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Bacterial Abundance and Production in July 1997 in the vicinity of Tokdo, East Sea

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Participating in a multi-disciplinary oceanography program in July 1997 in the vicinity of Tokdo, we studied the distributions of bacterial abundance and production along with those of phytoplankton and heterotrophic nanoflagellates. In the euphotic zone, chlorophyll *a* (chl *a*) concentrations ranged from 0.14 to 0.52 μg l^{-1}. Bacterial abundance in the euphotic zone (0.12—0.21×10^9 cells l^{-1}) in the study area was quite lower than that expected from the observed chl *a* concentration in the marine environment. The low bacterial abundance seemed to be due to active grazing pressure on bacteria. The fraction of primary production utilized by bacteria was also low (8—12%). Interestingly, surface water temperatures were lower at stations near islands compared to an offshore station located between Ulleungdo and Tokdo and the highest values of bacterial production and chl *a* were found at stations near islands, strongly indicating island mass effects.

INTRODUCTION

Many data on the distribution, activity and interactions of bacteria and other microbes have been accumulated from coastal waters surrounding the Korean Peninsula (Cho and Shim, 1992; Park *et al.*, 1993; Shim *et al.*, 1993, 1994, 1995a, 1995b; Cho *et al.*, 1994a, 1994b, 1996; Choi *et al.*, 1997). However, researches on marine microbial ecology are rare in the offshore regions. Recently, Tokdo has received much greater attentions than before due to increased national interests. In spite of the significance of Tokdo as a resource of tourism and bases of fishery and various marine researches, studies on aquatic sys tems near the island have been virtually non-existent.

A multi-disciplinary oceanography program has started in 1997 to understand physical, chemical and biological oceanographic processes in the vicinity of Tokdo and to use the results for the environmental conservation of the area. As a part of the program, we studied relationships between bacteria, phytoplankton and heterotrophic nanoflagellates and asked what environmental factors were important in regulating microbial interactions and variations of microbial parameters.

MATERIALS AND METHODS

Study area and sample collection

Three stations between Ulleungdo and Tokdo and one station (Stn 8) off Tokdo (Fig. 1) were occupied in July 29—31, 1997. Stn M was occupied in July 26, 1996. We employed the data from Stn M to compare with the data from the Tokdo area. Water samples were obtained with Niskin bottles at 3—5 depth intervals in the surface layer.

Bacterial abundance and production

Samples were fixed with 0.2 μm filtered neutrally buffered formalin (final conc. of 2%). Bacterial abundance was measured by epifluorescence microscopy after staining with DAPI (Porter and Feig, 1980). Bacterial production was measured according to the method of Duclow *et al.* (1992). [^{3}H] thymidine (^{3}H-TdR; Amersham Inc., specific activity = 84 Ci $mmol^{-1}$) was added to 10 ml water to give a final conc. of 10nM. The samples were incubated in the dark at *in situ* temperature for 1 h. The incorporated radioactivity was converted to the number of cells produced using a conversion factor of 2.65×10^{18} cells mol^{-1} of TdR incorporated into DNA (Duclow *et al.*, 1992). Bacterial biomass was calculated by using 20 fg C $bacterium^{-1}$ (Lee and Fuhrman, 1987). Bacterial biomass carbon was compared with phytoplankton biomass carbon assuming a C:chl *a* ratio of 50, and the use of the conversion factor has been known not to bias the analysis

