78 and 21 times within six days from December 14 to 20, 1988, and the highest concentrations observed were 0.51 μmol L^{-1} and 0.588 μmol L^{-1}, respectively, on January 3, 1989. The concentrations then decrease until reaching low levels as was found with the *P. pouchetii* cell numbers mentioned above. The changes of the both substances were 0.001 to 0.51 μmol L^{-1} for acrylic acid and 0.003 to 0.588 μmol L^{-1} for DMS during the *P. pouchetii* bloom (Table 1).

According to the results shown in Figs. 2 and 3, it seemed that the concentrations of acrylic acid and DMS were correlated with the cell numbers of *P. pouchetii* during the bloom. During the period of population increase of *P. pouchetii*, the cell numbers increased rapidly, accompanying the increases of both acrylic acid and DMS in sea water. In the senescence phase, the concentrations of acrylic acid and DMS decreased with the decrease of cell numbers, but the amplitudes of decrease of their concentrations were lower than that of cell numbers. There are strong positive correlations between acrylic acid, DMS and cell numbers, and polynomial curve fittings show that the relative coefficients (R) were both 0.998. This suggests that the source of acrylic acid and DMS was

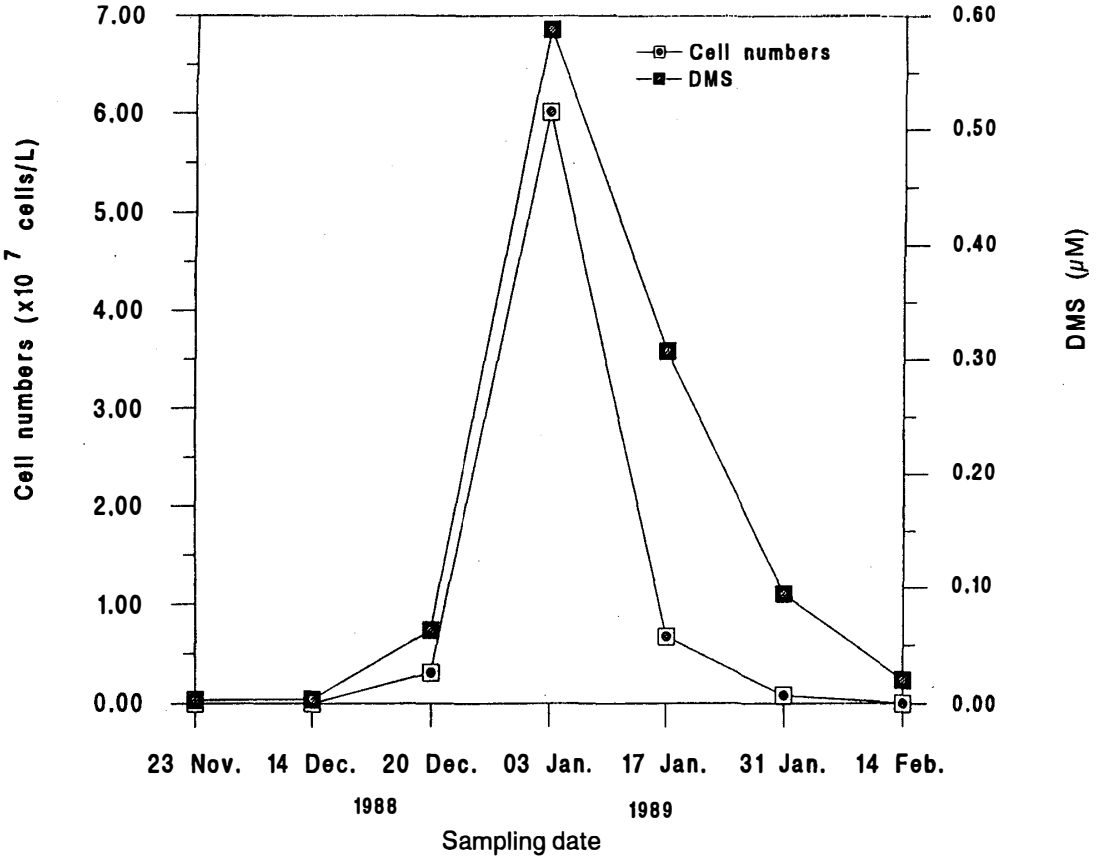

Fig. 3. Cell numbers of P. pouchetii *and DMS concentrations at 15 m depth in coastal Antarctic sea water.*

Table 1. Changes of algal cell numbers and concentrations of acrylic acid and DMS during a summer bloom of P. pouchetii.

