

A Microbiological Study on the Decline Process of a Phytoplankton Bloom in Aburatsubo Inlet, Kanagawa, Japan^{1), 2)}

KIMIO FUKAMI³⁾, USIO SIMIDU³⁾ AND NOBUO TAGA³⁾

Ocean Research Institute, University of Tokyo, Nakano, Tokyo 164

Abstract

The decline process of a phytoplankton bloom in Aburatsubo Inlet, Kanagawa, Japan, was investigated at one day intervals in July, 1978. The predominant organisms were *Prorocentrum* sp. In the surface layer, during the bloom, the fluctuation pattern of POC was similar to that of Chl *a*. In the 1 m and the bottom 4 m layers, however, the POC concentration showed different fluctuation patterns from that of Chl *a*. The maximum values of POC concentration were obtained on the 2nd day in the surface layer, on the 3rd day in the 1 m layer, and on the 4th day in the 4 m layer. The number of bacteria in both viable and direct counts showed almost constant values throughout the observation period in the surface and 1 m layers, whereas, in the 4 m layer both viable and direct counts of bacteria increased as the decline process of the bloom proceeded. These chemical and microbiological results suggest that the phytoplankton cells produced in the surface water gradually sank downward while being decomposed by microorganisms. The degradation process of the phytoplankton cells are also discussed from the results of microscopic observations.

Recently a number of studies and observations have been reported on phytoplankton blooms (HOLMES et al. 1967, TAKAHASHI et al. 1977, WATANABE et al. 1980). Most of these studies have dealt with the mechanisms of formation of the bloom and there are very few reports on the decline process of phytoplankton blooms. The decline process of the bloom is one good example of the decomposition of phytoplankton cells in the field.

Many laboratory studies on the decomposition of phytoplankton cells have been reported (e.g. GRILL & RICHARDS 1964, OTSUKI & HANYA 1972, MIYOSHI 1976). We also previously reported on the microbiological aspects of the decomposition of phytoplankton cells (FUKAMI et al. 1981) and indicated that there was a succession in the community of heterotrophic bacteria, especially among the communities attached to the detritus.

In this paper, we report the degradation process of a dinoflagellate, *Prorocentrum* sp., bloom which lasted for several days and describe the fluctuations of some chemical and microbiological parameters for 5 days. These results and microscopic observations enable us to adapt the results of laboratory experiments to field studies.

Materials and Methods

The dinoflagellate bloom took place in Aburatsubo Inlet, Kanagawa, Japan, on July 1, 1978.

¹⁾ Accepted 5 August 1981

²⁾ 油壺湾における植物プランクトンブルームの消滅過程についての微生物学的研究

³⁾ 深見公雄・清水 潮・多賀信夫, 東京大学海洋研究所

The predominant organisms were *Prorocentrum* sp.

Water samples were taken at the flood tide between 10:00 and 12:00 a.m. from one station where the water depth was about 5 m. As this station seemed to be the central part of the bloom throughout our observation, seawater samples were taken from this fixed station. Seawater samples for chemical analyses were collected from 0 m, 1 m, and 4 m depth using a Kitahara model sampler. Samples for microbiological analyses were also collected from the same depth as for chemical analyses with a sterile glass bottle or J-Z sampler (ZOBELL 1941).

The methods used for the determination of the concentrations of particulate organic carbon (POC), particulate organic nitrogen (PON), and dissolved organic carbon (DOC) were the same as those described previously (FUKAMI et al. 1981). The concentration of Chl *a* was determined by the method of SCOR/UNESCO (1966). The pheopigments and net Chl *a* concentrations were determined by the method of LORENZEN (1967). The numbers of viable heterotrophic bacteria were determined by the spread plate method on Medium 2216E. Direct counts of total bacteria and observations of the phytoplankton cells were made using an epifluorescent microscope following the modified method of HOBBIIE et al. (1977).

