Factors Relating to the Abundance of Bacteria in the Mangrove Estuary of Nakama River, Iriomote Island, Okinawa

HIDEO MIYOSHI¹, SOUWAPA ANGSPANICH², and YOSHIHIKO HATA²

1. *Usa Marine Biological Institute, Kochi University, Usa-cho, Tosa, Kochi 781-11, Japan*
2. *Faculty of Agriculture, Kochi University, Monobe, Nankoku, Kochi 783, Japan*

Abstract: Physical, chemical and bacteriological examinations of water and mud from the mangrove estuary of Nakama River were carried out. The numbers of total bacteria in the water were one order of magnitude larger in summer than in winter, on the average, and had close correlations with a lot of variables such as, chlorinity, concentrations of nitrate, nitrite, ammonia, total nitrogen, particulate carbohydrates, particulate amino acids and chlorophyll *a* in the water. The numbers of aerobic heterotrophic bacteria in the mud showed the same seasonal pattern as that for water bacteria, and had strong correlations with the concentrations of inorganic phosphate, ammonia and total nitrogen in the pore water as well as the contents of total carbohydrates and total amino acids in the mud. Interactions among water-related and mud-related variables were not so evident with few exceptions. The models for predicting bacterial abundance in the ecosystem are proposed by multivariate analysis, and the influence of environmental variables on the temporal and spatial distributions of bacteria in the estuary is discussed.

Key words: Bacterial distribution, multivariate analysis, Okinawa, mangrove estuary.

Introduction

Nakama River in Iriomote Island, Okinawa, has been totally undisturbed by pollutants resulting from human activity, and the middle estuary of the river has exuberant mangrove forest, which provides a source of organic matter.

Many workers have studied the relationships between bacterial populations or communities and biotic or abiotic variables in aquatic environments by multiple regression analysis (*e.g.*, Mahloch, 1974; Miyoshi and Nakamoto, 1975; Jones, 1977; Palumbo and Ferguson, 1978; Väättänen, 1982; Hazen, 1983). The characteristics of bacterial community in Nakama River estuary were already reported in the previous number of this bulletin (Souwapa *et al.*, 1989), but the influence of environmental variables on bacterial distribution in the mangrove estuary has not yet been elucidated.

We embarked on further study of physical and chemical variables relating to bacterial distribution in Nakama River estuary using multivariate analysis. The results obtained are reported herein.

Study area

Nakama River is located on the south-east coast of Iriomote Island (Fig. 1). The total length of the main stream is about 14 km and the daily tidal rise is observed up to Stn. 1. A small boat can pass upstream as far as 7.5 km at high tide, but only to Stn. 9 at neap tide, because the maximum tidal range is 1.2-2.5 m and the river is not very deep. Mangrove forest is most abundant in the middle estuary of the river where it covers about 300 hectares.

Fig. 1. Map showing the sampling stations in Nakama River.

Materials and methods

Samples

Water samples were collected with a liter bottle sampler at 1 m depth intervals throughout the entire water column. Particulate matter in the water was collected by filtration through precombusted glass fiber filters (Whatman GF/C). Mud was collected with a grab sampler and the uppermost layer (*ca.* 0–0.5 cm) was used as the sample. Pore water was obtained from the mud sample by pressure filtration. Dry mud was prepared by heating at 105°C for 1 hr.

Physical and chemical analyses

Temperature and pH were measured immediately after sampling with a mercury-in-glass thermometer and a portable pH meter (Toa Electronics HM-10K), respectively. Analyses of nitrate, nitrite, ammonia, inorganic phosphate, total phosphorous, total nitrogen and chlorinity were carried out according to the methods described by Grasshoff *et al.* (1983). Particulate carbohydrates and total carbohydrates were determined by the phenol sulfuric acid method (Koyama *et al.*, 1972). Particulate amino acids and total amino acids were determined by the ninhydrin method after being hydrolyzed with 6N HCl at 100°C for 24 hr. in a sealed tube (Koyama *et al.*, 1982). Fluorometric determination of chlorophyll *a* was performed on 90% acetone extracts with a spectrofluoro-

photometer (Shimadzu RF-500) (American Public Health Association, 1981).

