

Studies on Microbial Population in Coastal Waters— I  
Distribution of Nitrogen-cycle Bacteria in the Kumano Nada  
and its Adjacent Areas\*

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The occurrence and distribution of nitrogen-cycle bacteria in the Kumano Nada and its adjacent areas were studied during 1981-1983.

Heterotrophic bacteria in the water usually occurred at the level of  $10^0$ - $10^4$ /ml. The distribution of heterotrophic bacteria was uneven horizontally as well as vertically. The number of nitrate-reducing bacteria was in the order of  $10^{-1}$ - $10^3$  cells/ml, while denitrifying bacteria were in the range from  $10^{-1}$  to  $10^2$  cells/ml or sometimes lower than the value. The occurrence of nitrogen-fixing bacteria and nitrifying bacteria in the surface water was also very few ranging from  $10^0$  to  $10^2$  cells/l.

**Key words:** nitrogen-cycle bacteria, nitrate-reducing bacteria, denitrifying bacteria, nitrifying bacteria, nitrogen-fixing bacteria, microbial population, Kumano Nada

Organic substances or nutrient salts are brought with terrestrial runoff such as river water into neritic waters. Therefore, coastal waters are usually richer in biomass than the open sea. In general, productivity as well as biomass of community increases from the ocean to the shore.

Nitrogen is one of the most important bioelements, and its content is quite small in aquatic environments. Each of the reactions in the nitrogen cycle is mainly carried out by the action of microorganisms. In coastal waters, bacteria are thought to play an important role in the cycle of matter. The form and concentration of nitrogenous compounds sometimes control the productivity of community in aquatic environments.

The present paper deals with the occurrence and distribution of nitrogen-cycle bacteria in the Kumano Nada and its adjacent areas.

#### Methods

##### Collection of water and bottom sediment samples

The seawater and sediment samples from the Kumano Nada and its adjacent areas were collected during the cruises of 81-R-2, 81-R-6, 81-R-10, 82-R-2, 82-R-6, 82-R-9,

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\* Contribution from training ship Seisui-Marui, Mie University

83-R-2 and 83-R-5, respectively by the Seisui-Maru of Mie University. Fig. 1 shows the location of sampling stations.

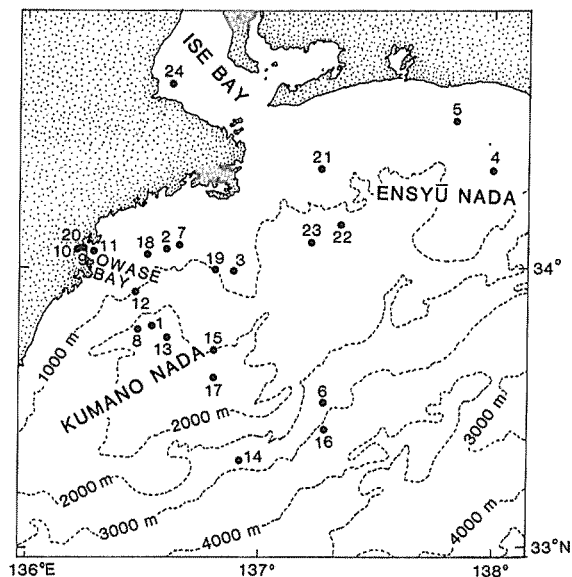


Fig. 1. Location of sampling stations in the Kumano Nada and its adjacent areas.

#### Enumeration of bacterial number

Viable counts of aerobic heterotrophic bacteria were determined by the spread plate method and the M. P. N. method. The composition of medium for culturing the heterotrophic bacteria is given as Medium I and Medium II of Table 1. All plates and M.

Table 1. Composition of media used in this study

Medium I (for heterotrophs, nitrate-reducers and denitrifiers)		Medium II (for heterotrophs)	
Polypepton (Daigo)	5.0 g	Polypepton (Daigo)	10.0 g
Yeast extract (Nakarai)	2.0 g	Yeast extract (Nakarai)	5.0 g
KNO <sub>3</sub>	1.0 g	Starch	2.0 g
Artificial seawater	1,000 ml	<i>Micrococcus luteus</i> cells	1.0 g
		Agar	15.0 g
		Tap water	100 ml
		Aged seawater	900 ml
Medium III (for nitrifiers)		Medium IV (for nitrogen fixers)	
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	30 mg	Sucrose	20.0 g
KH <sub>2</sub> PO <sub>4</sub>	100 mg	Glucose	10.0 g
EDTA-Fe	6 mg	KH <sub>2</sub> PO <sub>4</sub>	0.2 g
CaCO <sub>3</sub>	little	Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.01 g
Artificial seawater	1,000 ml	FeSO <sub>4</sub> ·7H <sub>2</sub> O	0.001 g
		MnSO <sub>4</sub>	0.001 g
		Artificial seawater	1,000 ml

Artificial seawater; NaCl 30.0 g, KCl 0.7 g, MgCl<sub>2</sub>·6H<sub>2</sub>O 10.8 g, MgSO<sub>4</sub>·7H<sub>2</sub>O 5.4 g, CaCl<sub>2</sub>·2H<sub>2</sub>O 1.0 g, Distilled water 1,000 ml.