Results and Discussion

Results of Chemical Analyses

The results of chemical and microbiological analyses just before this bloom on June 28, 1978 were shown in Table 1. The maximum values of POC and Chl *a* during this bloom were more than ten times greater than that of the ordinary level (Figs. 1, 2, and 3), so this bloom was relatively heavy for this inlet.

TABLE 1. CHEMICAL AND MICROBIOLOGICAL DATA JUST BEFORE THE PHYTOPLANKTON BLOOM ON JUNE 28, 1978, IN ABURATSUBO INLET. W.T.: WATER TEMPERATURE, CHROMO.: THE PERCENTAGE OF THE ORANGE-YELLOW PIGMENTED BACTERIA.

Depth (m)	W.T. (°C)	Chl <i>a</i> ($\mu\text{g}\cdot\text{l}^{-1}$)	POC ($\mu\text{g C}\cdot\text{l}^{-1}$)	DOC ($\text{mg C}\cdot\text{l}^{-1}$)	Viable Count ($\text{c.f.u}\cdot\text{ml}^{-1}$)	Chromo. (%)
0	20.1	1.57	216	1.99	8.50×10^3	11.8
4	19.2	1.26	320	1.49	1.05×10^3	23.8

TABLE 2. FLUCTUATIONS OF THE WEATHER AND THE WATER TEMPERATURE DURING THE PHYTOPLANKTON BLOOM IN ABURATSUBO INLET.

Day	Weather	Water Temperature (°C)		
		0 m	1 m	4 m
June 28	rain	20.1	—	19.2
July 1	rain	22.0	21.0	20.4
2	fine	23.1	21.8	20.5
3	fine	23.2	22.2	21.0
4	fine	25.1	24.6	23.7
5	fine	24.2	23.0	20.8

Fluctuations of the weather and the water temperature during the observation period were shown in Table 2. The water temperature increased gradually and the difference of water temperature between 0 m and 4 m depth became larger as the weather had gotten fine.

The Chl *a* concentration in the surface layer showed a peak value on the 2nd day (Fig. 1). The value decreased rapidly on the 3rd day and then remained nearly constant. In the 1 m layer, during the observation, the maximum value in the Chl *a* concentration occurred on the first day (Fig. 2). The value decreased rapidly on the 4th day. On the other hand, in the near bottom 4 m layer the high value in the Chl *a* concentration occurred two times: on the first day and on the 4th day (Fig. 3). These values in the 4 m layer, however, were relatively small compared with those in the upper layers.

In spite of the heavy bloom, the Chl *a* concentration decreased almost to the slightly high level of ordinary summer water after 5 days, indicating that the phytoplankton cells were decomposed very rapidly.

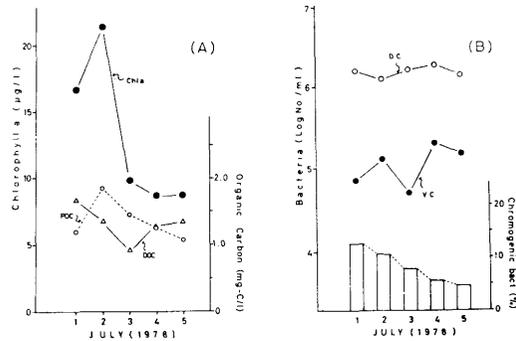


Fig. 1. Fluctuations of Chl *a*, POC, DOC concentrations (A) and bacterial numbers and the proportion of chromogenic bacteria (B) in the surface layer during the phytoplankton bloom in Aburatsubo Inlet. D. C. and V. C. represent the direct and viable counts of bacteria, respectively.

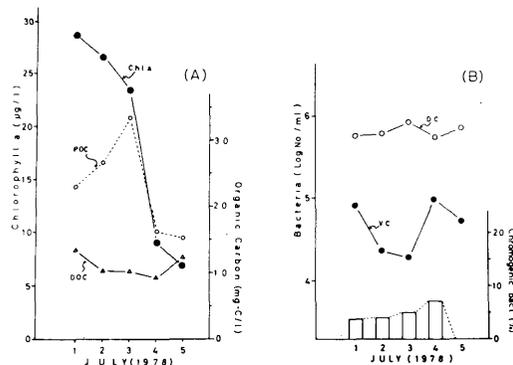


Fig. 2. Fluctuations of Chl *a*, POC, DOC concentrations (A) and bacterial numbers and the proportion of chromogenic bacteria (B) in the 1 m layer during the phytoplankton bloom in Aburatsubo Inlet. D. C. and V. C. represent the direct and viable counts of bacteria, respectively.