Counting of bacteria

Epifluorescent counting of total bacteria in the water (water bacteria) was made using the technique described by Nishino (1986). Aerobic heterotrophic bacteria in the mud (mud bacteria) were counted according to the spread plate method (Buck and Cleverdon, 1960) with the following modifications: Media; Modified media of Zo-Bell's 2216E were employed. Namely, peptone and yeast extract were reduced to one tenth of original recipe, and for samples with low original salinities, the 80% strength seawater of original recipe was replaced by 33% strength seawater or tap water. Procedures; The salinity of pore water of each mud sample was roughly estimated using a refractory salinometer (Atago T-100). When the salinity of pore water was between 0–5 ‰, 5–15 ‰, or >15 ‰, the following medium and diluent prepared respectively with tap water, 33% strength seawater or 80% strength seawater were employed, and spreading plates were made. After 2 weeks' incubation at 25°C, the colonies developed on each plate were counted.

Multivariate analysis

Correlation and regression relationships were analyzed by using commercially available programs (Fujitsu "Analyst").

Results

Physical, chemical and bacteriological examinations of water were carried out along a 7 km stretch of Nakama River estuary. Table 1 shows the results of these examinations. The numbers of water bacteria ranged from *ca.* 10^4 to 10^6 cells ml^{-1} . In terms of seasonal variation, the numbers of water bacteria were approximately one order of magnitude larger in summer than in winter. Particulate carbohydrates, particulate amino acids and chlorophyll were also more abundant in

summer. In addition, most levels of nitrogen components were slightly higher in summer. In terms of geographical distribution, chlorophyll, particulate carbohydrates and particulate amino acids were slightly abundant in the middle of estuary as compared with upper and lower parts of the stream, but the same pattern for the bacterial distribution was not evident.

The relationships among the variables related to the water are presented in Table 2. The log transformed counts of water bacteria had strong positive correlations with the concentrations of nitrate, nitrite, ammonia, total nitrogen, particulate carbohydrates, particulate amino acids, chlorophyll and temperature ($P < 0.01$), while the counts showed significant reverse correlation with chlorinity ($P < 0.01$). Excluding bacterial variable, there are a large number of significant correlations among the remaining variables. In particular, temperature, the concentrations of ammonia, particulate amino acids and chlorophyll had close correlations with 8 or more of the 13 variables ($P < 0.01$).

The possible relationships between the bacterial variable (dependent variable) and a series of environmental variables (independent variables) were examined by the multiple regression analysis, and the following model for predicting bacterial population in the water ($\log Y_w$, cells $\cdot\text{ml}^{-1}$) is obtained:

$$\begin{aligned}\log Y_w = & -0.087x_1 + 0.035x_2 - 0.018x_4 \\ & + 0.117x_5 - 0.044x_6 + 0.967x_8 \\ & + 0.216x_{12} + 4.867\end{aligned}\quad (1)$$

where x_1 =depth (m), x_2 =temperature ($^{\circ}\text{C}$), x_4 =chlorinity (‰), x_5 =inorganic phosphate concentration ($\mu\text{mol/l}$), x_6 =total phosphorous concentration ($\mu\text{mol/l}$), x_8 =nitrite concentration ($\mu\text{mol/l}$), x_{12} =particulate amino acid concentration (as mg glutamic acid/l). The coefficient of determination (R^2) of the model was 0.81.

The results of physical, chemical and bacteriological examinations of mud and overlying water are presented in Table 3. The correlation coefficients between the variables are presented in

Table 4. The numbers of mud bacteria ranged from *ca.* 10^5 to 10^7 colony forming units (cfu)/g wet mud. The numbers were higher in summer than in winter. The concentrations of inorganic phosphate, ammonia and total nitrogen in the pore water were also higher in summer. The log transformed counts of mud bacteria had significant positive correlations with the concentrations of inorganic phosphate, ammonia, total nitrogen, total carbohydrates and total amino acids in the pore water or mud ($P < 0.01$).

Excluding the bacterial variable, there were 9 significant correlations among the remaining variables. In particular, the concentrations of inorganic phosphate and total nitrogen in the pore water and the contents of carbohydrates in the mud had close correlations with 3 variables besides bacterial variable ($P < 0.01$).