P. N. tubes inoculated were incubated at 25°C for 3–14 days.

Nitrate-reducing bacteria, denitrifying bacteria, nitrifying bacteria and nitrogen-fixing bacteria were counted by the M. P. N. method. The composition of each medium for culturing these nitrogen-cycle bacteria is shown in Table 1. Cultivation was done at 25 °C for 2 weeks for nitrate-reducing bacteria and denitrifying bacteria, and for at least 2 months for nitrifying bacteria and nitrogen-fixing bacteria.

### Results and Discussion

#### Distribution of heterotrophic bacteria in the water

Table 2. Distribution of heterotrophic bacteria in the water of the Kumano Nada and its adjacent areas

Depth (m)	Temp. (°C)	Salinity (‰)	Heterotrophs (cfu/ml)	Depth (m)	Temp. (°C)	Salinity (‰)	Heterotrophs (cfu/ml)
Station 1 (33°47.2'N, 136°32.1'E)				Station 2 (34°03.9'N, 136°35.8'E)			
May 6, 1981; station depth 2,050 m.				May 8, 1981; station depth 497 m.			
0	18.9	—	3.5×10 <sup>2</sup>	0	20.44	—	4.2×10 <sup>2</sup>
200	11.664	34.431	2.3×10 <sup>2</sup>	50	17.697	34.652	4.5×10 <sup>2</sup>
400	6.954	34.230	4.0×10 <sup>2</sup>	100	15.649	34.633	6.4×10 <sup>2</sup>
600	5.348	34.293	4.4×10 <sup>2</sup>	150	13.717	34.544	4.4×10 <sup>2</sup>
800	4.197	34.356	5.0×10	200	12.198	34.469	1.3×10 <sup>2</sup>
1,000	3.319	34.440	5.0×10	250	10.884	34.406	1.4×10 <sup>2</sup>
1,200	2.934	34.480	2.3×10 <sup>2</sup>	300	8.879	34.323	8.7×10 <sup>2</sup>
1,400	2.555	34.524	1.6×10 <sup>2</sup>	350	7.713	34.278	3.7×10 <sup>2</sup>
1,600	2.332	34.552	1.6×10 <sup>2</sup>	400	7.103	34.283	2.7×10 <sup>2</sup>
1,800	2.202	34.572	7.0×10	450	6.375	34.284	1.2×10 <sup>2</sup>
2,000	2.006	34.601	1.0×10 <sup>2</sup>	470	5.665	34.299	3.7×10 <sup>2</sup>
Station 3 (33°59.5'N, 136°52.9'E)				Station 4 (34°20.5'N, 137°59.6'E)			
May 9, 1981; station depth 1,010 m.				Sept. 17, 1981; station depth 579 m.			
0	19.7	—	3.4×10 <sup>2</sup>	0	23.5	—	1.2×10 <sup>3</sup>
100	15.111	34.615	4.8×10 <sup>2</sup>	50	20.844	34.434	1.3×10 <sup>2</sup>
200	11.824	34.468	3.8×10 <sup>2</sup>	100	16.056	34.595	1.6×10 <sup>2</sup>
300	9.702	34.350	2.4×10 <sup>2</sup>	150	13.455	34.521	1.1×10 <sup>2</sup>
400	7.759	34.286	1.5×10 <sup>2</sup>	200	12.538	34.475	8.0×10
500	6.404	34.270	5.0×10	250	11.651	34.438	1.8×10 <sup>2</sup>
800	4.208	34.363	4.5×10 <sup>2</sup>	300	9.995	34.360	8.0×10
1,000	3.237	34.449	5.0×10	350	9.248	34.329	7.0×10
				400	7.628	34.272	1.0×10
				450	6.629	34.272	7.0×10
				550	5.270	34.305	5.0×10
				575	5.159	34.242	0
Station 5 (34°30.9'N, 137°49.7'E)				Station 6 (33°31.3'N, 137°16.3'E)			
Sept. 18, 1981; station depth 468 m.				Nov. 6, 1981; station depth 3,150 m.			
0	23.4	—	1.1×10 <sup>3</sup>	0	22.5	—	1.1×10 <sup>3</sup>
4	23.438	34.187	1.9×10 <sup>2</sup>	100	21.887	34.592	1.0×10 <sup>2</sup>
50	23.129	34.246	1.4×10 <sup>3</sup>	200	12.917	34.495	3.9×10 <sup>2</sup>

100	17.297	34.568	$1.7 \times 10^2$	300	9.832	34.327	$7.8 \times 10^2$
125	16.273	34.584	$4.6 \times 10^2$	400	7.091	34.276	$1.2 \times 10^3$
150	14.420	34.556	$2.9 \times 10^2$	500	5.035	34.265	$3.7 \times 10^2$
200	12.504	34.478	$2.0 \times 10$	600	4.386	34.328	$1.6 \times 10^2$
250	10.339	34.378	$2.0 \times 10$	800	3.556	34.415	$2.8 \times 10^2$
300	9.443	34.336	$2.7 \times 10^2$	1,000	3.119	34.458	$1.3 \times 10^2$
350	7.610	34.278	$1.7 \times 10^2$	1,500	2.315	34.553	$9.0 \times 10^2$
400	6.558	34.273	$1.0 \times 10^2$	2,000	1.979	34.601	$7.0 \times 10$
450	6.139	34.283	$1.3 \times 10^2$	2,500	1.684	34.643	$1.2 \times 10^2$
454	6.077	34.285	$3.7 \times 10^2$				

## Station 7 (34°04.7'N, 136°39.1'E)

Nov. 7, 1981; station depth 564 m.