The fluctuation of the POC concentration in the surface layer showed the same trend as that of Chl *a* (Fig. 1). On the other hand, in the 1 m and the 4 m layers, the POC concentrations showed different fluctuation patterns from that of Chl *a* (Figs. 2 and 3) and the maximum values of POC occurred progressively later as the depth increased. These results suggest that the particulate matter produced by the phytoplankton in the surface water sank gradually downward.

HOLMES et al. (1967) reported that the maximum concentrations of DOC were measured as the bloom declined. In our observations, the concentrations of DOC decreased at the onset

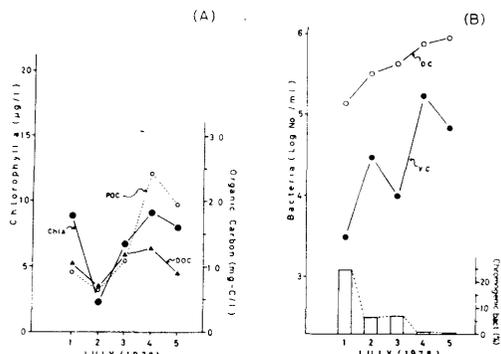


Fig. 3. Fluctuations of Chl *a*, POC, DOC concentrations (A) and bacterial numbers and the proportion of chromogenic bacteria (B) in the 4 m layer during the phytoplankton bloom in Aburatsubo Inlet. D. C. and V. C. represent the direct and viable counts of bacteria, respectively.

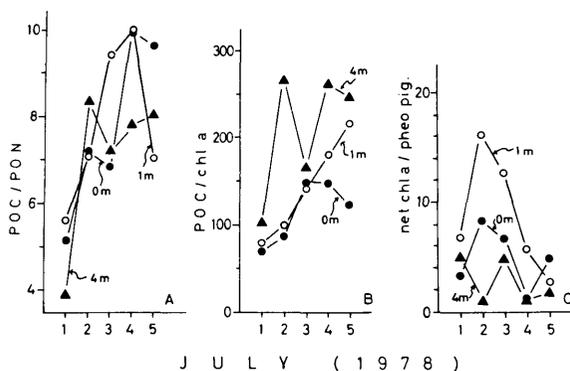


Fig. 4. Fluctuations of the ratios of POC : PON (A), POC : Chl *a* (B), and net Chl *a* : pheopigments (C) during the phytoplankton bloom in Aburatsubo Inlet.

Explanation of Plate I

A-G. Changes of the phytoplankton cells under microscopic observation during the decline process of the bloom. These monochromatic photographs are the prints from the color negative film. (See text for the detailed description).

