Bacterial Abundance in the Sea of the Hiuchi-Nada Area

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Abstract : As a part of the JIBP-PM programme, bacteriological examinations were carried out to provide information on bacterial abundance in the Hiuchi-Nada area. Bacterial countings were done by the following three methods: direct microscopic method, extinction dilution method; and agar pour plate method.

As a whole, the apparent number of bacteria decreased according to the method employd in the following order; direct microscopic count; extinction dilution count; agar pour plate count. Significant correlations were observed among the corresponding counts. Irrespective of counting methods employed, during the warm water period, the bacterial population was large especially in the topmost few meters of water; but during the cold water period, the vertical localization of bacteria was less pronounced. The bacterial population was large in polluted waters.

The volume of these cells fell within the range of 0.1−0.4 μ^3 with an average volume of 0.26 μ^3. Based on these data, the bacterial biomass in the waters was estimated.

Introduction

As a part of the JIBP-PM programme, the Hiuchi-Nada area, the central part of the Seto Inland Sea, was investigated from an ecological viewpoint. The waters had been contaminated by increasing effluents from the surrounding land, and bacterial biomass seemed to be imporant in view of the nutrient budget of the waters. Then attempts were made to estimate the number and volume of bacteria in the waters. As a matter of course, apparent number of bacteria was influenced greatly by the counting method employed[1]. Thus two cultural and one direct microscopic methods were employed for estimating the number of bacteria.

The outline of this work has been reported[2], and the present paper describes the details of this work.

Materials and Methods

Sampling Water samples were collected at 5 *m* depth intervals from surface to bottom by using Van Dorn samplers.

Bacterial counting After collecting each sample, bacterial counting was undertaken within 1 hour by the following three methods:

Direct microscopic method - An appropriate dilution of 2 *l* sample was filtered through a membrane filter of 0.2 μ porosity, and the cells retained on the filter were counted by the procedures described by Lumpkins and Arveson[3].

Extinction dilution method - An appropriate series of decimal dilutions of 2 *l* sample was inoculated into 5 replicate tubes containing 2216 E broth. After 2−3 weeks of incubation at 30°C, a most probable number count was estimated for the combination of positive and negative tubes from the table of MPN index.

Agar pour plate method - Appropriate dilutions of 2 *l* sample were transferred to sterile Petri

dishes, and the dishes were poured with molten 2216 E agar medium and mixed thoroughly. After 2−3 weeks of incubation at 30°C, an agar pour plate count was estimated from the colonies developed.

Estimation of bacterial biomass The measurement of bacterial size was done under a microscope with the aid of conventional micrometers, and the volume of cells was calculated in the same manner as described in "Methods in Aquatic Microbiology"[4]. The specific gravity of the bacterial cells was presumed to be about 1, so the wet weight of the bacterial cells can be readily estimated from the volume of cell. Accordingly, the dry weight of bacteria can be caluculated from the wet weight by assuming that an average wet cell contains 20 % dry matter [5,6].

Results and Discussion

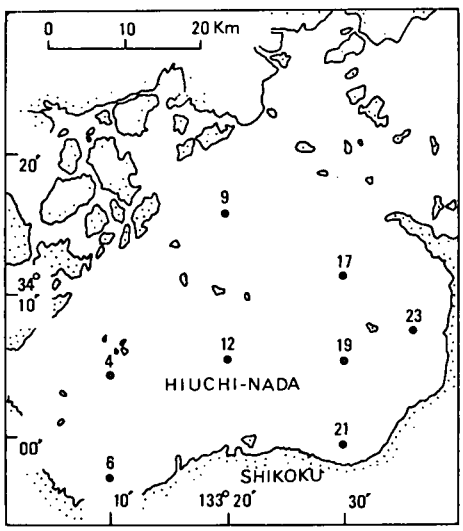

Fig. 1. The Hiuchi-Nada area in the Seto Inland Sea, showing locations of sampling.

Bacterial countings of each sample collected from the sea of the Hiuchi-Nada area (Fig. 1) were carried out by the direct microscopic method, the extinction dilution method, and the agar pour plate method respectively. The results are shown in Table 1.

Irrespective of counting method employed, during the warm water period, bacterial population was large especially in the topmost few meters of water; but during the cold water period, the vertical localization of bacteria was less pronounced. Bacterial population was large in the coastal regions especially in area adjacent to Shikoku Island.

