赤道海域における海面皮層中のバクテリアおよび植物プランクトン

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Neustonic Bacteria and Phytoplankton in Surface Microlayers of the Equatorial Waters* **

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Abstract

In September 1969, the community of bacteria and nanoplankton in surface microlayers on the equator of the Pacific Ocean was surveyed. The population of the surface film was remarkably large, as compared with that of the subsurface layers, but most of cells in the film was dead or nearly dead. Such film biota seems to be not in good physiological conditions owing to the effect of strong solar radiation, and the community is considered to be formed rather by physical accumulation than by propagation.

In 1941, Miyazaki revealed his interesting view that the surface seawater in the depths of 0 to 10 cm might be physico-chemically specific, and thereby exists a rather different biological environment or biota in this layer as compared with the subsurface layer. He suggested the necessity of further oceanographical studies with respect to such a surface layer (Miyazaki, 1941). In these several years, new findings have been brought about on the

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neustonic community in seawater of surface film. Dense populations of bacteria in surface layer were reported by many authors (Sieburth, 1965 ; Harvey, 1966 ; Tsyban, 1971). In the film water, abundant inhabitations of *Prorocentrum micans* and minute flagellates in association with nearly dead and dying diatoms and dinoflagellates were observed by Harvey (1966), and he (1970) recently reported the existence of undescribed and rare species there.

The present paper reports the results of studies in order to know if the former findings could be applicable to the community of bacterial and nanoplankton in the surface layer of the Pacific equatorial waters, where the solar radiation in the daytime seems to be too strong and rather harmful to organisms inhabiting in the surface film water.

Table 1. Number of plankton per liter at St. 14 (KH-69-4).

Depth	Sampler	Bacillariophyceae (cell)	*Trichodesmium Thiebautii* (filament)	Dinoflagellata (cell)	Silicoflagellata (cell)	Radiolaria (cell)	Helizoa (cell)	Foraminifera (cell)	Tintinnoinea (cell)	Copepoda (individual)	T (°C)
Surface film	Metal screen	9988	124	16	2				34	2	28.3
5 cm	Siphon	2056		2			12		10	6	
10 cm	〃	948		4			12	2	6	8	
0 m	Bucket	52				2					28.3
10 m	Van Dorn sampler	74			2						26.58
20 m	〃	170				10					26.30
30 m	〃	118			4	6					26.02
50 m	〃	200				10					25.50
75 m	〃	168			2	2					25.00
100 m	〃	54									22.75
125 m	〃	42			2						19.10
150 m	〃	18									17.30
178 m	〃	8			2						
200 m	〃	2			2						13.90

Counting was done by a sedimentation and centrifugal method for metal screen and siphon samples and by a Millipore® filter method for bucket and Van Dorn samples.

Methods and materials

Sample waters were collected from three layers : film, 5-cm and 10-cm layes for plankton, and film, 10-cm and 50-cm layers for bacteria, at station 14 of KH-69-4 Cruise, 154° 53′E, on the equator of the Pacific Ocean, at 1545–1710 on September 30, 1969 (Ocean Research Institute, 1970) on a small boat which was lowered on the sea from the mother ship, Hakuhō Maru, Ocean Research Institute, University of Tokyo. During the sampling the boat was in the distances of 500 to 1000 m south of the mother ship. The sea was almost calm with gentle east wind of 2 m/sec and long swell of SE-2. The weather was fine and strongly shiny. The sun set at 1813. Transparency was 29 m.

Collection and counting of plankton : The surface film water was taken with a round-framed metal screen sampler (50 cm in diameter and 16 meshes per inch), which was originally designed by Garrett (1965). The sampler was dipped vertically into the sea and turned parallel in the water and then pulled up gently. Fragments of jelly fish captured on the screen wire were removed. Sample water was poured into a bottle by inclining of the sampler. Five-hundred ml of sample water were obtained by repeating this operation. Subsurface samples from 5-cm and 10-cm layers were taken with a siphon. Samples were also taken from various depths with Van Dorn samplers at this station in order to compare them with film samples. Organisms in these samples were fixed with neutralized formalin immediately after collecting. Identification and counting were done by using a Sedgwick-Rafter cell.