Based on the multiple regression analysis, the population of mud bacteria ($\log Y_m$, cfu/g wet mud) can be predicted by the following model:

$$\log Y_m = 0.0385x'_7 + 0.9668 \quad (2)$$

where x'_7 =total nitrogen concentration in the pore water ($\mu\text{mol}\cdot\text{l}^{-1}$). The R^2 value of the model was 0.73.

The relevant correlation coefficients, which were calculated by using the data shown in Tables 1, and 3, are given in Table 5. The result indicated that the log transformed counts of mud bacteria had significant correlations with the concentrations of ammonia, particulate amino acids and nitrite in the water ($P < 0.05$), while the log transformed counts of water bacteria had significant correlations with the concentrations of total nitrogen, inorganic phosphate and ammonia in the pore water ($P < 0.05$). Excluding bacterial variables, the concentrations of both ammonia and total nitrogen in the pore water had significant correlations with 4 or more out of the 9 variables related to water.

Table 1. Data on the physical, chemical and bacteriological

Date	Stn.	Depth (m)	Temperature (°C)	pH	Chlorinity (‰)	Inorganic phosphate ($\mu\text{mol/l}$)	Total phosphorus ($\mu\text{mol/l}$)	Nitrate ($\mu\text{mol/l}$)
Feb 25, 1987 (low tide)	3	0	17.2	6.9	0.5	0	0.6	2.6
		1	17.8	6.9	2.4	0	0.5	2.5
	5	0	18.5	6.9	8.0	0	1.0	2.7
	7	0	18.8	7.3	12.0	0.1	0.6	4.8
	9	0	19.0	7.7	16.6	0	0.3	3.1
	11	0	18.5	8.0	18.5	0	0.3	1.2
		1	19.0	8.0	18.7	0.1	1.8	0.8
	13	0	19.0	8.1	18.9	0.1	0.5	0.6
		1	19.0	8.1	18.9	0.1	0.5	0.4
Feb 26, 1987 (high tide)	1	0	16.5	7.1	0.3	0.1	0.6	3.0
	3	0	17.5	7.1	0.7	1.2	1.6	2.6
		1	17.0	7.2	3.0	1.2	1.6	2.8
	5	0	17.3	7.5	8.4	1.8	1.9	2.8
		1	17.0	7.5	8.5	1.7	2.1	1.4
	7	0	17.0	8.2	17.5	2.5	3.0	1.2
		1	17.0	8.2	17.3	3.0	3.0	1.2
	9	0	17.0	7.7	18.2	3.0	3.3	0.6
		1	17.0	8.2	18.2	2.0	2.5	1.2
	11	0	17.5	8.2	18.7	1.8	2.5	0.8
		1	17.5	8.2	18.9	1.7	2.7	0.5
	13	0	18.2	8.2	19.4	2.3	2.5	0.7
		1	17.5	8.2	19.3	1.6	1.9	0.4
Aug 26, 1987 (low tide)	3	0	30.0	8.1	2.4	0.2	1.2	4.0
		1	30.0	8.0	3.6	0.2	1.0	3.6
	5	0	32.0	8.0	4.0	0	0.8	2.7
		1	31.8	8.0	5.2	0.2	1.0	2.6
	7	0	32.0	8.2	9.6	0.1	1.2	3.6
	9	0	31.2	8.0	12.4	0.1	1.2	3.0
	11	0	31.3	8.4	15.0	0.1	1.8	2.1
	13	0	31.6	8.5	16.4	0	1.0	1.3
		1	32.0	8.5	16.5	0.2	1.1	2.0
Aug 27, 1987 (high tide)	1	0	26.5	8.2	0.8	2.2	8.8	4.7
		1	29.5	8.1	2.5	1.2	8.5	3.4
	3	0	29.5	8.3	4.6	1.2	8.0	3.8
		1	30.0	8.2	5.4	0.8	4.1	4.0
	5	0	29.8	8.4	12.8	1.0	5.8	3.4
		1	29.8	8.5	14.4	2.9	6.0	3.2
	7	0	30.0	8.5	17.5	4.5	8.5	0.7
		1	30.0	8.4	18.3	0.6	2.5	0.5
	9	0	30.3	8.5	18.3	0.4	2.7	0.5
		1	30.0	8.5	18.3	0.3	4.1	0.4
		3	29.8	8.5	18.3	0.4	0.8	0.4
	11	0	30.0	8.5	18.5	0.5	3.0	0.4
		1	29.9	8.5	18.5	0.2	0.8	0.5
	13	0	30.0	8.5	19.0	0.4	2.3	0.1
		1	30.0	8.5	19.0	0.4	1.9	0.2