0	22.3	—	$4.6 \times 10^2$
25	22.323	34.290	$1.0 \times 10$
50	22.362	34.379	$2.4 \times 10^2$
100	19.347	34.830	$9.0 \times 10$
150	16.781	34.743	$1.1 \times 10^3$
200	15.238	34.627	$1.7 \times 10^2$
250	13.922	34.543	$1.8 \times 10^2$
300	12.529	34.455	$3.5 \times 10^2$
350	10.245	34.351	$3.9 \times 10^2$
400	8.808	34.240	$2.9 \times 10^2$
500	7.060	34.199	$1.5 \times 10^2$
550	6.161	34.211	$1.9 \times 10^2$

## Station 8 (33°46.3'N, 136°28.9'E)

Apr. 25, 1982; station depth 2,050 m.

0	19.4	—	$2.2 \times 10^3$
100	16.240	34.633	$8.0 \times 10$
200	12.492	34.478	$2.5 \times 10^3$
400	7.106	34.225	$1.3 \times 10^3$
600	5.166	34.270	$2.0 \times 10^2$
800	4.474	34.312	$9.5 \times 10^2$
1,000	3.551	34.398	$3.1 \times 10^2$
1,200	3.034	34.456	$1.2 \times 10^3$
1,400	2.662	34.503	$4.6 \times 10^3$
1,600	2.385	34.539	$1.2 \times 10^3$
1,800	2.196	34.564	$1.2 \times 10^2$
2,000	1.986	34.594	$2.1 \times 10^2$

## Station 9 (34°04.1'N, 136°13.1'E)

Apr. 24, 1982; Owase Bay.

0	18.2	—	$2.1 \times 10^3$
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## Station 10 (34°04.4'N, 136°14.3'E)

Apr. 24, 1982; Owase Bay.

0	16.5	—	$1.6 \times 10^3$
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## Station 11 (34°03.9'N, 136°16.7'E)

Apr. 24, 1982; Owase Bay.

0	16.1	—	$< 1.0 \times 10^2$
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## Station 12 (33°54.6'N, 136°28.0'E)

Apr. 24, 1982

0	18.3	—	$< 1.0 \times 10$
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## Station 14 (33°18.3'N, 136°55.3'E)

May 22, 1982; station depth 2,850 m.

0	20.0	—	$6.0 \times 10^2$
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## Station 20 (34°04.3'N, 136°13.4'E) Owase Bay.

Apr. 25, 1983; station depth 25 m.

0	—	—	$6.4 \times 10^4$
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## Station 13 (33°44.8'N, 136°36.3'E)

May 21, 1982; station depth 2,030 m.

0	20.4	—	$2.6 \times 10^2$
100	15.636	34.645	$1.4 \times 10^2$
200	11.927	34.435	$1.1 \times 10^4$
400	6.670	34.269	$7.1 \times 10^2$
600	4.405	34.305	$1.2 \times 10^3$
800	3.712	34.387	$1.2 \times 10^3$
1,000	3.295	34.432	$3.8 \times 10^2$
1,200	2.893	34.479	$1.2 \times 10^2$
1,400	2.514	34.524	$3.0 \times 10^2$
1,600	2.313	34.551	$8.2 \times 10^2$
1,800	2.070	34.585	$1.1 \times 10^3$
2,000	1.941	34.602	$7.5 \times 10^2$

## Station 15 (33°42.1'N, 136°48.3'E)

Sept. 9, 1982; station depth 2,030 m.

0	25.7	—	$9.0 \times 10$
50	—	—	$3.0 \times 10^2$
100	—	—	$1.5 \times 10^2$
200	—	—	$1.6 \times 10^3$
400	—	—	$< 1.0 \times 10$
600	—	—	$1.0 \times 10$
800	—	—	$9.1 \times 10^2$
1,000	—	—	$8.1 \times 10^2$
1,200	—	—	$3.1 \times 10^3$
1,400	—	—	$2.1 \times 10^3$
1,600	—	—	$3.0 \times 10$
1,800	—	—	$1.0 \times 10$
2,000	—	—	$1.0 \times 10$