Collection and counting of bacteria : Water sample for the estimation of bacteria in the surface film was collected in the same way as plankton collection by using the screen sampler. Film water on the screen was gathered into a sterilized bottle by inclining the screen. By repeating this operation several times, finally was obtained the sample of about 300 ml. Subsurface sample from 10-cm depth was collected repeatedly into a sterilized bottle with a sterile pipette. Water sample from the depth of about 50 cm was also collected with the J-Z bacteriological sampler (ZoBell, 1941). A portion of each sample of 20 to 100 ml was filtered through a sterile Millipore® filter (GS-25 mm, porosity 0.22 μ) for the estimation of the total number of bacteria and the number of detritus smaller than 50 μ. The filter was dried at 80°C after rinsing salt on it and mounted by a small amount of the Cargille's immersion oil (R. P. Cargille Labo. Inc.) on a slide glass, then the bacteria and detritus on it were directly counted by the phase-contrast microscope. For the viable counting of heterotrophic and nitrogen-fixing bacteria in surface and subsurface waters, duplicate 0.1-ml portions of variously diluted samples were spread on agar plates of Medium PPES-II (Taga, 1968) and Non-nitrogenous Medium N and A (Maruyama *et al.*, 1970). The inoculated plates were incubated at 18°C for two weeks before colonies on them were counted. While, duplicate 10 to 50-ml portions of 50-cm sample were filtered through sterilized Miilipore® filters (HA-45 mm, porosity 0.45 μ). These inoculated filters were also placed on the above-mentioned agar plates, and bacterial colonies on the filters were counted after keeping the plates for two weeks at 18°C.

Results and consideration

Diatoms were most predominant in each of the film and subsurface samples (Table 1). Diatom cell number reached about 10^4 per liter in the film samples, while it decreased to

2×10^3 in 5-cm layer sample and 10^3 in 10-cm layer sample. It was very few, namely 50-200 per liter in the samples of lower layers collected with Van Dorn sampler. For each of surface microlayers *Nitzschia* sp. was most important and *Planktoniella sol, Rhizosolenia,* and *Thalassiothrix delicatula* were next to it, while *Bacteriastrum comosum* and *Chaetoceros* were found almost only in the subsurface samples (Table 2). Any rare or undescribed species were not found in these layers. According to microscopic observation, chromatophores of *Planktoniella sol* and *Rhizosolenia* were already vanished in the film sample, while most of them were held in the subsurface samples. Thus, diatom community of the film water was composed of a large number of dead or half-dead cells.

Table 2. Number of diatom cells per liter of surface microlayers at St. 14 (KH-69-4).

	Surface film	5 cm	10 cm
Bacillariophyceae (Total)	9988	2056	948
Bacteriastrum comosum		18	36
Chaetoceros atlanticus v. *neapolitana*	6	126	12
C. peruvianus		24	10
C. coarctatus		32	
C. curvisetus		8	8
C. lorenzianus			14
C. pseudocurvisetus		18	
Coscinodiscus sp.	2		
Nitzschia sp.	9870	1652	788
Planktoniella sol (a)	22	16	8
Planktoniella sol (b)	12	2	2
Rhizosolenia acuminata	8		2
R. alata		6	
R. bergonii	16	8	6
R. castracanei	4		12
Thalassiothrix delicatula	48	146	50

Chromatophores were contained in the cells of (a) and not of (b).

Trichodesmium Thiebautii, a pelagic blue-green alga, was found only in the film water not as colony but as solitary filament (Table 1). This might indicate that this alga is not in good physiological conditions.

A small number of thecate dinoflagellates appeared in these three layers, but any minute flagellates as reported by Harvey (1966) were never observed. It is not known whether these flagellates had been destroyed by fixation after collecting or they had never inhabited in the original water. *Ceratium furca* in the film layer contained numerous chromatophores. Thirty-two cells of *Tintinnus lusus-undae* in the film sample were considerably broken.