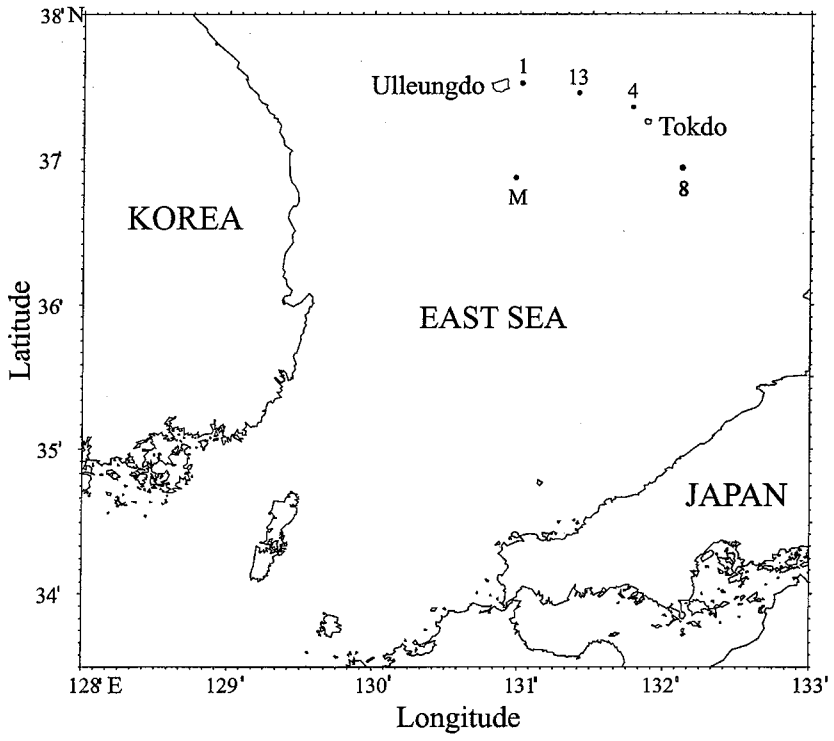

Fig. 1. A map showing sampling stations in the vicinity of Tokdo, East Sea.

(Simon *et al.* 1992). Bacterial doubling time was calculated by dividing bacterial abundance with the bacterial production rate.

Heterotrophic nanoflagellates (HNF) abundance

Samples were fixed with both Lugol solution and 3% formalin, and were kept refrigerated. HNF abundance was measured by epifluorescence microscopy after staining with primulin (Caron, 1983).

Other analytical methods

Chl *a* concentrations and rates of primary production were measured according to the method of Parsons *et al.* (1984). Water temperature and salinity were measured with a CTD system (SBE 25, Seabird). Transparency of waters was measured with a Secchi disk. Linear regression analysis was performed using a "Minitab" statistical software package (Minitab Inc., PA, USA).

RESULTS

The water column was stratified and the thermoclines were found at *ca.* 10—15 m depth at four stations (Fig. 2) in July 1997. Water column at Stn M in July 1996 also showed stratification with a thermocline at *ca.* 15 m depth (Fig. 2). Surface water temperature and salinity at the five stations varied within narrow ranges. Surface water temperature ranged from 23.2 to 24.3°C in Tokdo area, but it was somewhat lower at Stn M (21.2°C). Below thermocline, water temperature decreased almost linearly to 3.5—10.8°C at 100 m depth. At Stn 8, water temperature at 100 m depth was much higher than the other 4 stations. Surface salinity ranged from 33.3 to 33.8‰ at the five stations. Below thermocline, salinity varied a little (Fig. 2). The depth of the euphotic zone ranged from 30 m at Stn 1 to 54 m at Stn 13 (Fig. 3).

Chl *a* concentrations in the euphotic zone ranged from 0.14 to 0.52 μg l^{-1} in Tokdo area, and chl *a* concentrations at Stn M were within the above range (Fig. 3). Two highest chl *a* concentrations were found at Stns 1 and 4 near islands. Subsurface chl *a* maxima were found below the euphotic zone at Stns 1 and 4, and within the euphotic zone at Stns 8 and 13. At Stn M, the location of subsurface chl *a* maximum was hard to tell due to the limited number of samples for the depth profile. Substantial chl *a* concentrations (0.07—0.19 μg l^{-1}) were found at 100 m depth in Tokdo area.