As shown in Figs. 2 and 3, significant correlations were observed between the direct microscopic counts and the two cultural

Table 1. *Number and biomass of the bacteria detected by direct microscopic method, extinction dilution method, or agar pour plate method. Samples were collected from the Sea of the Hiuchi-Nada area*

Date	Station	Depth (m)	Bacterial numbers estimated by: pour plate method (c. f. u. /ml)	Extinction dilution method (MPN/ml)	Direct microscopic method (cells/ml)	Bacterial dry weights calculated from: Pour plate count (g/m^3)	Extinction dilution count (g/m^3)	Direct microscopic count (g/m^3)
June 25 1972	4	0	5.7×10^4	2.4×10^5	3.6×10^6	3.0×10^{-3}	1.3×10^{-2}	1.9×10^{-1}
		5	6.4×10^3	9.0×10^3	2.2×10^6	3.3×10^{-4}	4.7×10^{-4}	1.1×10^{-1}
		10	4.0×10^3	4.3×10^3	3.0×10^4	2.1×10^{-4}	2.2×10^{-4}	1.6×10^{-3}
		15	1.6×10^3	2.3×10^3	2.4×10^4	8.3×10^{-5}	1.2×10^{-4}	1.3×10^{-3}
		20	1.0×10^3	2.3×10^3	2.4×10^4	5.2×10^{-5}	1.2×10^{-4}	1.3×10^{-3}
		25	1.3×10^3	1.5×10^3	5.7×10^3	6.8×10^{-5}	9.8×10^{-5}	3.0×10^{-4}
		30	7.0×10^2	4.3×10^3	6.6×10^3	3.6×10^{-5}	2.2×10^{-4}	3.4×10^{-4}
	6	0	6.0×10^4	2.4×10^4	1.2×10^6	3.1×10^{-3}	1.3×10^{-3}	6.2×10^{-2}
		5	9.0×10^3	4.3×10^3	4.5×10^5	4.7×10^{-4}	2.2×10^{-4}	2.3×10^{-2}

Table 1. (*Continued*)