examinations of water from Nakama River estuary

Nitrite ($\mu\text{mol/l}$)	Ammonia ($\mu\text{mol/l}$)	Total nitrogen ($\mu\text{mol/l}$)	Particulate carbohydrates (as mg glucose /l)	Particulate amino acids (as mg glutamic acid/l)	Chlorophyll <i>a</i> (mg/m ³)	Water bacteria (cells/ml)
0.05	0.2	30.5	0.04	0.13	1.1	5.4×10^5
0.05	0.3	31.0	0.08	0.36	3.0	2.3×10^5
0.08	0.4	28.0	0.33	1.21	7.1	2.8×10^5
0.05	0.2	27.0	0.11	0.51	1.6	4.1×10^5
0.06	0.2	23.5	0.10	0.20	1.0	2.0×10^5
0.03	0.1	22.0	0.06	0.14	0.8	1.7×10^5
0.03	0.2	23.5	0.06	0.20	0.6	1.7×10^5
0	0.1	22.0	0.07	0.15	0.9	2.0×10^5
0	0.1	23.4	0.05	0.22	0.9	1.1×10^5
0	0.7	23.5	0.03	0.11	1.2	6.3×10^4
0	0.7	29.0	0.06	0.29	3.2	5.2×10^5
0	0.6	29.0	0.13	0.51	1.4	4.7×10^5
0	0.4	30.5	0.05	0.27	2.4	5.8×10^5
0	0.4	30.0	0.12	0.43	1.6	5.4×10^5
0	0.3	22.0	0.08	0.19	0.9	2.3×10^5
0	0.3	34.5	0.04	0.23	1.0	2.0×10^5
0	0.2	21.2	0.04	0.24	1.4	2.4×10^5
0	0.2	22.0	0.06	0.26	0.7	1.5×10^5
0	0.2	19.0	0.03	0.11	0.8	2.2×10^5
0	0.3	27.5	0.02	0.16	0.7	1.8×10^5
0	0.2	21.0	0.02	0.12	0.9	2.1×10^5
0	0.4	19.1	0.02	0.14	0.9	1.1×10^5
0.18	3.8	40.0	0.10	0.64	5.3	1.4×10^6
0.19	3.1	37.0	0.22	0.89	6.2	1.3×10^6
0.06	3.1	28.0	0.13	0.64	7.7	1.4×10^6
0.25	2.7	36.0	0.15	0.78	6.3	1.6×10^6
0.07	1.3	38.0	0.17	0.74	4.3	1.6×10^6
0.23	1.0	35.0	0.17	0.71	3.2	1.1×10^6
0.26	1.1	36.0	0.21	0.93	3.3	1.5×10^6
0.21	0.3	22.5	0.15	0.55	2.5	1.0×10^6
0.22	0.6	35.3	0.15	0.83	1.7	7.8×10^5
0.11	2.7	48.0	0.05	0.22	1.4	6.1×10^5
0.20	3.8	41.0	0.15	0.77	6.5	6.3×10^5
0.23	2.4	68.0	0.14	0.73	7.4	1.2×10^6
0.10	3.5	38.0	0.13	0.77	4.2	1.2×10^6
0.20	2.7	31.0	0.08	0.49	2.6	1.2×10^6
0.20	3.0	35.2	0.07	0.54	1.4	8.9×10^5
0.10	1.4	35.2	0.09	0.64	4.1	6.1×10^5
0.02	1.3	70.0	0.07	0.46	1.5	3.5×10^5
0	0.8	31.0	0.09	0.42	2.8	5.1×10^5
0	0.9	38.5	0.09	0.56	1.5	3.4×10^5
0	1.4	28.0	0.07	0.49	1.3	3.5×10^5
0	0.4	35.0	0.05	0.23	1.9	4.2×10^5
0	0.5	35.7	0.08	0.37	1.0	2.7×10^5
0.02	0.3	38.0	0.06	0.38	2.7	3.8×10^5
0.02	0.6	61.0	0.06	0.40	1.6	2.6×10^5