Sampling date	Cell numbers cell L^{-1}	Acrylic acid μmol L^{-1}	DMS μmol L^{-1}
Nov. 23, 1988	471×10	0.0010	0.0030
Dec. 14, 1988	137×10^{2}	0.0015	0.0033
Dec. 20, 1988	311×10^{4}	0.1880	0.0630
Jan. 3, 1989	601×10^{5}	0.5100	0.5880
Jan. 17, 1989	667×10^{4}	0.3190	0.3070
Jan. 31. 1989	792×10^{3}	0.0830	0.0940
Feb. 14, 1989	139×10	0.0190	0.0200

related to *P. pouchetii*. Acrylic acid produced by marine algae has been found with a broad spectrum of bacteriocides (SIEBURTH, 1961). A cellular product of dimethylsulfonium propionate (DMSP) was decomposed to acrylic acid and DMS (SIEBURTH, 1961), DMSP was most probably formed from methionione. This compound efficiently supplies sulphur, methylcarbon and hydrogen required for DMSP formation (GREEN, 1962), although glycine has also been considered as a

methyl donor (VAIRAVAMURTHY *et al.*, 1985). Our study indicates that dissolved free amino acids in the sea water decreased with the increase of *P. pouchetii* population (YANG *et al.*, 1990). It is suggested that acrylic acid and DMS are produced by *P. pouchetii* which utilizes the dissovled free amino acids during the bloom season.

Table 2 shows the possible natural productivity of acrylic acid and DMS during the increase and decline phases of the *P. pouchetii* population. In the early phase of population increase between November 23 and December 14, 1988, the total increase of cell number was 482 cells L^{-1} per day, the estimated rate of change (r^*) was 0.051/day. Possible natural productivity of acrylic acid and DMS, calculated on the basis of concentrations of acrylic acid and DMS and their rates of change compared to cell numbers of *P. pouchetii* population with their rate of change, were 7.90×10^{-8} μmol $cell^{-1}$ and 6.23×10^{-8} μmol $cell^{-1}$, respectively. Rates of increase (r^*) of both substances during the period were 0.019 and 0.005/day respectively. After that, increase of cell numbers was 4.4×10^5 cells per day from December 14 to December 20, and its rate of increase (r^*) was 0.904/day. This rate is 17.7 times that during the previous time period. Possible natural productivities of acrylic acid and DMS were 9.75×10^{-8} μmol $cell^{-1}$ and 13.09×10^{-8} $cell^{-1}$, and their rate of increase (r^*) were 0.805 and 0.491/day, respectively. Thus natural productivity was only 1.23 and 2.09 times, respectively, compared to the previous period. However, the estimated rates of increases were extremely large, reaching 42.3 times and 83.8 times for acrylic acid and DMS, respectively. From December 20, 1988 to January 3, 1989, cell numbers increased to 3.8×10^6 cells L^{-1} per day, but the rate of increase (r^*) of cells, $r^*=0.21$/day, decreased to 4.26 times, and the natural productivity of both compounds decreased to 2.0×10^{-8} μmol $cell^{-1}$ and 1.5×10^{-8} μmol $cell^{-1}$, respectively, 4.79 times less and 8.60 times less compared to the early phase. Their rates of increase (r^*) were 0.07 and 0.15/day, which decreased to 11.3 times and 3.1 times compared to the early phase.

Table 2. Increased rate and possible natural productivity of acrylic acid and DMS during a summer bloom of P. pouchetii.

Duration	Population		Acrylic acid		DMS	
	changing rate r^*day^{-1}	change cells/day	changing rate r^*day^{-1}	producitvity μ mol/cell	changimg rate r^*day^{-1}	productivity μ mol/cell
Nov. 23−Dec. 14	0.051	482	0.019	7.90×10^{-8}	0.005	6.23×10^{-8}
Dec. 14−Dec. 20	0.904	441×10^3	0.805	9.75×10^{-8}	0.491	13.09×10^{-8}
Dec. 20−Jan. 3	0.212	379×10^4	0.071	2.03×10^{-8}	0.159	1.52×10^{-8}
Jan. 3−Jan. 17	-0.157	-355×10^4	-0.034	1.83×10^{-8}	-0.047	2.93×10^{-8}
Jan. 17−Jan. 31	-0.152	-392×10^3	0.096	3.01×10^{-8}	-0.084	2.54×10^{-8}
Jan. 31−Feb. 14	-0.454	-527×10^2	-0.104	2.40×10^{-8}	-0.111	2.10×10^{-8}

These results show that the cell numbers increased with time, but the rate varied with time. Concentrations of acrylic acid and DMS showed increases in time similar to those of the cell numbers as well as to their rates of increase and natural productivities.