Bacterial abundance in the euphotic zone varied from 0.12 to 0.21 $\times 10^9$ cells l^{-1} and showed the < 2 fold vertical variation in Tokdo area (Fig. 3). At Stn

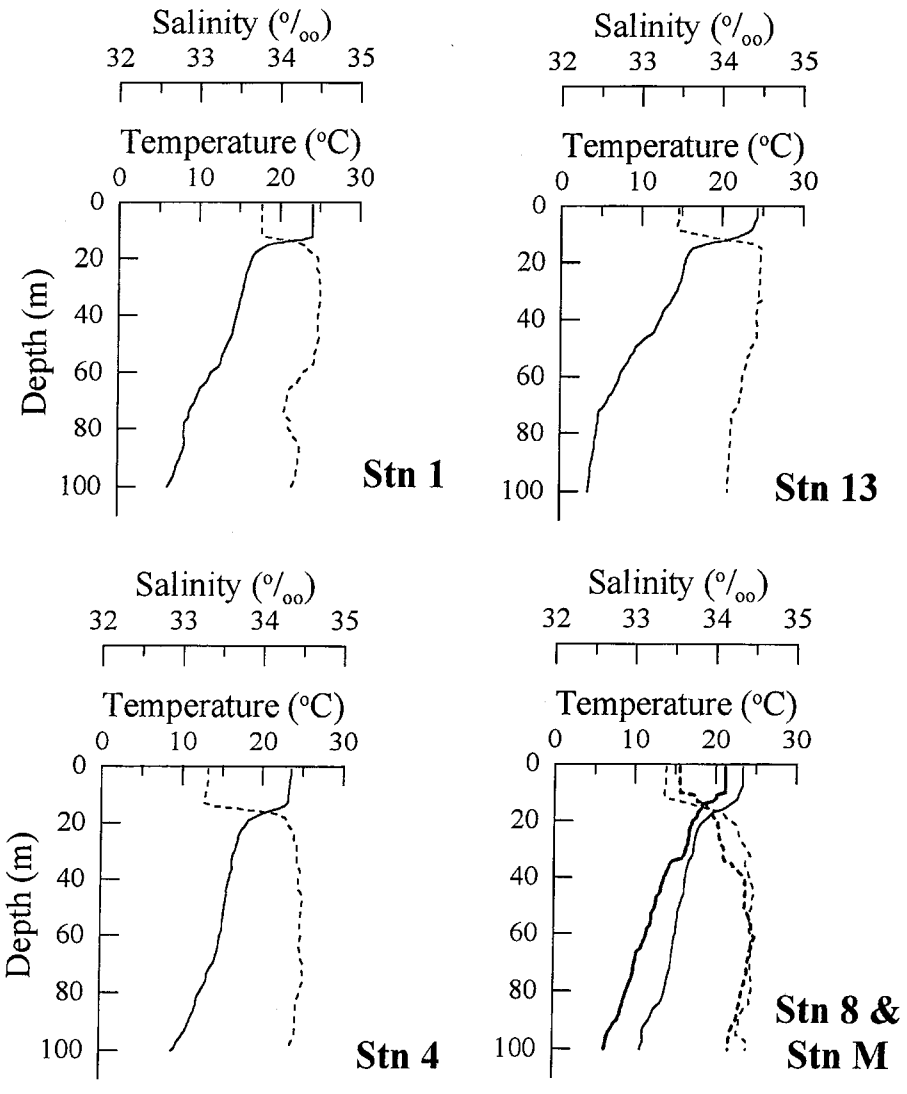

Fig. 2. Depth profiles of temperature (solid line) and salinity (dashed line) in surface layers in the vicinity of Tokdo in July 1997 and at Stn M in July 1996 (lines in bold).

M, however, bacterial abundance in the euphotic zone was at least 4.6 fold higher (0.97 to 1.24×10^9 cells l^{-1}) than that in Tokdo area (Fig. 3). Bacterial abundance in the surface layer showed significant positive correlations with water temperature and bacterial production in Tokdo area (Table 1). However, when data of Stn M were included, bacterial abundance was insignificantly ($p > 0.05$) correlated with water temperature and bacterial production in surface layer (data not shown). Bacterial abundance in the surface layer showed a significant positive correlation with chl *a* concentration in Tokdo area (Table 1). However, when data from Stn M were included, bacterial abundance showed insignificant ($p > 0.05$) correlation with chl *a* concentration in the surface layer (data not shown).