Table 2. Correlation matrix showing the degree of linear

Variables	x_1	x_2	x_3	x_4	x_5	x_6
Depth (x_1)	1.000					
Temperature (x_2)	0.093	1.000				
pH (x_3)	0.191	0.641 ^a	1.000			
Chlorinity (x_4)	0.140	-0.011	0.609 ^a	1.000		
Inorganic phosphate (x_5)	-0.024	-0.242	0.168	0.168	1.000	
Total phosphorus (x_6)	-0.066	0.272	0.361	-0.069	0.625 ^a	1.000
Nitrate (x_7)	-0.237	0.130	-0.390 ^a	-0.784 ^a	-0.141	0.182
Nitrite (x_8)	-0.105	0.615 ^a	0.208	-0.328	-0.150	0.284
Ammonia (x_9)	0.105	0.613 ^a	0.232	-0.529 ^a	0.019	0.483 ^a
Total nitrogen (x_{10})	0.108	0.569 ^a	0.293	-0.200	-0.053	0.420 ^a
Particulate carbohydrates (x_{11})	-0.062	0.398 ^a	-0.123	-0.320	-0.360	-0.072
Particulate amino acids (x_{12})	0.082	0.625 ^a	0.074	-0.319	-0.238	0.126
Chlorophyll (x_{13})	-0.159	0.491 ^a	0.076	-0.564 ^a	-0.188	0.201
Total bacteria (log Y_w)	-0.144	0.715 ^a	0.194	-0.428 ^a	-0.110	0.244

^a, significant at 0.01% level

Discussion

The numbers of water bacteria had close correla-

tions with as many as 9 out of the 13 environmental variables related to water (Table 2). Especially, tight couplings were observed among the

Table 3. Data on the physical, chemical and bacteriological examinations of mud and overlying

Date	Stn.	Temperature ^a (°C)	Chlorinity ^a (‰)	Inorganic phosphate ^b ($\mu\text{mol/l}$)	Nitrate ^b ($\mu\text{mol/l}$)	Nitrite ^b ($\mu\text{mol/l}$)	Ammonia ^b ($\mu\text{mol/l}$)	Total nitrogen ^b ($\mu\text{mol/l}$)
Feb 26, 1987	1	16.5	0.3	1.2	11.0	0.3	9.0	126
	3	17.0	3.0	1.7	8.8	0.5	6.6	130
	5	17.0	8.5	3.2	1.1	0.2	6.8	140
	7	17.0	17.3	3.0	1.7	0.1	5.6	144
	9	17.0	18.2	2.1	1.4	0.1	5.1	135
	11	17.5	18.9	1.8	1.8	0.1	4.9	130
	13	17.5	19.3	1.2	1.9	0.1	4.9	133
Aug 27, 1987	1	29.5	2.5	4.2	2.8	0.2	14.3	143
	3	30.0	5.4	3.3	2.1	0.2	10.7	156
	5	29.8	14.4	7.1	1.9	0.1	9.3	165
	7	30.0	18.3	7.1	1.6	0.1	8.1	163
	9	29.8	18.3	4.7	1.5	0.1	7.4	140
	11	29.9	18.5	4.5	1.4	0.1	6.9	138
	13	30.0	19.0	4.3	1.4	0.1	6.0	137

^a, determined by using overlying water (The same as in Table 1);^b, determined by using pore water.

relationships between the variables related to water of Nakama River estuary

x_7	x_8	x_9	x_{10}	x_{11}	x_{12}	x_{13}	$\log Y_w$
1.000							
0.549 ^a	1.000						
0.594 ^a	0.625 ^a	1.000					
0.191	0.332	0.443 ^a	1.000				
0.425 ^a	0.588 ^a	0.320	0.191	1.000			
0.429 ^a	0.691 ^a	0.541 ^a	0.385 ^a	0.907 ^a	1.000		
0.494 ^a	0.577 ^a	0.669 ^a	0.359	0.698 ^a	0.770 ^a	1.000	
0.543 ^a	0.742 ^a	0.681 ^a	0.412 ^a	0.560 ^a	0.707 ^a	0.661 ^a	1.000

levels of water bacteria, chlorophyll, particulate carbohydrates and particulate amino acids. This trend strongly suggested that the numbers of water

bacteria had a close correlation with the biomass of phytoplankton. Similar tendencies have been confirmed by many other workers (*e.g.*, Straskrbova and Komerkova, 1979; Pedros-Alio and Brock; 1982; Bird and Kalff, 1984; Nagata, 1984). That is, the growth of water bacteria may be supported by the pool of organic matter derived from both living and dead phytoplankton. In addition, the growth of water bacteria seemed to be controlled by the pool of particulate organic matter originating probably from mangrove litter. Detailed discussion on this problem will be described elsewhere.