<b>Station 16 (33°25.3' N, 137°17.2' E)</b>				<b>Station 17 (33°36.3' N, 136°48.1' E)</b>			
Apr. 24, 1983; station depth 3,170m.				Apr. 24, 1983; station depth 2,060 m.			
0	17.6	—	$2.0 \times 10^2$	0	18.1	—	$2.8 \times 10^2$
100	14.267	34.505	$3.0 \times 10$	50	16.167	34.549	$1.0 \times 10^2$
200	10.053	34.285	$1.0 \times 10$	100	14.205	34.509	$2.5 \times 10$
400	6.046	34.183	$8.5 \times 10$	200	9.825	34.286	$5.5 \times 10$
600	4.289	34.257	$1.5 \times 10$	400	6.156	34.185	$1.0 \times 10^2$
800	3.516	34.331	$8.5 \times 10$	600	4.576	34.232	$3.5 \times 10$
1,000	3.120	34.376	$8.5 \times 10$	800	3.659	34.315	$1.0 \times 10$
1,200	2.836	34.407	$2.5 \times 10$	1,000	3.159	34.372	$8.5 \times 10$
1,600	2.355	34.468	$1.5 \times 10$	1,200	2.832	34.410	$5.5 \times 10$
1,800	2.150	34.496	$< 1.0 \times 10$	1,400	2.557	34.445	$1.0 \times 10$
2,000	1.994	34.518	$4.0 \times 10$	1,600	2.333	34.474	$< 1.0 \times 10$
3,000	1.557	34.579	$7.0 \times 10$	1,800	2.214	34.492	$5.0 \times 10$
				2,000	2.061	34.513	$1.0 \times 10$
<b>Station 18 (34°02.4' N, 136°31.4' E)</b>				<b>Station 21 (34°21.0' N, 137°15.0' E)</b>			
Apr. 25, 1983; station depth 549 m.				May 22, 1983; station depth 155 m.			
0	17.5	—	$6.7 \times 10^2$	0	20.6	—	$1.0 \times 10^2$
25	16.405	34.356	$7.0 \times 10$	10	20.263	34.252	$1.7 \times 10^2$
50	15.850	34.498	$1.0 \times 10$	30	19.241	34.403	$2.3 \times 10^2$
100	13.951	34.486	$1.0 \times 10$	40	19.229	34.403	$2.4 \times 10^2$
150	12.841	34.415	$2.0 \times 10$	50	18.899	34.420	$1.4 \times 10^2$
200	10.238	34.277	$1.0 \times 10$	60	18.486	34.434	$3.1 \times 10^2$
250	8.816	34.222	$< 1.0 \times 10$	70	16.693	34.527	$1.2 \times 10^2$
300	7.809	34.198	$2.0 \times 10$	80	16.203	34.542	$2.2 \times 10^2$
350	7.261	34.189	$8.0 \times 10$	90	15.961	34.548	$1.6 \times 10^2$
400	6.538	34.178	$4.0 \times 10$	100	15.649	34.547	$2.1 \times 10^2$
450	5.960	34.184	$5.0 \times 10$	145	14.113	34.331	$1.1 \times 10^2$
500	5.389	34.194	$7.0 \times 10$				
540	5.126	34.203	$5.0 \times 10$				
<b>Station 19 (33°59.9' N, 136°48.1' E)</b>				<b>Station 24 (34°38.2' N, 136°37.1' E) Ise Bay.</b>			
Apr. 25, 1983; station depth 1,045 m.				May 23, 1983; station depth 22.0 m.			
0	18.6	—	$2.4 \times 10^3$	0	20.9	—	$5.2 \times 10^3$
100	14.785	34.553	$7.0 \times 10$				
200	10.961	34.324	$1.0 \times 10$				
400	6.357	34.185	$2.0 \times 10$				
600	4.633	34.236	$1.0 \times 10$				
800	3.886	34.295	$< 1.0 \times 10$				
1,000	3.482	34.338	$< 1.0 \times 10$				
<b>Station 22 (34°08.9' N, 137°20.7' E)</b>				<b>Station 23 (34°05.4' N, 137°13.2' E)</b>			
May 22, 1983; station depth 1,285 m.				May 23, 1983; station depth 1,770 m.			
0	21.1	—	$6.6 \times 10^2$	0	20.9	—	$5.5 \times 10$
100	15.520	34.500	$1.6 \times 10^2$	100	15.433	34.518	$1.2 \times 10^2$
200	11.622	34.357	$1.5 \times 10$	200	11.136	34.323	$3.5 \times 10$
300	9.978	34.271	$4.0 \times 10$	300	9.698	34.270	5.0
400	7.923	34.192	$5.5 \times 10$	400	7.586	34.183	$1.5 \times 10$
500	6.512	34.178	$3.0 \times 10$	500	6.126	34.175	$5.0 \times 10$
600	5.311	34.197	$< 1.0 \times 10$	600	5.198	34.198	$2.5 \times 10$
700	4.668	34.230	$< 1.0 \times 10$	800	4.110	34.273	$< 1.0 \times 10$

800	4.279	34.260	$5.0 \times 10$	1,000	3.449	34.339	$< 1.0 \times 10$
900	3.838	34.297	$< 1.0 \times 10$	1,200	3.015	34.390	$1.0 \times 10$
1,000	3.544	34.327	$1.0 \times 10$	1,400	2.661	34.432	$< 1.0 \times 10$
1,100	3.301	34.354	$< 1.0 \times 10$	1,600	2.376	34.469	$1.0 \times 10$
1,250	2.966	34.394	$3.0 \times 10$	1,736	2.240	34.483	$< 1.0 \times 10^2$

Table 2 shows the number of heterotrophic bacteria counted by the spread plate method. Heterotrophic bacteria in the water usually occurred at the level of  $10^0 - 10^3$  cfu/ml, except for  $6.4 \times 10^4$  cfu/ml in Owase Bay (station 20). Although the maximum bacterial number was observed in the surface water at each station, the vertical distribution of heterotrophic bacteria had no distinct tendency.