PLATE I

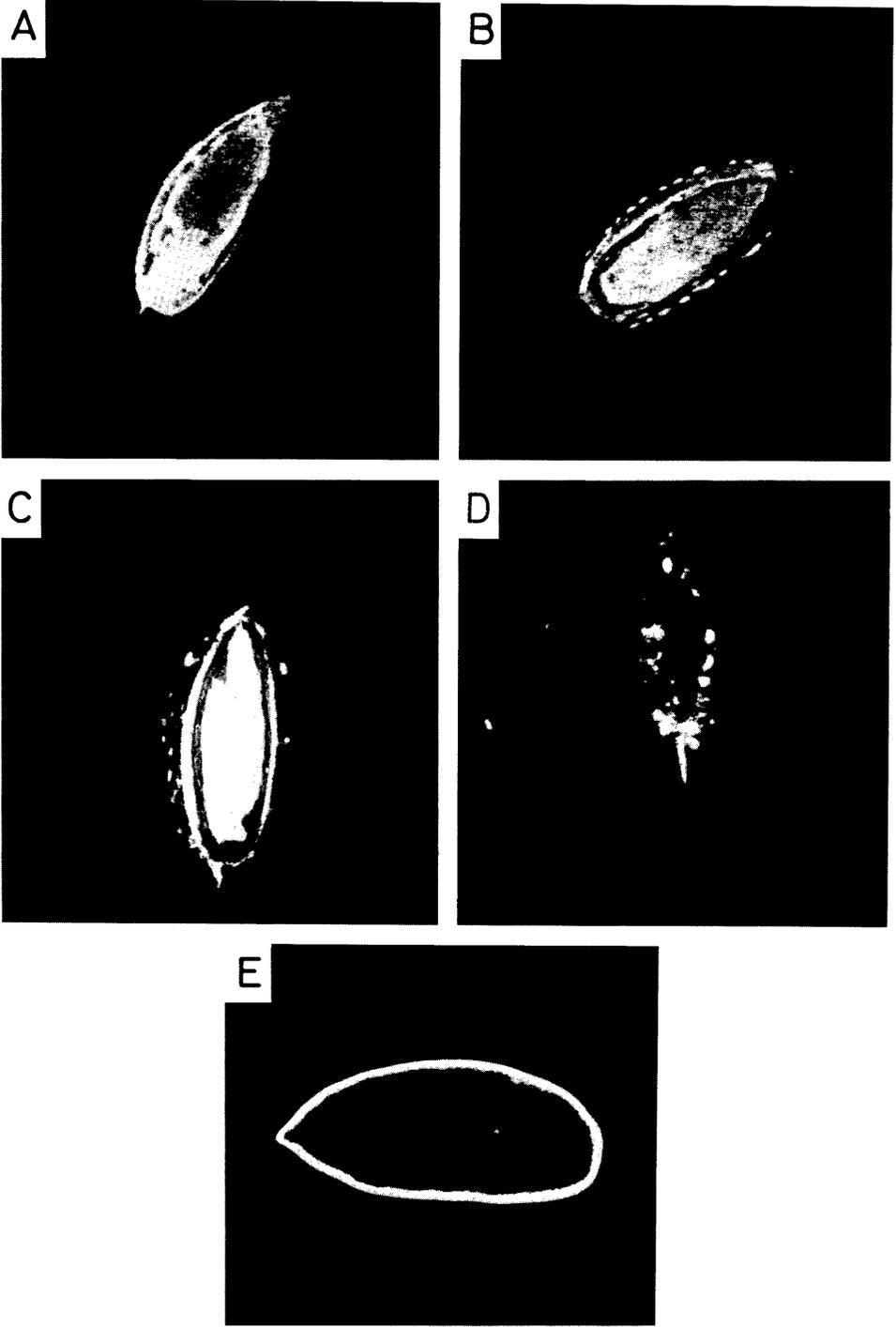
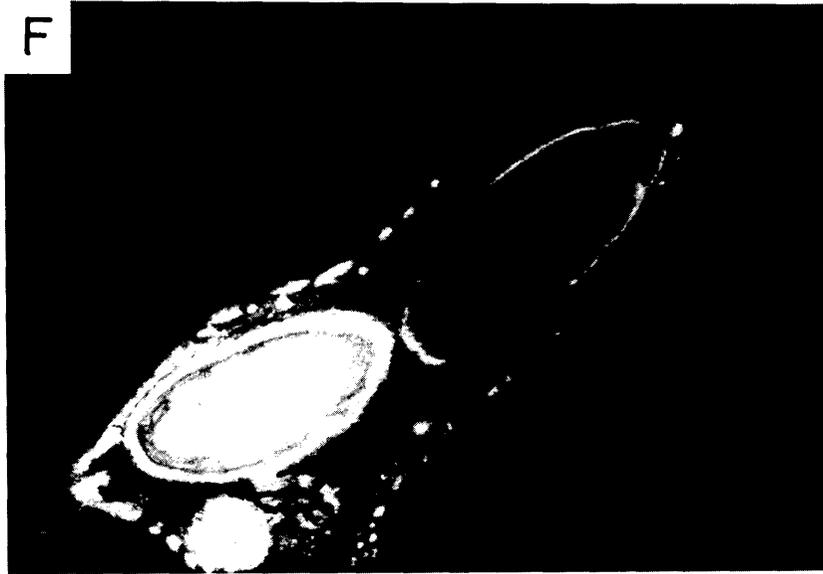