Bacterial production ranged from 0.8 to 7.9×10^6 cells l^{-1} h^{-1} in the euphotic zone at five stations (Fig. 3). High values (7.9×10^6 and 10.1×10^6 cells l^{-1} h^{-1}) of bacterial production, like chl *a*, were found at Stns 1 and 4 near islands. At Stn 1, subsurface maximum of bacterial production (10.1×10^6 cells l^{-1} h^{-1}) was found below the euphotic zone. The depth-integrated bacterial production (36—46 mg C m^{-2} d^{-1}) over the euphotic zone comprised a small fraction (8—12%) of primary production (377—481 mg C m^{-2} d^{-1}) in the study area, including Stn M (Table 2).

HNF abundance in the surface layer varied from 0.39 to 0.64×10^6 cells l^{-1} in Tokdo area and showed vertically < 2 fold variations (Fig. 3). At Stn M, HNF abundance in the surface layer was higher ($1.2—1.9 \times 10^6$ cells l^{-1}; Fig. 3) than that observed in Tokdo area. HNF abundance apparently covaried with bacterial abundance, but correlation between two variables was insignificant. HNF abundance in the surface layer significantly correlated

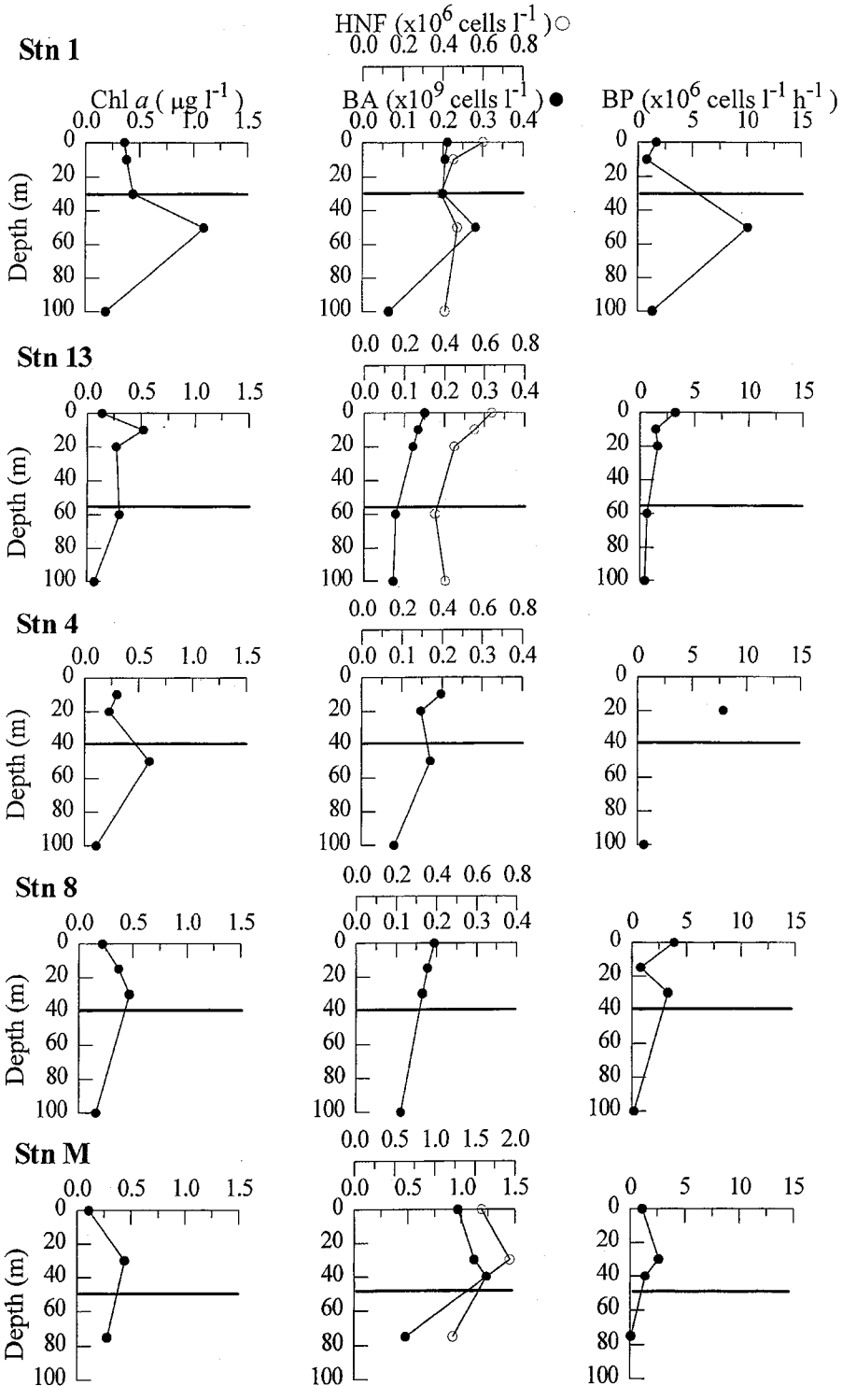

Fig. 3. Depth profiles of chlorophyll *a* (Chl *a*), bacterial abundance (BA), heterotrophic nanoflagellate (HNF) abundance and bacterial production (BP) in surface layer in the vincinity of Tokdo in July 1997 and at Stn M in July 1996. Lines parallel to the *x*-axis indicate the bottom of the euphotic zone.

only with water temperature (Table 1). However, when data from Stn M were included, HNF abundance showed significant positive correlations with bacterial abundance and bacterial turnover time, but an insignificant correlation with water temperature in the surface layer (data not shown).