Figures 4 and 5 show the correlation between rates of increase of acrylic acid and DMS, and of *P. pouchetii* cells. Polynomial correlations of rates of increase of acrylic acid and DMS with the rate of increase of *P. pouchetii* cells are as follows:

$$Y=-1.5891\times10^{-2}+3.4781\times10^{-1}X+4.2786\times10^{-1}X^2+2.1193\times10^{-1}X^3,\ (r=0.996)$$

where Y and X are the rates of increase of acrylic acid and number of cells, respectively;

$$Y=2.8349\times10^{-3}+5.8768\times10^{-1}X+4.8683\times10^{-1}X^2-5.9619\times10^{-1}X^3,\ (r=0.992)$$

where Y and X are the rates of increase of DMS and number of cells, respectively.

These excellent correlations suggest that acrylic acid and DMS were produced by cells during the growth of *P. pouchetii*.

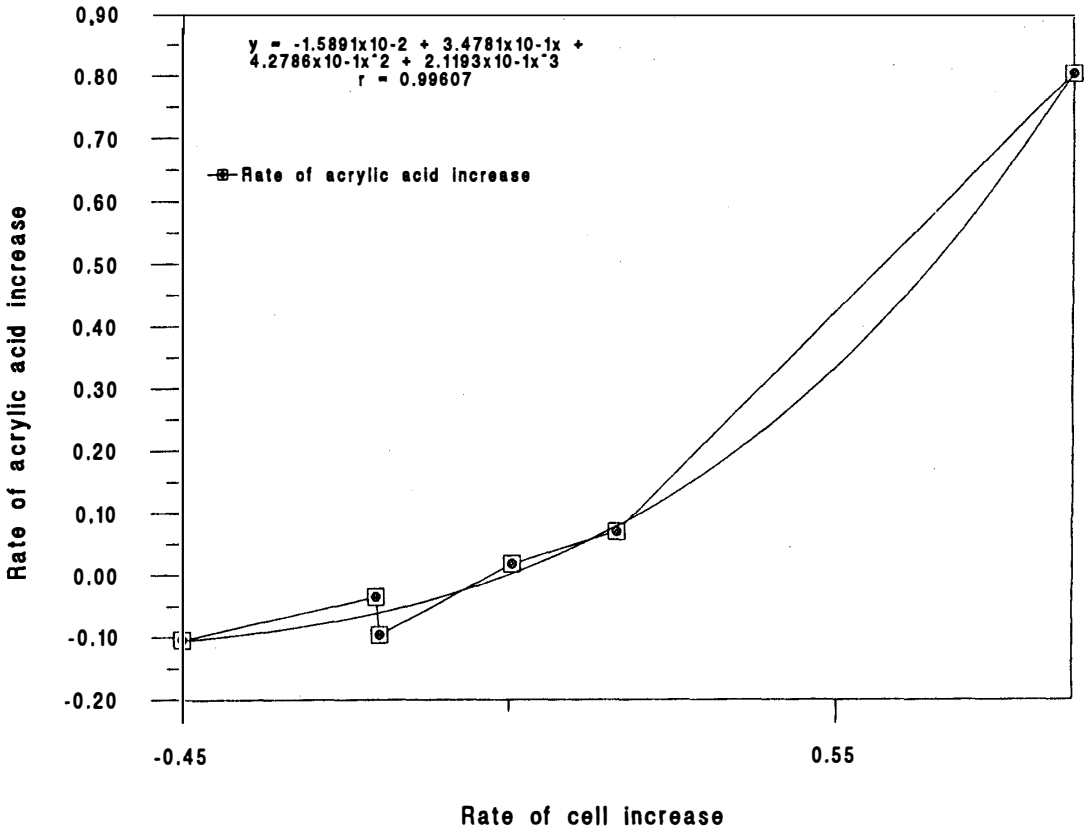

Fig. 4. Correlation of rate of acrylic acid increase with rate of increase of P. pouchetii *cell.*