PLATE I (Continued)



of bloom, and also showed the tendency to increase when the bloom declined (Table 1. Figs. 1, 2, and 3). These results are consistent with those of laboratory experiments (OTSUKI & HANYA 1972, FUKAMI et al. 1981), indicating that the rapidly decreased POC, which had been decomposed by microorganisms, turned into DOC.

The fluctuations of the POC : PON ratio, the POC : Chl *a* ratio and the net Chl *a* : pheopigments ratio are shown in Fig. 4. In general, the ratios of POC : PON and POC : Chl *a* tended to increase up to the 5th day. The net Chl *a* : pheopigments ratio in the upper two layers decreased on the 4th day, and this ratio in the bottom 4 m layer had a relatively low value throughout the observation period. Assuming that Chl *a* was degraded rapidly after the phytoplankton had died and it resulted in the production of pheopigments, the fact that POC : Chl *a* ratio increased and net Chl *a* : pheopigments ratio tended to decrease would indicate that the proportion of dead phytoplankton, that is detritus, in particulate organic matter increased and decomposition of plankton cells proceeded as the bloom was declining. This is also suggested from the results of microscopic observation (shown afterward).

Results of Microbiological Analyses

Both viable and direct counts of bacterial number in the surface and the 1 m layers showed almost constant values (Figs. 1 and 2). In the 4 m layer, however, the bacterial number increased in both viable and direct counts as the degradation process of the bloom proceeded (Fig. 3). If the bacteria grew on the nutrient of dead phytoplankton cells, the number of bacteria in both viable and direct counts must have increased at each depth. The fact that the number of bacteria increased only in the bottom 4 m layer suggests that the bacteria growing in the upper layers were attached to the dead plankton cell and sank gradually downward.

According to the results of our previous experiment (FUKAMI et al. 1981), in the early stage of the decomposition process of phytoplankton cells most of the colonies on the plate media were colorless and the proportion of orange-yellow pigmented bacteria (mainly composed of *Flavobacterium* spp. and *Cytophaga* spp.) was very low. During our observation of the phytoplankton bloom, the proportion of the orange-yellow pigmented bacteria in the viable counts decreased. In the 5th day these chromogenic bacteria almost disappeared (Table 1, Fig. 1, 2, and 3). This was the result of the growth of non-pigmented bacteria rather than being due to a decrease in the number of pigmented bacteria in the early stage of the degradation process of phytoplankton bloom. These results coincide with those of our laboratory experiment and suggest that during the decomposition process of phytoplankton cell the orange-yellow pigmented bacteria are not concerned with the decomposition of "fresh" organic matters.

Microscopic Observations

The visual changes of the phytoplankton cells in the decomposition process of the bloom are shown in Plate I. On the first two days in the upper layers, the cells of *Prorocentrum* sp. had clear shapes and displayed the orange-red color of fluorescence from the chloroplast (Plate I, A). On the 3rd day, it was observed that a number of bacteria were attached to the

surface of the plankton cells (Plate I, B). The red color inside the plankton cells diminished gradually and the growth of bacteria on and in the cells of *Prorocentrum* sp. was seen (Plate I, C and D). Finally only the greenish "shells" of *Prorocentrum* sp. remained (Plate I, E). On the 4th or 5th day in the 4 m layer, it was observed that several cells of *Prorocentrum* sp. were connected by slime-like material containing numerous bacteria (Plate I, F).