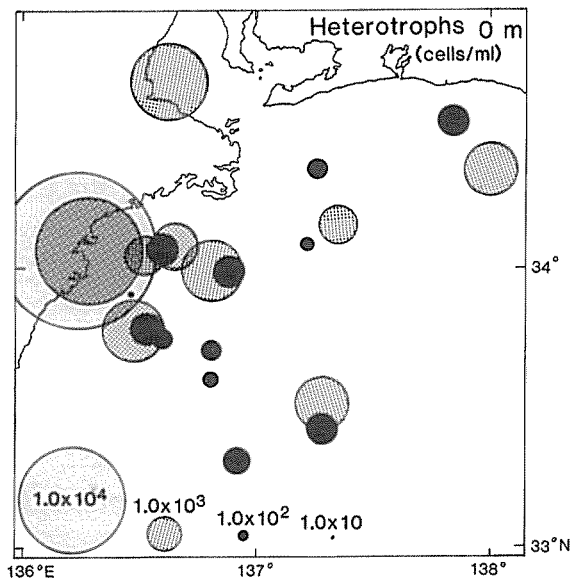


Fig. 2. Distribution of heterotrophic bacteria in the surface water of the Kumano Nada and its adjacent areas.

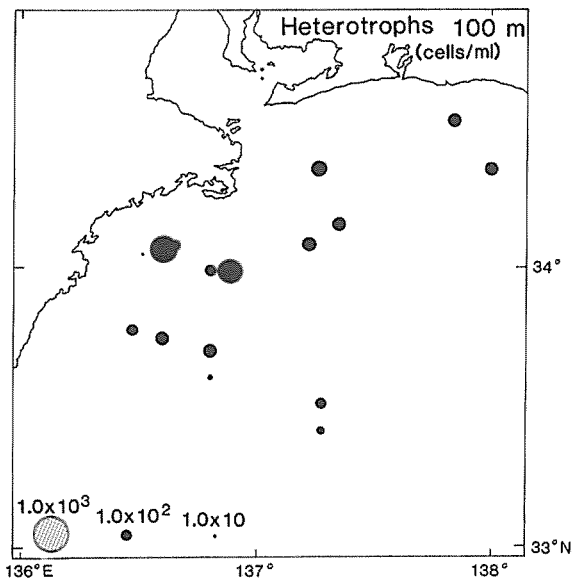


Fig. 3. Distribution of heterotrophic bacteria in the water at the depth of 100 m.

Figs. 2-5 show the distribution of heterotrophic bacteria enumerated by the M. P. N. method. The results in Fig. 2 indicated that heterotrophic bacteria in the surface water were generally in the order of  $10^1 - 10^3$  cells/ml. High count ( $2.4 \times 10^4$  cells/ml) was detected in Owase Bay (station 20). As shown in Figs. 3, 4 and 5, the bacterial counts in the water were at most  $10^2$  cells/ml at 100 m layer and  $10^1 - 10^3$  cells/ml at 400 m layer, while the number ranged from  $10^0$  to  $10^4$  cells/ml at 200 m layer. The distribution of heterotrophic bacteria was uneven horizontally as well as vertically.

The heterotrophic bacteria in the water were only  $10^0 - 10^2$  cells/ml in offshore regions such as Suruga Bay and Sagami Bay, while the number was in the range from

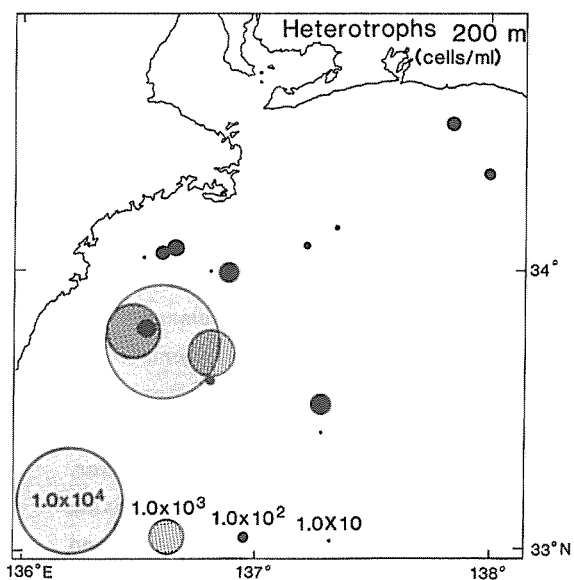


Fig. 4. Distribution of heterotrophic bacteria in the water at the depth of 200 m.