Bacterial population, most of the nitrogen components and all of the particulate components in the water increased with increasing temperature (Table 2). However, direct coupling between bacterial population and temperature was thought to be doubtful in marine environments (ZoBell, 1946). In this light, it seems reasonable to consider that the seasonality of bacterial population in the water is also brought about indirectly by changes in the phytoplankton biomass.

The possible explanations for the close relationships between bacterial numbers and inorganic

water from Nakama River estuary

Total carbohydrates (as mg glucose /g dry mud)	Total amino acids (as mg glutamic acid/g dry mud)	Mud bacteria (cfu/g wet mud)
0.13	1.1	1.7×10^6
0.09	0.5	1.7×10^6
0.11	0.9	2.0×10^6
0.42	2.4	2.4×10^6
0.12	0.8	9.0×10^5
0.06	0.7	5.0×10^5
0.06	0.8	9.0×10^5
0.07	0.7	7.1×10^6
0.54	1.6	9.5×10^6
0.98	2.7	1.8×10^7
0.57	2.0	2.2×10^7
0.44	1.1	5.8×10^6
0.15	0.8	9.1×10^5
0.16	0.8	5.5×10^5

Table 4. Correlation matrix showing degree of linear relationships between variables related to mud

Variables	x'_1	x'_2	x'_3	x'_4	x'_5	x'_6	x'_7	x'_8	x_{9min}	$\log Y_m$
Temperature (x'_1) ^a	1.000									
Chlorinity (x'_2) ^a	0.139	1.000								
Inorganic phosphate (x'_3) ^b	0.805 ^c	0.270	1.000							
Nitrate (x'_4) ^b	0.392	-0.715 ^c	-0.453	1.000						
Nitrite (x'_5) ^b	0.340	-0.805 ^c	-0.410	0.822 ^c	1.000					
Ammonia (x'_6) ^b	0.537	-0.652	0.364	0.164	0.212	1.000				
Total nitrogen (x'_7) ^b	0.615	0.122	0.844 ^c	-0.432	-0.340	0.425	1.000			
Total carbohydrates (x'_8)	0.520	0.147	0.743 ^c	-0.243	-0.285	0.259	0.877 ^c	1.000		
Total amino acids (x'_9)	0.265	0.162	0.593	-0.211	-0.335	0.153	0.792 ^c	0.896 ^c	1.000	
Mud bacteria ($\log Y_m$)	0.552	-0.240	0.708 ^c	-0.082	-0.014	0.662 ^c	0.853 ^c	0.782 ^c	0.670 ^c	1.000

^a and ^b, the same as in Table 3; ^c, significant at 0.01% level.

nitrogen variables are as follows: the inorganic components may control bacterial growth indirectly by changes in the phytoplankton biomass, or the inorganic nitrogen components may be produced from organic nitrogen compounds by the catalytic action of bacteria, or both.

In view of geographical distribution, the levels

of several variables such as chlorophyll and particulate amino acids in the midestuarine water were higher than those in the lower and upper estuaries, *i.e.* the midestuarine peaks of these variables were observed. These phenomena can be ascribed to the prolonged residence of water in the estuary which causes the accumulation of dissolved nutri-

Table 5. Relevant correlation coefficients between the variables related to the water and the mud from Nakama River estuary