Date	Station	Depth (m)	Bacterial numbers estimated by: Pour plate method (c. f. u. /ml)	Extinction dilution method (MPN/ml)	Direct microscopic method (cells/ml)	Bacterial dry weights calculated from: Pour plate count (g/m^3)	Extinction dilution count (g/m^3)	Direct microscopic count (g/m^3)
June 25 1972	9	0	3.9×10^{4}	4.3×10^{4}	3.3×10^{6}	2.0×10^{-3}	2.2×10^{-3}	1.7×10^{-1}
		5	2.8×10^{3}	2.3×10^{3}	1.7×10^{5}	1.5×10^{-4}	1.2×10^{-4}	8.8×10^{-3}
		10	6.9×10^{3}	1.5×10^{4}	2.2×10^{4}	3.6×10^{-4}	7.8×10^{-4}	1.1×10^{-3}
		15	2.2×10^{3}	4.6×10^{3}	1.3×10^{4}	1.1×10^{-4}	2.4×10^{-4}	6.8×10^{-4}
	12	0	1.6×10^{4}	4.6×10^{4}	2.1×10^{5}	8.3×10^{-4}	2.4×10^{-3}	1.1×10^{-2}
		5	2.5×10^{3}	4.3×10^{3}	6.3×10^{4}	1.3×10^{-4}	2.4×10^{-4}	3.3×10^{-3}
		10	1.3×10^{3}	1.5×10^{3}	4.5×10^{4}	6.8×10^{-5}	7.8×10^{-5}	2.3×10^{-3}
		15	2.1×10^{3}	9.3×10^{2}	9.0×10^{3}	1.1×10^{-4}	4.8×10^{-5}	4.7×10^{-4}
	17	0	2.7×10^{4}	9.3×10^{4}	6.3×10^{6}	1.4×10^{-3}	4.8×10^{-3}	3.3×10^{-1}
		5	6.3×10^{3}	4.3×10^{3}	6.3×10^{5}	3.3×10^{-4}	2.2×10^{-4}	3.3×10^{-2}
		10	2.3×10^{3}	3.9×10^{3}	1.4×10^{4}	1.2×10^{-4}	2.0×10^{-4}	7.3×10^{-4}
		15	1.0×10^{3}	9.0×10^{2}	1.5×10^{4}	5.2×10^{-5}	4.7×10^{-5}	7.8×10^{-4}
	19	0	4.4×10^{4}	9.3×10^{4}	3.0×10^{6}	2.3×10^{-3}	4.8×10^{-3}	1.6×10^{-1}
		5	4.3×10^{3}	4.6×10^{3}	5.1×10^{4}	2.3×10^{-4}	2.4×10^{-4}	2.7×10^{-3}
		10	1.1×10^{3}	1.5×10^{3}	6.0×10^{3}	5.7×10^{-5}	7.8×10^{-5}	3.1×10^{-4}
		15	2.0×10^{2}	9.0×10^{2}	4.5×10^{3}	1.0×10^{-5}	4.7×10^{-5}	2.3×10^{-4}
	21	0	5.0×10^{4}	4.6×10^{4}	8.7×10^{6}	2.6×10^{-3}	2.4×10^{-3}	4.5×10^{-1}
		5	1.2×10^{4}	9.5×10^{3}	4.5×10^{5}	6.2×10^{-4}	4.9×10^{-4}	2.3×10^{-2}
		10	8.0×10^{2}	4.3×10^{3}	6.0×10^{4}	4.2×10^{-5}	2.2×10^{-4}	3.1×10^{-3}
	23	0	3.6×10^{4}	2.4×10^{4}	1.6×10^{6}	1.9×10^{-3}	1.3×10^{-3}	8.3×10^{-2}
		5	9.0×10^{3}	2.4×10^{3}	1.2×10^{5}	4.7×10^{-4}	1.3×10^{-4}	6.2×10^{-3}
		10	2.3×10^{3}	2.3×10^{3}	6.9×10^{4}	1.2×10^{-4}	1.2×10^{-4}	3.6×10^{-3}
		15	1.4×10^{3}	9.0×10^{2}	6.1×10^{4}	7.3×10^{-5}	4.7×10^{-5}	3.2×10^{-3}
Jan. 26/27 1973	4	0	1.4×10^{3}	3.4×10^{3}	1.7×10^{4}	2.1×10^{-4}	1.8×10^{-4}	8.8×10^{-4}
		5	9.0×10^{2}	3.4×10^{3}	1.9×10^{4}	4.7×10^{-5}	1.8×10^{-4}	9.9×10^{-4}
		10	8.0×10^{2}	1.5×10^{3}	1.8×10^{4}	4.2×10^{-5}	7.8×10^{-5}	9.4×10^{-4}
		15	7.0×10^{2}	1.5×10^{3}	2.4×10^{4}	3.6×10^{-5}	7.8×10^{-5}	1.3×10^{-3}
		20	8.0×10^{2}	1.6×10^{3}	1.5×10^{4}	4.2×10^{-5}	8.3×10^{-5}	7.8×10^{-4}
		25	3.1×10^{2}	1.0×10^{3}	2.4×10^{4}	1.6×10^{-5}	5.2×10^{-5}	1.3×10^{-3}
		30	6.6×10^{2}	1.6×10^{3}	1.5×10^{4}	3.4×10^{-5}	8.3×10^{-5}	7.8×10^{-4}
	6	0	1.6×10^{4}	3.4×10^{4}	3.8×10^{4}	8.3×10^{-4}	1.7×10^{-3}	2.0×10^{-3}
		5	1.5×10^{4}	2.6×10^{4}	4.5×10^{4}	7.3×10^{-4}	1.4×10^{-3}	2.3×10^{-3}
		10	6.2×10^{3}	2.6×10^{4}	2.6×10^{4}	3.2×10^{-4}	1.4×10^{-3}	1.4×10^{-3}
	9	0	1.0×10^{4}	2.2×10^{4}	6.1×10^{4}	5.2×10^{-4}	1.1×10^{-3}	3.2×10^{-3}
		5	9.9×10^{3}	2.2×10^{4}	6.2×10^{4}	5.2×10^{-4}	1.1×10^{-3}	3.2×10^{-3}
		10	7.0×10^{3}	9.9×10^{3}	6.2×10^{4}	3.6×10^{-4}	5.2×10^{-4}	3.2×10^{-3}
		15	6.2×10^{3}	9.9×10^{3}	5.7×10^{4}	3.2×10^{-4}	5.2×10^{-4}	3.0×10^{-3}
	12	0	1.4×10^{3}	6.6×10^{3}	1.5×10^{4}	7.3×10^{-5}	3.4×10^{-4}	7.8×10^{-4}
		5	8.0×10^{2}	2.2×10^{3}	1.8×10^{4}	4.2×10^{-5}	1.1×10^{-4}	9.4×10^{-4}
		10	5.0×10^{2}	1.5×10^{3}	1.3×10^{4}	2.6×10^{-5}	7.8×10^{-5}	6.8×10^{-4}
		15	8.0×10^{2}	9.8×10^{2}	1.5×10^{4}	4.2×10^{-5}	5.1×10^{-4}	7.8×10^{-4}
	17	0	2.4×10^{3}	9.8×10^{3}	2.0×10^{4}	1.3×10^{-4}	5.1×10^{-4}	1.0×10^{-3}
		5	3.6×10^{3}	9.8×10^{3}	8.8×10^{3}	1.9×10^{-4}	5.1×10^{-4}	4.6×10^{-4}
		10	2.4×10^{3}	6.6×10^{3}	1.1×10^{4}	1.3×10^{-4}	3.4×10^{-4}	5.7×10^{-4}
		15	3.4×10^{3}	6.6×10^{3}	1.6×10^{4}	1.8×10^{-4}	3.4×10^{-4}	8.3×10^{-4}
		20	3.4×10^{3}	6.6×10^{3}	9.3×10^{3}	1.8×10^{-4}	3.4×10^{-4}	4.8×10^{-4}
	19	0	8.5×10^{2}	1.6×10^{3}	3.8×10^{4}	4.4×10^{-5}	8.3×10^{-5}	2.0×10^{-3}
		5	1.4×10^{3}	3.4×10^{3}	2.4×10^{4}	7.3×10^{-5}	1.8×10^{-4}	1.3×10^{-3}
		10	6.0×10^{2}	1.6×10^{3}	1.7×10^{4}	3.1×10^{-5}	8.3×10^{-5}	8.8×10^{-4}
		15	6.0×10^{2}	2.6×10^{3}	2.0×10^{4}	3.1×10^{-5}	1.4×10^{-4}	1.0×10^{-3}
	21	0	8.0×10^{3}	9.8×10^{3}	2.6×10^{4}	4.2×10^{-4}	5.1×10^{-4}	1.4×10^{-3}
		5	7.7×10^{3}	9.8×10^{3}	1.2×10^{4}	4.0×10^{-4}	5.1×10^{-4}	6.2×10^{-4}
		10	6.2×10^{3}	9.8×10^{3}	1.6×10^{4}	3.2×10^{-4}	5.1×10^{-4}	8.3×10^{-4}
		15	7.3×10^{3}	1.7×10^{4}	3.1×10^{4}	3.8×10^{-4}	8.8×10^{-4}	1.6×10^{-3}

