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Bacterial abundance and production and heterotrophic nanoflagellate abundance in subarctic coastal waters (Western North Pacific Ocean)

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ABSTRACT: We studied the temporal variations of bacterial abundance and production in subarctic Funka Bay (Hokkaido, Japan) for a 1 yr period, and related this data to chlorophyll *a* (chl *a*) concentration (highest conc. = 5.9 µg l^{-1}), particulate organic carbon (POC) concentration (ranging from 60 to 360 µg C l^{-1}), heterotrophic nanoflagellate (HNF) abundance (<0.5 to 5.6 × 10^3 cells ml^{-1}), and temperature. Bacterial abundance ranged from 2.6 to 9.1 × 10^5 cells ml^{-1}, whereas bacterial production ranged from 0.1 to 22.9 µg C l^{-1} d^{-1}. The calculated bacterial growth rate ranged from 0.02 to 2.57 d^{-1}, with an average turnover time of 1.6 d, and 'attached' bacteria (>1 µm fraction) contributed more than half the total bacterial production (mean ± 95% CL = 54 ± 12%). Bacterial abundance was relatively stable throughout the year (coefficient of variation, CV = 20%) even though the CV for bacterial production was high (CV = 100%). Grazing (top-down) is an important control mechanism in Funka Bay, and HNF seemed to be directly cropping bacterial production. Annual bacterial production was estimated at 140 g C m^{-2} yr^{-1}, and was relatively high compared to the annual primary production (100 to 170 g C m^{-2} yr^{-1}), indicating the importance of the microbial loop in Funka Bay. After bacterial respiration loss was accounted for, bacterial carbon demand was higher than primary production. The inflow of Tsugaru warm water, a branch of the Kuroshio Current brought organic matter, but the concentration of POC and the timing of the inflow suggested that this organic matter addition was insignificant, and we concluded that other sources, including riverine input, are more important sources of organic matter.

KEY WORDS: Bacterial production · Funka Bay · Heterotrophic nanoflagellate · Particulate organic matter · Tsugaru warm water

INTRODUCTION

Bacteria are responsible for much of the carbon and nutrient cycling in aquatic systems (e.g. Ducklow & Carlson 1992, Simon et al. 1992). Bacteria act as an important link between detritus, dissolved organic matter and higher trophic organisms, especially heterotrophic nanoflagellates (HNF), which are the major consumers of bacteria (Sherr & Sherr 1984). Currently, we know that substrate supply, grazing, temperature and viruses are the principal factors that control both bacterial abundance and production (Sherr & Sherr 1984, Fuhrman & Noble 1995, Felip et al. 1996). However, among these factors, the most critical one differs at different locations and at different periods of time (Shiah & Ducklow 1994, Goosen et al. 1997, Ferrier-Pagès & Gattuso 1998).

This study aimed at determining the influence of bacteria on the carbon budget of Funka Bay. Funka Bay is located in the subarctic region, southwest of

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Hokkaido, Japan; it covers an area of about 2300 km^2 and has a maximum depth of about 100 m (see Fig. 1). The ecology of both micro- and net-plankton have been well studied in this bay (Odate 1992), but the role played by bacteria has remained uncertain. This study had 2 goals: (1) to measure the bacterial production rates in this region for the first time, and (2) to address the potential mechanisms of control for bacterial abundance and production.

We investigated the temporal variations of both bacterial abundance and production in a 1 yr study, and related this to both chlorophyll *a* concentration and HNF abundance. Since typically more than half the phytoplankton productivity is in the form of particulate (rather than dissolved) organic matter (Baines & Pace 1991), attached bacteria may play a significant role in the breakdown of particulate matter. Although free-swimming bacteria (or bacterioplankton) may be able to graze on particulate substrates without becoming firmly attached (Kefford et al. 1982), it is generally assumed that detritus is degraded primarily by bacteria attached to the particulate material (Hoppe 1991). Our results indicated that more than half of the bacterial production could be attributed to 'attached' bacterial (>1 µm fraction) production. The estimated annual bacterial production was also high relative to annual primary production, suggesting the importance of the microbial loop in Funka Bay.