TANAKA & KADOTA (1980) reported that bacterial density increased during and immediately after the bloom and that the bacterial population decreased due to the grazing of zooplankton. In our observations, at the end of the bloom the existence of small microflagellates was observed in the sample (Plate I, G). This suggests that after the growth of bacteria, these microflagellates grazed and replaced the bacteria. FENCHEL (1970) and HARRISON & MANN (1975) reported the succession of detritus decomposers from bacteria to microflagellates then finally to ciliates in a laboratory experiment. We must confirm Fenchel's suggestion in the field in future studies.

Concluding Remarks

There have been many arguments about the fate of phytoplankton cell. In the field study, however, there are very few reports on the decomposition process of phytoplankton cells even in the decline process of the bloom, and it has not been clear yet whether phytoplankton cells which had grown explosively and then declined were grazed directly by the zooplankton or were decomposed by microorganisms.

From the results and observations in the present study, it is suggested that at least at one bloom in Aburatsubo Inlet the phytoplankton cells produced in the surface water were decomposed by the attack of the bacterial community after death, and then several cells were aggregated by slime-like material composed of microorganisms, forming larger particles that sank gradually downward.

Acknowledgement

We would like to express our thanks to the staff of the Misaki Marine Biological Station, University of Tokyo, for their kind help in collecting samples.

Literature Cited

- FENCHEL, T., 1970. Studies on the decomposition of organic detritus derived from the turtle grass *Thalassia testudinum*. *Limnol. Oceanogr.*, **15**: 14-20.
- FUKAMI, K., U. SIMIDU & N. TAGA, 1981. Fluctuation of the communities of heterotrophic bacteria during the decomposition process of phytoplankton. *J. exp. mar. Biol. Ecol.*, (in press).
- GRILL, E. V. & F. A. RICHARDS, 1964. Nutrient regeneration from phytoplankton decomposing in seawater. *J. mar. Res.*, **22**: 51-69.
- HARRISON, P. G. & K. H. MANN, 1975. Detritus formation from eelgrass (*Zostera marina* L.): The relative effects of fragmentation, leaching, and decay. *Limnol. Oceanogr.*, **20**: 924-934.
- HOBBIE, J. E., R. J. DALEY & S. JASPER, 1977. Use of nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. environ. Microbiol.*, **33**: 1225-1228.
- HOLMES, R. W., P. M. WILLIAMS & R. W. EPPLEY, 1967. Red water in La Jolla Bay, 1964-1966. *Limnol. Oceanogr.*, **12**: 503-512.

- LORENZEN, C. J., 1967. Determination of chlorophyll and pheo-pigments: spectrophotometric equations. *Limnol. Oceanogr.*, **12**: 343-346.
- MIYOSHI, H., 1976. Decomposition of marine plankton under laboratory conditions. *Bull. Japan. Soc. sci. Fish.*, **42**: 1205-1211.
- OTSUKI, A. & T. HANYA, 1972. Production of dissolved organic matter from dead green algal cells. I. Aerobic microbial decomposition. *Limnol. Oceanogr.*, **17**: 248-257.
- SCOR/UNESCO, 1966. *Determination of Photosynthetic Pigments in Seawater*. (Monographs on Oceanographic Methodology (1) UNESCO, Paris, 69 pp.
- TAKAHASHI, M., D. L. SEIBERT & W. H. THOMAS, 1977. Occasional blooms of phytoplankton during summer in Sannich Inlet, B. C., Canada. *Deep-Sea Res.*, **24**: 775-780.
- TANAKA, N. & H. KADOTA, 1980. Bacterial densities and their growth rates during *Uroglena* blooms in Lake Biwa. *Bull. natl. Res. Inst. Aquaculture*, **1**: 87-93.
- WATANABE, K., A. HINO & R. HIRANO, 1980. Some observations on 1978 red tide blooms in Shonai Estuary, Shizuoka Prefecture. *Bull. Plankton Soc. Japan*, **27**: 87-98.
- ZOBELL, C. E., 1941. Apparatus for collecting water samples from different depths for bacteriological analysis. *J. mar. Res.*, **4**: 173-188.