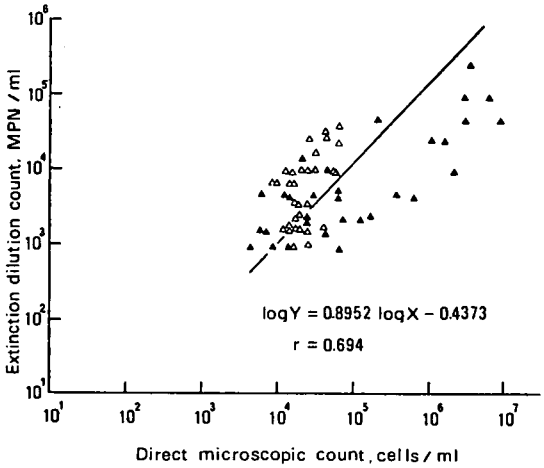

Fig. 2. Relationship between the direct microscopic counts and the extinction dilution counts.
▲: June 1972, △: Jan. 1973

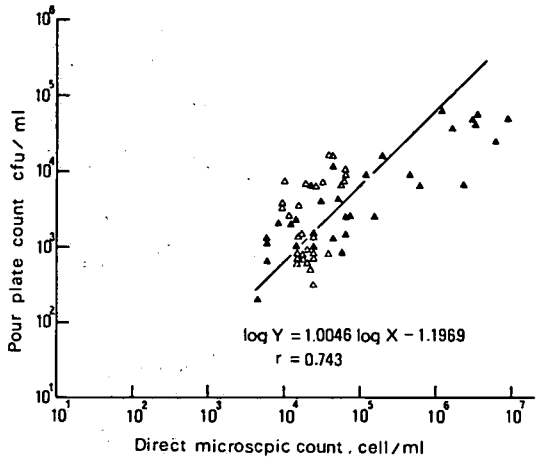

Fig. 3. Relationship between the direct microscopic counts and the agar pour plate counts.
▲: June 1972, △: Jan. 1973.

method counts (the extinction dilution counts and the agar pour plate counts), but the direct microscopic counts were larger than the cultural method counts. This latter tendency seemed to be especially true for the water samples of high bacterial density.