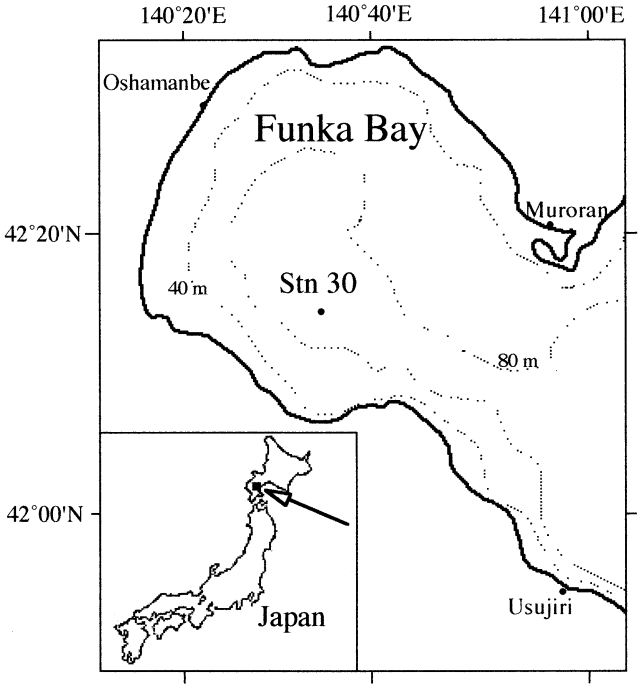

Fig. 1. Location of Sampling Stn 30 (42° 16.2′ N, 140° 36.0′ E) in Funka Bay, south-western Hokkaido, Japan. Isobaths for 40 and 80 m depths are delineated by dotted lines. Inset: location of Funka Bay in Japan

MATERIALS AND METHODS

Sampling was carried out on 9 occasions at Stn 30 (average depth of 92 m) in Funka Bay (42° 16.2′ N, 140° 36.0′ E) (Fig. 1) from February until December 1999. Seawater samples were collected at intervals of 10 m down to 90 m depth using 5 l Niskin samplers; samples 30 cm above the bottom were collected with a devised water sampler. Subsamples for the determination of microbial abundance were preserved with filtered (Nuclepore filter, 0.2 µm pore size) 1% glutaraldehyde (final conc.). The remaining samples were kept in a cooler box for no more than 5 h until processing in the laboratory. Pre-combusted (450°C for 5 h) Whatman GF/F filters (nominal pore size 0.7 µm) were also used to collect samples for both chlorophyll *a* (chl *a*) and particulate organic matter (POM) determinations, and were stored at –30°C until analysis.

The temperature-salinity profile at the time of sampling was obtained with a CTD profiler (SeaBird SBE 19); photosynthetically active radiation (PAR) and light attenuation were measured with an underwater quantum sensor (LI-193SB, Li-Cor). The 1% light-level depth ranged from 20 to 50 m, average (±95% confidence limits [CL]) = 32 ± 8 m. In this study, for analysis and discussion purposes, the euphotic layer was defined as the upper 30 m depth. Chl *a* was extracted with N, N-dimethylformamide (Suzuki & Ishimaru 1990), and measured with a spectrofluorometer (F2000, Hitachi). For POM analysis, filters were dried for 5 h in a freeze-dryer (FDU-830, Eyela) and then analysed with an elemental analyser (CHN-Corder MT-5, Yanaco).

Bacterial abundance was determined by epifluorescence microscopy on samples stained with 4′6-diamidino-2-phenylindole (DAPI) (0.1 µg l^{-1} final conc.) for 7 min (Kepner & Pratt 1994). More than 300 cells were counted for each sample. Some samples were duplicated (n = 50), and the CV ranged from 2 to 21%. Bacterial abundance was converted into biomass using the 'constrained' conversion factor of 15 fg C bacterium^{-1} (Caron et al. 1995). For nanoflagellates, a 20 ml sample was filtered onto a dyed 1.0 µm pore size Nuclepore filter, and then stained with the fluorochrome primulin, and nanoflagellates without chl *a* fluorescence were counted as HNF (Bloem et al. 1986). About 50 cells were counted for each sample. Several samples were duplicated (n = 12); CV ranged from 1 to 6%.

Bacterial production was measured using the radiolabelled thymidine (TdR) incorporation method (Fuhrman & Azam 1982). [Methyl-^{3}H]thymidine (final conc. = 10 nM; specific activity 3.1 to 3.3 TBq mmol^{-1}; New England Nuclear, USA) was added to triplicate 10 ml samples. The CV ranged from 2 to 42%; the average was 14%. Samples were incubated for 1 h in the dark

at the ambient temperature of the depth of collection (±2°C). Incubation was terminated by the addition of formaldehyde (10% final conc.). Bacteria were collected onto a 0.2 µm Millipore filter, and washed 5 times with 2 ml ice-cold 5% trichloroacetic acid. The filter was immediately placed into a vial filled with 8 ml liquid scintillation solution and subsequently assayed for TdR incorporation. All samples were corrected for abiotic incorporation by subtracting the radioactivity in a formaldehyde-killed control. TdR incorporation rates were converted to bacterial production using a theoretical conversion factor of 2×10^{18} cells mol^{-1} (Fuhrman & Azam 1982).