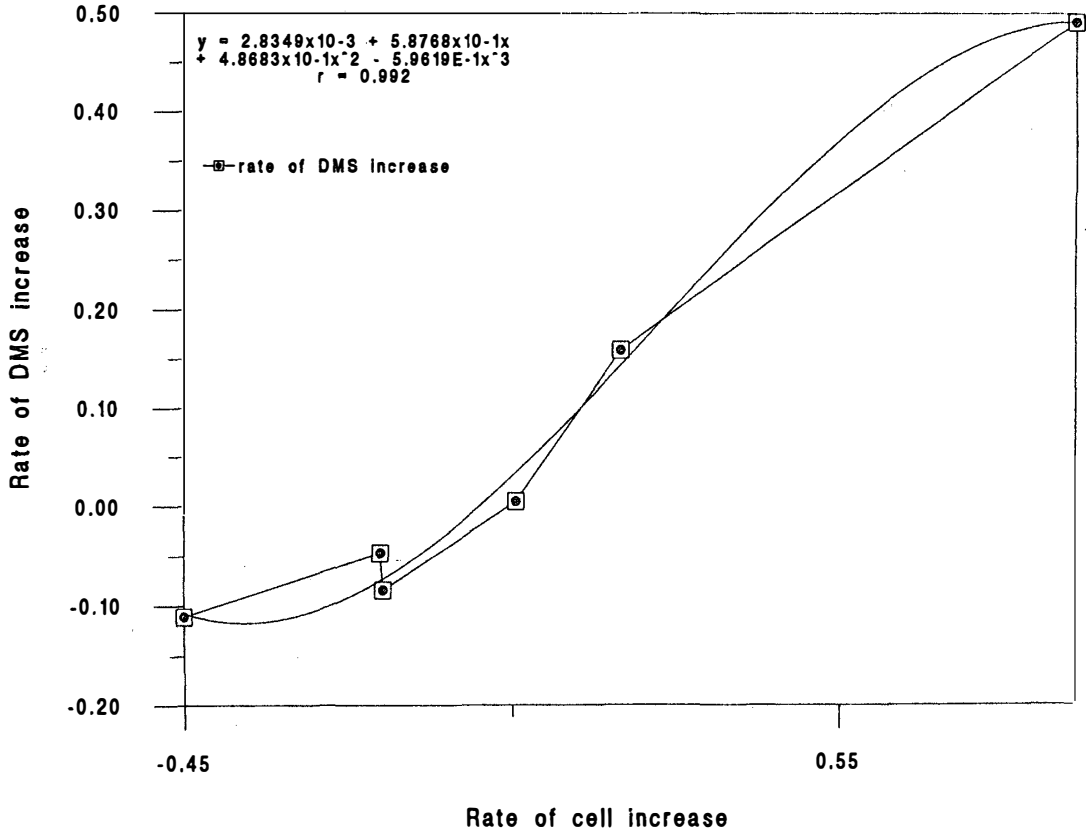

Fig. 5. Correlation of rate of DMS increase with rate of increase of P. pouchetii *cells.*

There exist some differences in variation of number of cells, rates of decrease of acrylic acid and DMS between the increasing phase and decreasing phase of *P. pouchetii* population. These variations were smoother during the decreasing phase. Total cell numbers decreased to 6.7×10^6 cells L^{-1} during the first declining phase from January 3 to 17, 1989, in which the rate of change of population was -0.157/day. Cell numbers decreased to 7.9×10^5 cells L^{-1} from January 17 to January 31, when the rate of change of population was -0.152/day, which was similar to that in the first declining phase on January 3. In the last declining phase the rate of change of -0.454/day was higher than that of the early phase.

The concentration of acrylic acid and DMS decreased with the decrease of cell numbers during the declining phase, although productivities of both substances increased. From January 3 to 17, the concentration of acrylic acid decreased from 0.51 μmol L^{-1} to 0.319 μmol L^{-1}, and the rate of change (r^*) was -0.034/day. The concentration of DMS also decreased from 0.588 μmol L^{-1} to 0.307 μmol L^{-1}, rate of change (r^*) was -0.047/day. Low productivities of both substances, 1.87×10^{-8} μmol $cell^{-1}$ and 2.93×10^{-8} μmol $cell^{-1}$, respectively, were found during this period. After that, the concentrations and rates of change both continuously decreased. The rates of change (r^*) of acrylic acid and DMS were -0.096 and -0.084/day respectively, their natural productivities were 3.018×10^{-8} μmol $cell^{-1}$ and 2.54×10^{-8} μmol $cell^{-1}$ from January 17 to 31, 1989. This rate of

change of acrylic acid was reduced by 2.824 times compared to that in the early phase, but the natural productivity increased by 1.64 times. The rate of change of DMS was 1.78 times and its productivity increased by 0.87 times relative to the early phase. During the last declining phase of the *P. pouchetii* population, from January 31 to February 14, the concentrations and rates of change (r^*) continuously decreased. The concentrations of acrylic acid and DMS decreased by 0.23 and 0.27 times, and the rates of change (r^*) wee reduced by 1.08 times and 1.32 times, respectively. The productivity of acrylic acid also decreased to 2.40×10^{-8} μmol cell^{-1}, but that of DMS decreased to 2.10×10^{-8} μmol cell^{-1}. However, the concentrations and rates of increase of acrylic acid and DMS were reduced with the decay of the cells, and their productivities varied during all declining phases of *P. pouchetii* (Table 2). This may be related to the rate of diffusion loss of both compounds in water except for material released by cell division (diffusive coefficient of DMS K=0.133 d^{-1}).

4. Conclusion

Acrylic acid and DMS were found to parallel the population change of *P. pouchetii* produced by cell division during a possible bloom. Rates of increase of cells showed a proportional relation to natural productivity of acrylic acid and DMS, concentrations of acrylic acid and DMS were controlled by total numbers of cells of *P. pouchetii* in sea water.

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