The counts obtained by the direct microscopic method should correspond to the total bacterial cells, but are obliged to include the numbers of dead cells and non-biological particles indistinguishable from bacteria. The counts obtained by the cultural methods should correspond to those of heterotrophic members of bacteria. Part of aquatic bactera are believed to be inactive[1], and all members of heterotrophic bacteria cannot always proliferate under a given cultrual condition. Therefore, the differences between the direct microscopic counts and the cultural method counts were unavoidable.

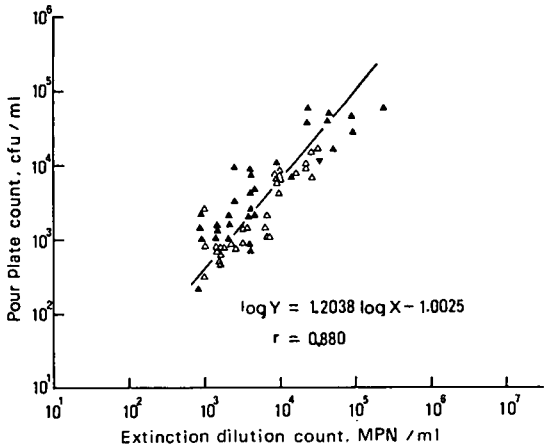

Fig. 4. Relationship between the extinction dilution couuts and the agar pour plate counts.
▲: June 1972, △: Jan. 1973.

As shown in Fig. 4, there was a close correlation between the agar pour plate counts and the extinction dilution counts. As a whole, the agar pour plate counts were a little smaller than the extinction dilution counts. It is noteworthy that the correlation coefficient between the agar pour plate counts and the extinction dilution counts was significantly higher than the coefficients between the direct microscopic counts and the cultural method counts.

The underestimation in agar pour plate counts was mainly due to the thermal

sensitivity of bacteria[7]. The difference between the paired cultural method counts was not so large as compared with a previous report[1] which was carried out tentatively with various types of seawater samples. The discrepancy may depend on the difference in bacterial flora.

Thirty-three hundred and ten cells of bacteria were chosen randomly from various depths of water in the whole waters of the Hiuchi-Nada area, and the volumes of these cells were determined. As illustrated in Fig. 5, the volume of most bacterial cells fell within the range of 0.1−0.4 μ^3 with an average volume of 0.26 μ^3. The average volume was somewhat larger than that of a previous report[5]. The larger value may be associated with the fact that the waters contained a relatively large amount of nutrients available for bacteria.

Based on the average volume, the dry weights of bacteria in each sample are also shown in Table 1.

Of course, the distribution and the abundance of bacterial biomass should vary directly with the bacterial counts. Irrespective of counting methods employed, the waters had a relatively abundant biomass of bacteria. High values of bacterial biomass seemed to be associated with the "eutrohpic" conditions of the waters.

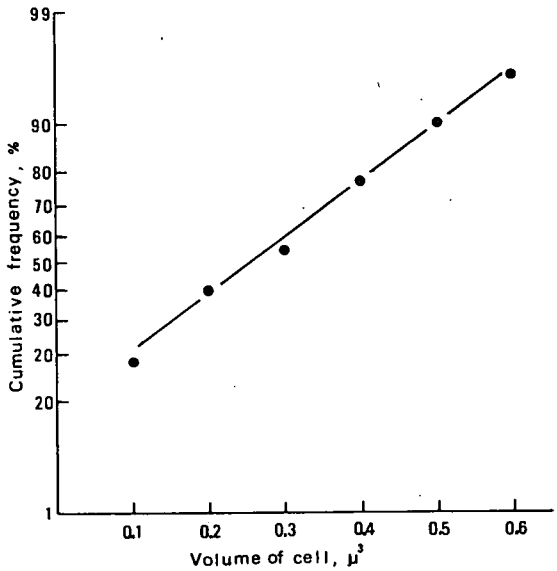

Fig. 5. Cumulative frequency distribution of bacterial volumes. Samples were collected from the sea of the Hiuchi-Nada area at June '72, Aug. '72, and Jan. '73.

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