Bacterial biomass, estimated by the direct microscopic counting, was obviously abundant rather in surface film than in the subsurface seawater, as shown in Table 3. The total number (T) of bacterial cells per 100-ml seawater, which is the sum of the number of bacteria in a free state (F) and the number of bacteria in a state attached to or aggregated on detritus (A), was 5.5×10^7 in surface film (S), whereas it decreased to 2.5×10^6 in

Table 3. Number of bacterial cells and detritus per 100 ml of surface microlayers at St. 14 (KH-69-4).

Depth	Direct counts				Viable counts of heterotrophic bacteria	Ratio		
	Total bacteria (T)	Free bacteria (F)	Attached bacteria (A)	Detritus (D)	(H)	F/A	A/D	T/H
Surface film (S)	5.5×10^{7}	5.36×10^{7}	2.03×10^{6}	1.1×10^{5}	–	26.4	18	–
10 cm (B)	2.5×10^{6}	2.3×10^{7}	1.5×10^{5}	0.8×10^{4}	–	15.3	17.3	–
About 50 cm (C)*	1.7×10^{6}	1.2×10^{6}	4.4×10^{5}	0.7×10^{4}	7.2×10^{2}	2.95	62.9	2300
Ratio S/B	22	23	13.5	13.7				
Ratio S/C	32	44.6	4.6	15.7				

* Water sample was collected with the J-Z sampler from this depth.

the 10-cm depth (B) and to 1.7×10^{6} in the depth of about 50 cm (C). This value in surface film (S) corresponds to 22 and 32 times as many as those in 10-cm depth (B) and 50-cm depth (C), respectively as shown in the ratios of S/B and S/C. Numbers of free bacteria (F), attached bacteria (A) and detritus (D) showed also the same tendency, as that of total bacteria, to be abundant rather in surface film than in the subsurface seawater.

However, as shown in the ratios of F/A and A/D at three layers in Table 3, the former ratio decreased remarkably from the value of 26.4 in surface film to that of 2.95 in 50-cm layer, whereas the latter increased fairly from the value of 18 in surface film to that of 62.9 in 50-cm layer. This result seems to indicate that the bacterial cells in surface film and/or 10-cm layer as compared with 50-cm layer were relatively more abundant in a free state (F) than in a state attached to or aggregated on detritus (A). On the other hand, the above phenomenon of relative decrease of attached or aggregated cells in the surface layer of the equatorial waters may imply somewhat physiologically harmful effect of strong solar radiation in the daytime upon the bacterial viability. In fact, as shown in Table 4, viable counts of heterotrophic and nitrogen-fixing bacteria were smallest in surface film as com-

Table 4. Viable counts of bacteria per 0.1 ml of surface microlayers at St. 14 (KH-69-4).

Depth	Viable counts of bacteria on different media		
	Non-nitrogenous medium		Heterotrophic medium
	(N)	(A)	(PPES-II)
Surface film	0	0	undetectable*
10 cm	25	0	3
About 50 cm**	0.5	0.2	0.7

* Bacteria were undetectable in 0.001 ml of seawater sample.

** Water sample was collected with the J-Z sampler from this depth.

pared with 10-cm and 50-cm layers. This result appears also to endorse the harmful effect of solar radiation upon the microbial viability in surface film.

Although we usually assume that ultraviolet rays are not a natural environmental factor, this is not so, as near ultraviolet light is present in significant amounts, especially at high altitudes (Brock, 1969). In his review, Brock (1969) has cited the Findenegg's findings (1966) that algal photosynthesis in high alpine lakes was definitely inhibited in surface waters by near ultraviolet light from the sun. Thus, in reference to the former findings, it would be conceivable also that viability of microbial biota in surface layer might be efficiently inhibited in the equatorial waters by near ultraviolet light in the daytime.

In conclusion, as far as our result obtained in the equatorial waters is concerned, the biomass of bacteria and phytoplankton in surface film is remarkably large, but surface biota seems to be not in good physiological conditions owing to the effect of strong solar radiation. Accordingly, the abundant community in the film is considered to be formed rather by physical accumulation than by propagation.

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