DISCUSSION

Surface water was warm (23.2—24.3°C), and stratification was well developed within the euphotic zone in the vicinity of Tokdo in July 1997. Chl *a* concentrations in the vicinity of Tokdo (0.14—0.52

Table 1. Correlation coefficients of the relationships between parameters observed in the surface layers in the vicinity of Tokdo

	Temperature (°C)	Chlorophyll *a* ($\mu g\ l^{-1}$)	Bacterial abundance ($\times 10^9\ l^{-1}$)	Bacterial production ($\times 10^6\ l^{-1}\ h^{-1}$)	Turnover time (day)	Heterotrophic nanoflagellate ($\times 10^6\ l^{-1}$)
Temperature (°C)	1.000					
Chlorophyll *a* ($\mu g\ l^{-1}$)	0.382	1.000				
Bacterial abundance ($\times 10^9\ l^{-1}$)	0.756[3]	0.702[3]	1.000			
Bacterial production ($\times 10^6\ l^{-1}\ h^{-1}$)	0.413	0.502	0.584[1]	1.000		
Turnover time (day)	−0.116	−0.263	−0.213	−0.918[3]	1.000	
Heterotrophic nanoflagellate ($\times 10^6\ l^{-1}$)	0.787[2]	0.082	0.463	0.502	−0.224	1.000

[1]$p<0.05$, [2]$p<0.01$, [3]$p<0.001$

$\mu g\ l^{-1}$) in July 1997 were somewhat lower than the earlier summer observations (mean of 0.52 $\mu g\ l^{-1}$; Kang and Kang, 1992). Chl *a* concentrations during the study period indicated that the area was close to oligotrophic, although surface chl *a* concentrations (0.30—0.36 $\mu g\ l^{-1}$) at stations near islands seemed to be rather high. Typical chl *a* concentrations in the oligotrophic Pacific Ocean in summer range from < 0.1 to 0.4 $\mu g\ l^{-1}$ in the euphotic zone and surface chl *a* concentrations of ≥ 0.2 $\mu g\ l^{-1}$ have been rarely found (Eppley *et al.*, 1988).