Variables		Mud-related variables							
		Inorganic phosphate	Nitrate	Nitrite	Ammonia	Total nitrogen	Total carbohydrates	Total amino acids	Mud bacteria
Water-related variables	Inorganic phosphate	0.002	-0.288	-0.157	-0.219	0.224	0.287	0.486	0.174
	Total phosphorous	0.350	-0.231	0.018	0.711 ^a	0.502	0.300	0.294	0.486
	Nitrate	-0.051	0.478	0.672 ^a	0.728 ^a	0.240	0.255	0.209	0.421
	Nitrite	0.441	-0.100	0.110	0.796 ^a	0.556 ^b	0.471	0.362	0.532 ^b
	Ammonia	0.471	-0.083	0.164	0.897 ^a	0.626 ^b	0.506	0.329	0.626 ^b
	Total nitrogen	0.695 ^a	-0.273	-0.174	0.241	0.562 ^b	0.321	0.272	0.255
	Particulate carbohydrates	0.226	0.024	0.533 ^b	0.645 ^b	0.277	0.028	-0.148	0.370
	Particulate amino acids	0.516	-0.174	0.290	0.753 ^a	0.579 ^b	0.372	0.127	0.559 ^b
	Chlorophyll <i>a</i>	0.178	-0.024	0.248	0.880 ^a	0.279	0.004	-0.067	0.348
	Water bacteria	0.569 ^b	-0.321	0.190	0.541 ^b	0.680 ^a	0.520	0.292	0.512

The basic data for the analysis were given in Tables 1 and 3.

^a, significant at 0.01% level; ^b, significant at 0.05% level.

ents released from mud, as suggested by Wright and Coffin (1984). The resulting nutrients may support the growth of phytoplankton.

With the aim to select some important environmental variables for bacterial distribution, the regression analysis was carried out, and the predictive model including 7 environmental variables was obtained. Therefore, water as a bacterial habitat seems to be so diversified that there are no dominant variables controlling bacterial number. In other words, bacterial number in the estuary seems to be controlled by a lot of variables. Considering the R^2 value of equation 1, the environmental variables included in the model can explain up to 81% of the variance of bacterial numbers.

The numbers of mud bacteria were slightly higher in summer than in winter just like that of water bacteria. These tendencies might be attributed mainly to the summer increase of organic matter supplied by the water column. Midestuarine peaks were observed for the levels of mud bacteria, inorganic phosphate, total nitrogen, total carbohydrates and total amino acids in the pore water or mud.

Pravdič (1970) showed that marine sediments were negatively charged in seawater, but most of them were positively charged in freshwater and a reversal of charge occurred between 2 and 6‰ salinity. Accordingly, particle aggregation due to the neutralization of surface charge should occur in the midestuary, and a relatively large part of the resulting aggregates should settle onto the bottom surface within the midestuary. Naturally, co-sedimentation of these aggregates and their attached bacteria should take place, and mud becomes rich in organic nutrients available for bacteria. This may be the reason why midestuarine peak is evident for mud bacteria. Close correlations between the populations of mud bacteria and the concentrations of inorganic components such as phosphate and ammonia can be also explained as follows: these components may control bacterial growth directly, or these components

may be produced by the catalytic action of bacteria, or both.

The populations of mud bacteria had close correlations with as many as 5 out of the 9 environmental variables related to mud. Nevertheless, the predictive model for describing the abundance of mud bacteria contained only one variable: total nitrogen concentration in the pore water. This suggests that the total nitrogen concentration is a critical parameter for controlling bacterial density in the mud. Judging from the R^2 value of equation 2, 73% of the variance of bacterial populations can be explained by the environmental variable included in the model.

Exchange of nutrients between mud and overlying water has been studied extensively (e.g., Meyer-Reil *et al.*, 1978; Golterman *et al.*, 1983; Ramohr *et al.*, 1987), but it is not so easy to detect water-mud interaction in the estuary due to tidal current. An attempt was made to evaluate the interaction by computing the degree of correlations between the variables related to water and those related to mud. The population of water bacteria correlated with 3 out of the 8 variables related to mud at the 0.05% level or better, while the population of mud bacteria correlated with 3 out of the 10 variables related to water at the 0.05% level (Table 5). On the whole, these correlations were not as good as in the foregoing analyses shown in Tables 2 and 4. This may be because our analysis shown in Table 5 is not sensitive enough to detect such a slow reaction as that catalyzed by the natural microbial populations.

The present study has been designed to analyze the bacterial variables, but the results obtained make it possible to elucidate some of the interrelationships among the environmental variables. In particular, a broad spectra of interactions were noted for some environmental variables. This fact strongly suggests that these variables may have great influences on the ecosystem and seem to be richer in ecological information.

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