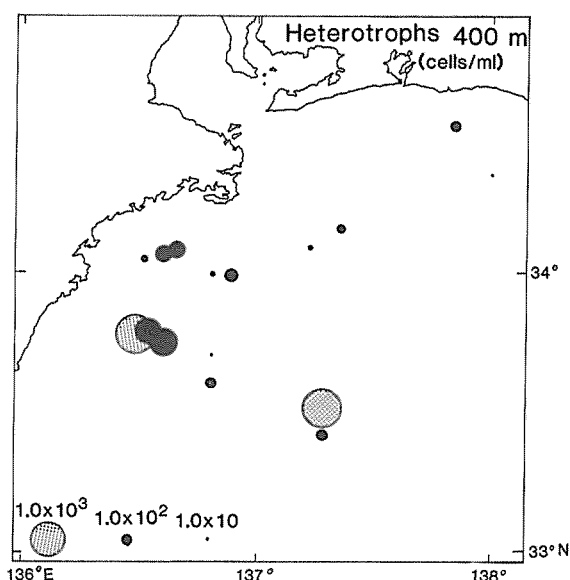


Fig. 5. Distribution of heterotrophic bacteria in the water at the depth of 400 m.

$10^2$  to  $10^4$  cells/ml in coastal regions such as Kumihama Bay, Maizuru Bay, Mikawa Bay, Fukuyama Bay and the Hibiki Nada (KAWAI and SUGAHARA 1971a, 1971b, MURAKAMI and FUJISAWA 1978, FUJISAWA *et al.* 1978, KAWAKAMI *et al.* 1981).

#### Distribution of nitrogen-cycle bacteria in the Kumano Nada and its adjacent areas

Table 3. Distribution of nitrogen-cycle bacteria in the surface water and bottom sediment of the Kumano Nada and its adjacent areas

Date	Station		Heterotrophs		Nitrate reducers cells/ml, g	Denitrifiers cells/ml, g	N <sub>2</sub> fixers cells/l, g	Nitrifiers cells/l, g
			cfu/ml, g	cells/ml, g				
1983.4.25	St. 20 (Owase Bay)	Water	$6.4 \times 10^4$	$2.4 \times 10^4$ (100.0%)	$1.4 \times 10^3$ (5.8%)	$2.0 \times 10^2$ (0.83%)	200 (0.0009%)	330 (0.0014%)
1983.5.22	St. 21	Water	$1.0 \times 10^2$	$3.3 \times 10^2$ (100.0%)	$1.3 \times 10$ (3.9%)	2.0 (0.61%)	170 (0.0515%)	4.5 (0.0014%)
1983.5.22	St. 22	Water	$6.6 \times 10^2$	$1.3 \times 10^3$ (100.0%)	$1.3 \times 10^2$ (10.0%)	$4.9 \times 10$ (3.8%)	81 (0.0062%)	240 (0.0185%)
1983.5.23	St. 23	Water	$5.5 \times 10$	$1.3 \times 10^2$ (100.0%)	3.3 (2.5%)	<0.2 (<0.15%)	27 (0.0208%)	7.8 (0.0060%)
1983.5.23	St. 24 (Ise Bay)	Water	$5.3 \times 10^3$	$4.9 \times 10^3$ (100.0%)	$1.2 \times 10^2$ (2.4%)	< $2.0 \times 10$ (<0.41%)	23 (0.0005%)	<20 (<0.0004%)
	Average			100.0%	4.9%	1.7%	0.016%	0.007%
1983.4.24	St. 16(3270m) (Kumano Nada)	Sediment	$2.1 \times 10^4$	$2.4 \times 10^4$ (100.0%)	$1.5 \times 10^3$ (6.3%)	$1.2 \times 10^3$ (5.0%)	$2.1 \times 10$ (0.09%)	$1.7 \times 10$ (0.07%)

Values in parentheses show the ratio (%) of the number of each of nitrogen-cycle bacteria to that of heterotrophs.

As shown in Table 3, in the surface water of bays such as Owase Bay and Ise Bay, aerobic heterotrophic bacteria were abundant, i.e.  $10^3$ – $10^4$  cells/ml as compared with those of offshore regions (stations 21, 22 and 23), i.e.  $10^1$ – $10^3$  cells/ml. Nitrate-reducing bacteria in the surface water occurred at the level of  $10^0$ – $10^3$  cells/ml, whereas denitrifying bacteria were in the range from  $10^{-1}$  to  $10^2$  cells/ml or sometimes lower than the value. However, nitrogen-fixing bacteria and nitrifying bacteria in the surface water were very few ranging from  $10^0$  to  $10^2$  cells/l.

On the other hand, the number of aerobic heterotrophic bacteria in the bottom sediment (0–5 cm layer) collected at station 16 (3,270 m in depth) of the Kumano Nada was in the order of  $10^4$  cells per 1 g of wet sediment. Nitrogen-fixing bacteria and nitrifying bacteria were in the order of 10 cells/g, while nitrate-reducing bacteria and denitrifying bacteria occurred at the level of  $10^3$  cells/g. Nitrogen-fixing bacteria and nitrifying bacteria occurred more abundantly in the bottom sediment than in the water.