A notable result from our study was that bacterial abundance was strikingly low ($0.12—0.21 \times 10^9\ l^{-1}$) in the euphotic zone. Even in the oligotrophic Pacific gyre, bacterial abundance range from *ca.* 0.3 to $1.0 \times 10^9\ l^{-1}$ in the euphotic zone (Cho and Azam, 1990). The low bacterial abundance in Tokdo area is close to the lower limit ($0.09—0.23 \times 10^9\ l^{-1}$) reported for oligotrophic areas (Cho, 1991). A comparison of the bacterial carbon (BOC) vs. phytoplankton carbon (phyto-C) relationship of Simon *et al.* (1992) with our data (Fig. 4A) shows that bacterial abundance in the vicinity of Tokdo is almost one order of magnitude lower than the expected abundance from chl *a* concentration for the marine environment. A comparison of the data with bacterial abundances from Stn M, only *ca.* 80 km from Ulleungdo, indicates unusually low bacterial abundance in Tokdo area in July 1997. A study done in the Tokdo area in summer 1991 (Lee, 1997) reported that bacterial abundances in the euphotic zone were usually $1 \times 10^9\ l^{-1}$ and occasionally (4 out of 12 samples within 50 m) low ($1 \times 10^8\ l^{-1}$). It seems that bacterial abundance in the vicinity of Tokdo in summer is highly vulnerable to grazing pressures and thus kept low (see below).

Is the unexpectedly low abundance of bacteria due to an unusually slow growth of bacteria in the area? To answer the question, we compared our plot of bacterial abundance and bacterial production with the relationship of White *et al.* (1991) (Fig. 4B). In the surface layer, most values of bacterial production were either within the expected range or above the upper limit of the expected range, indicating that bacterial production was compatible to the observed bacterial abundance or more active than expected from bacterial abundance. Thus, the low abundance of bacteria in the study area seems to be related to active consumption of bacteria by grazers. In fact, HNF abundance in surface layer in Tokdo area was higher than that expected from the relationship of Sanders *et al.* (1992), especially when bacterial abundance was lowest (Fig. 4C). Further study on the growth physiology of HNF in low bacterial abundance in the study area is to be open.

The observed ratios (8—12%) of depth-integrated bacterial production to primary production are low, though within the reported range from the marine environment (Cole *et al.*, 1988). Values of primary production in Tokdo area (mean of 405 mg C m^{-2} d^{-1}) were close to the earlier measurements of 350 mg C m^{-2} d^{-1} around Tokdo in June (Kang and Kang, 1992), and somewhat lower than 456 mg C m^{-2} d^{-1} observed in the oligotrophic Pacific gyre (Cho and Azam, 1988). Thus, the low ratios of depth-integrated bacterial production to primary production suggest that mechanisms of dissolved organic matter like exudation and sloppy feeding (Azam and Cho, 1987) are not working well, utilization efficiency of dissolved organic matter by bacteria is low, or bacterial growth might be limited by nutrient(s), causing low bacterial production (Carlson *et al.*, 1998).

Interestingly, the highest values of bacterial production and chl *a* during the study were observed below the euphotic zone at stations near islands. These observations suggest that enhanced production of organic matter occurred sporadically in the euphotic zone and was subsequently utilized by