According to KAWAI and SUGAHARA (1971a, 1971b), the population of nitrogen-fixing bacteria in the water was  $10^1$ – $10^2$  cells/ml in enclosed bays such as Maizuru Bay and Kumihama Bay in Kyoto Prefecture, while the number was quite small in offshore waters in Suruga Bay and Sagami Bay, i.e. 0–800 cells/l. In the open sea such as the East China Sea, the Japan Sea and the Mid-Pacific Ocean, nitrogen-fixing bacteria were at most 400 cells/l. However, nitrogen-fixing bacteria occurred abundantly in the bottom sediments, namely 1–10 cells per 1 g of wet mud in offshore regions and open sea such as Suruga Bay, Sagami Bay and the East China Sea.

KIMATA *et al.* (1963) reported that nitrifying bacteria in the seawater of Maizuru Bay were hardly found, i.e. ca. 1 cell/ml or less and the bacterial counts in the bottom sediments were very large i.e.  $10^2$ – $10^5$  cells/g. YOSHIDA and KIMATA (1967) also demonstrated that the counts of nitrifying bacteria were very low, i.e.  $10^0$ – $10^2$  cells/l and ca.  $10^1$  cells/g or less in Wakasa Bay and its offshore region.

The results in Table 3 were comparable to the data of KAWAI and SUGAHARA (1971a, 1971b), KIMATA *et al.* (1963) and YOSHIDA and KIMATA (1967).

Vertical distribution of nitrate-reducing bacteria and denitrifying bacteria in the water column

Table 4. Vertical distribution of nitrate-reducing bacteria and denitrifying bacteria in the water of the Kumano Nada and its adjacent areas

Station	Depth (m)	Heterotrophs (cells/ml)	Nitrate reducers (cells/ml)	Denitrifiers (cells/ml)
Station 6 (Nov. 6, 1981)	0	$2.2 \times 10^3$	$4.9 \times 10$ (2.2 %)	22 (1.0 %)
	100	$1.7 \times 10$	$1.3 \times 10$ (76.5 %)	1.3 (7.6 %)
	200	$> 1.6 \times 10^2$ ( $3.9 \times 10^2$ )*	$2.8 \times 10$ (7.2 %)	2.6 (0.67%)
	300	$> 1.6 \times 10^2$ ( $7.8 \times 10^2$ )*	$5.4 \times 10$ (6.9 %)	0.93 (0.12%)
	400	$> 1.6 \times 10^2$ ( $1.2 \times 10^3$ )*	$> 1.6 \times 10^2$	2.8 (0.23%)
	500	$> 1.6 \times 10^2$ ( $3.7 \times 10^2$ )*	$9.2 \times 10$ (24.9 %)	4.7 (1.3 %)



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800	$> 1.6 \times 10^2$ ( $2.8 \times 10^2$ )*	$1.6 \times 10^2$ (57.1 %)	22 (7.9 %)
1,000	$1.6 \times 10^2$	$5.4 \times 10$ (33.8 %)	5.6 (3.5 %)
1,500	$> 1.6 \times 10^2$ ( $9.0 \times 10^2$ )*	$9.2 \times 10$ (10.2 %)	3.4 (0.38%)
2,000	$> 1.6 \times 10^2$	$1.1 \times 10$	11

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Station 8 (Apr. 25, 1982)	0	$3.5 \times 10^3$	$3.3 \times 10^2$ (9.4 %)	330 (9.4 %)
	100	$1.4 \times 10^2$	$6.4 \times 10$ (45.7 %)	<0.2
	200	$> 1.6 \times 10^3$ ( $2.5 \times 10^3$ )*	$3.3 \times 10$ (1.3 %)	0.2 (0.01%)
	600	$3.5 \times 10^2$	$4.9 \times 10$ (14.0 %)	4.8 (1.4 %)
	1,000	$> 1.6 \times 10^3$	$7.0 \times 10$	0.4-4.0
	1,600	$> 1.6 \times 10^3$	$4.6 \times 10$	0.4
	2,000	$3.5 \times 10^2$	$3.5 \times 10^2$ (100.0 %)	2.0 (0.57%)

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Station 13 (May 21, 1982)	0	$3.3 \times 10^2$	$4.9 \times 10$ (14.8 %)	22 (6.7 %)
	100	$4.0 \times 10$	$1.3 \times 10$ (32.5 %)	0.4 (1.0 %)
	200	$1.8 \times 10^4$	$6.4 \times 10$ (0.36%)	24 (0.13%)
	400	$1.3 \times 10^3$	$1.7 \times 10$ (1.3 %)	1.1 (0.08%)
	600	$7.9 \times 10^2$	$1.4 \times 10$ (1.8 %)	0.78 (0.10%)
	800	$2.2 \times 10^3$	$1.1 \times 10^3$ (50.0 %)	47 (2.1 %)
	1,000	$3.5 \times 10^3$	4.0 (0.11%)	2.4 (0.07%)
	1,200	$7.0 \times 10^2$	$7.9 \times 10$ (11.3 %)	33 (4.7 %)
	1,400	$3.3 \times 10^2$	$2.1 \times 10$ (6.4 %)	1.1 (0.33%)
	1,600	$9.2 \times 10^3$	$2.4 \times 10$ (0.26%)	13 (0.14%)
	1,800	$2.4 \times 10^3$	$1.3 \times 10^2$ (5.4 %)	130 (5.4 %)
2,000	$1.7 \times 10^3$	$4.9 \times 10$ (2.9 %)	33 (1.9 %)	