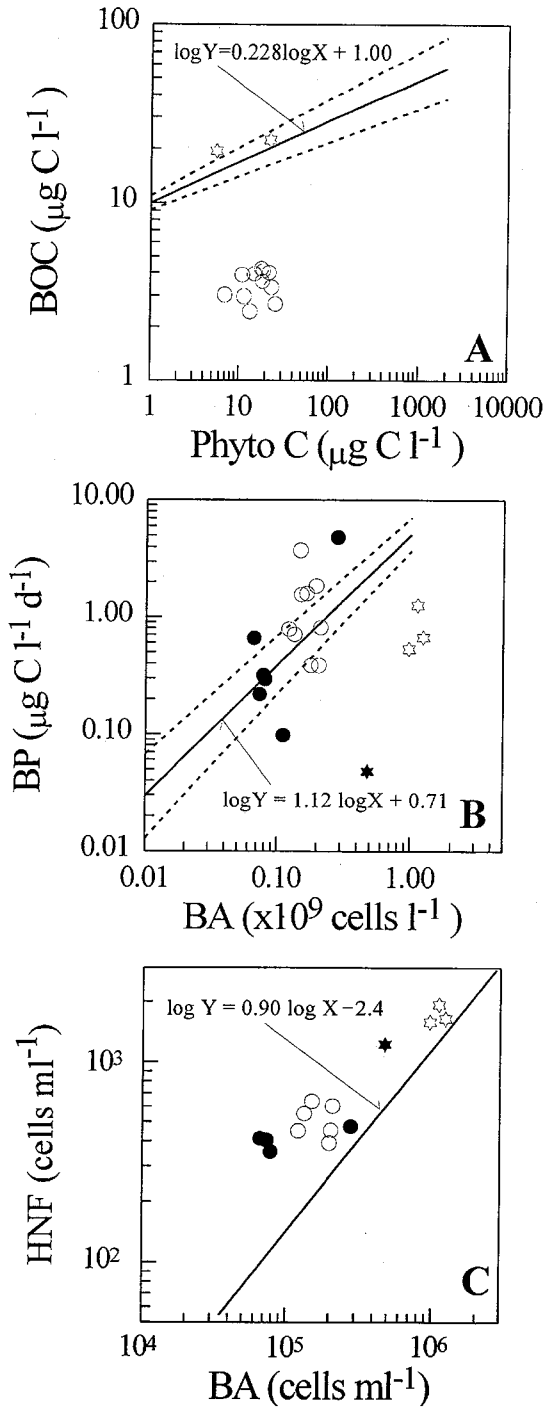

Fig. 4. (**A**) Plots of phytoplankton vs. bacterial biomass carbon in the euphotic zone (circles: Tokdo area; stars: Stn M). A regression line and 95% confidential limits for marine environments from Simon *et al.* (1992) were shown. (**B**) Comparison of measured bacterial production (BP) with expected BP based on bacterial abundance (BA) according to White *et al.* (1991). Dotted lines represent upper and lower limits for the expected BP, and solid line the relationship. Closed symbols are data from the aphotic zone. (**C**) Comparison of the plot of observed heterotrophic nanoflagellate (HNF) abundance vs. bacterial abundance with the relationship of Sanders *et al.* (1992).

Table 2. Depth-integrated bacterial production (BP) and primary production (PP) over the euphotic zone, and the ratio of BP to PP in the vicinity of Tokdo and Stn M

	Stn 1	Stn 8	Stn 13	Stn M
BP (mg C m^{-2} d^{-1})	36	46	38	42
PP (mg C m^{-2} d^{-1})	377	384	455	481
BP/PP (%)	10	12	8	9

bacteria after being exported from the euphotic zone. Thus, near islands, sporadic events of nutrient supply to phytoplankton production in the euphotic zone would be expected. In fact, surface water temperature of 24.0°C at Stn 1 near Ulleungdo was 0.3°C lower than that at Stn 13 (between Ulleungdo and Tokdo). Also, surface water temperature at Stn 4 (23.2°C) near Tokdo was 0.2—1.1°C lower than those at Stns 13 and 8. Thus, distribution of surface water temperature also strongly indicates the island mass effects, presumably on a small scale. To confirm the island mass effects, such as dynamic variations of Ulleung warm eddy (Shin *et al.*, 1995) near Tokdo, on distributions of microbial activity, further detailed studies on physical and chemical oceanographic processes are needed.

In conclusion, the very low abundance of bacteria and the relatively high abundance of HNF compared to the observed bacterial abundance in the vicinity of Tokdo suggest an interesting type of bacteria-HNF interaction. Further investigations in the study area will provide insights into prime oceanographic processes regulating microbial interactions and conservation of the pristine marine environment.

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