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Station 15 (Sept. 9, 1982)	0	$3.3 \times 10^2$	$2.6 \times 10$ (7.9 %)	1.8 (0.55%)
	50	$1.1 \times 10^2$	4.5 (4.1 %)	<2.0
	100	$2.2 \times 10^2$	$3.3 \times 10$ (15.0 %)	0.18 (0.08%)
	200	$1.8 \times 10^3$	$7.9 \times 10$ (4.4 %)	49 (2.7 %)
	400	3.3	3.3 (100.0 %)	<0.2
	600	$1.3 \times 10$	2.3 (17.7 %)	<0.2
	800	$2.4 \times 10^3$	$3.1 \times 10$ (1.3 %)	2.2 (0.09%)
	1,000	$9.5 \times 10^2$	$2.2 \times 10$ (2.3 %)	<0.2
	1,200	$1.6 \times 10^4$	$4.9 \times 10^2$ (3.1 %)	2.1 (0.01%)
	1,400	$3.5 \times 10^3$	$4.9 \times 10^2$ (14.0 %)	490 (14.0 %)
	1,600	$1.3 \times 10^2$	2.2 (1.7 %)	0.18 (0.14%)
	1,800	1.7	1.7 (100.0 %)	<0.2
2,000	7.9	0.45 (5.7 %)	<0.2	

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Station 16 (Apr. 24, 1983)	0	$7.9 \times 10^2$	$1.4 \times 10^2$ (17.7 %)	2.0 (0.25%)
	100	$3.3 \times 10$	4.9 (14.8 %)	<0.2
	200	$7.9 \times 10$	$1.1 \times 10$ (13.9 %)	<0.2
	400	$3.3 \times 10$	$1.1 \times 10$ (33.3 %)	0.45 (1.4 %)
	600	7.9	2.7 (34.2 %)	0.2 (2.5 %)
	800	$2.8 \times 10$	$1.1 \times 10$ (39.3 %)	<0.2
	1,000	$1.1 \times 10$	$1.1 \times 10$ (100.0 %)	<0.2
	1,200	$1.7 \times 10$	$1.1 \times 10$ (64.7 %)	<0.2
	1,600	4.9	4.9 (100.0 %)	<0.2
	1,800	1.7	1.7 (100.0 %)	<0.2
	2,000	5.6	1.3 (23.2 %)	<0.2
3,000	$1.1 \times 10^2$	$2.6 \times 10$ (23.6 %)	<0.2	

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Station 17 (Apr. 24, 1983)	0	$2.2 \times 10^2$	$7.9 \times 10$	(35.9 %)	4.5	(2.0 %)
	1,000	$7.9 \times 10$	$2.2 \times 10$	(27.8 %)	0.45	(0.57%)
	2,000	4.6	3.3	(71.7 %)	<0.2	
Station 23 (May 23, 1983)	0	$1.3 \times 10^2$	3.3	(2.5 %)	<0.2	
	100	$7.9 \times 10$	$1.7 \times 10$	(21.5 %)	<0.2	
	200	$1.3 \times 10^2$	4.9	(3.8 %)	<0.2	
	300	$3.3 \times 10$	7.9	(23.9 %)	<0.2	
	400	$1.7 \times 10$	3.3	(19.4 %)	<0.2	
	500	$9.2 \times 10$	4.9	(5.3 %)	<0.2	
	600	$4.9 \times 10$	$1.3 \times 10$	(26.5 %)	2.3	(4.7 %)
	800	$2.3 \times 10$	4.9	(21.3 %)	<0.2	
	1,000	3.1	0.78	(25.2 %)	<0.2	
	1,200	$1.3 \times 10$	1.4	(10.8 %)	<0.2	
	1,400	4.9	0.78	(15.9 %)	<0.2	
1,600	$1.1 \times 10$	4.6	(41.8 %)	<0.2		
1,750	$1.3 \times 10^2$	$2.0 \times 10$	(15.4 %)	<20		

\* Heterotrophs ; values in parentheses show bacterial number counted by the spread plate method. Nitrate reducers and denitrifiers ; values in parentheses show the ratio (%) of the number of nitrate reducers or denitrifiers to that of heterotrophs.

The results in Table 4 indicated that the population of nitrate-reducing bacteria ranged from  $10^{-1}$  to  $10^3$  cells/ml, while the occurrence of denitrifying bacteria was very poor, i. e.  $10^{-1}$  -  $10^2$  cells/ml or less. The ratio of the number of nitrate-reducing bacteria or denitrifying bacteria to that of heterotrophic bacteria varied considerably with the depths of water column. However, there was no distinct tendency of the vertical distribution of nitrate-reducing bacteria and denitrifying bacteria; The depth of water column showing maximum bacterial occurrence and maximum ratio varied from one station to another. Relatively high counts ( $10^2$  celles/ml) of denitrifying bacteria were observed at stations 8, 13 and 15, respectively.

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