In this study, we determined the contribution of attached bacteria indirectly by subtracting the bacterial production in the <1 µm fraction from the total. We defined operationally that the TdR incorporation in the >1 µm fraction was due to bacteria attached to larger particles. Samples were filtered using 1.0 µm Nuclepore filters before incubating with TdR. The procedures for extraction and radioassay were as described above. The grazing potential of HNF was also estimated in August and December. The change in bacterial abundance for both the <10 and <0.7 µm fractions (10 and 90 m depth samples) was determined in a single 24 h culture, and the bacterial growth rate in each fraction was estimated. Bacterial activity was assumed the same for both the <10 and <0.7 µm fractions, and that bacteria grew exponentially during the incubation period (1 d). HNF grazing potential was then estimated as the difference between bacterial growth rates in the <0.7 µm fraction (assumed free of grazing), and in the <10 µm fraction which presumably represented the product of both growth and grazing (McManus 1993).

All data are reported as means ±95% confidence limits, and all other variables except for temperature were log-transformed to equalize variance (homoscedasticity) (Zar 1996). Bacterial growth rate (BGR) was calculated as bacterial production (BP) divided by abundance (BA), and error propagation analysis was carried out according to Day & Underwood (1980) using the following equation:

$$(\sigma^2_{BGR} / BGR) = (\sigma^2_{BP} / BP) + (\sigma^2_{BA} / BA)$$

RESULTS

From temperature-salinity profiles, 2 external water masses were observed in Funka Bay, the Oyashio water after March, and the Tsugaru warm water (a branch of the Kuroshio Current) after August (Fig. 2). The Oyashio water is important to the timing of the spring bloom (Kudo & Matsunaga 1999). Particulate organic carbon (POC) concentration ranged from 60 to 360 µg C l^{-1} (Fig. 3A), particulate organic nitrogen (PON) concentration from 10 to 60 µg N l^{-1} (Fig. 3B). The POC:PON ratio for the particulate organic matter ranged from 4 to 10 (Fig. 3C).

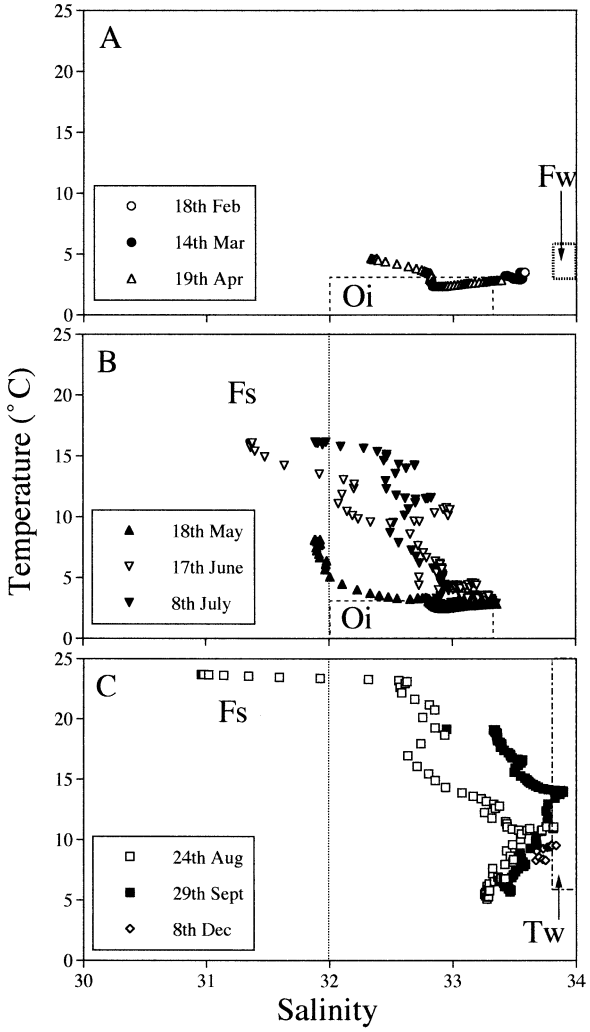

Fig. 2. Temperature-salinity profiles on each sampling occasion at Stn 30, Funka Bay. Temperature-salinity boundaries for the water masses are drawn according to Ohtani (1971). Fw: Funka Bay winter water (salinity >33.8, temperature >3°C < 6°C); Fs: Funka Bay summer water (salinity <32); Oi: Oyashio water (salinity >32.0 < 33.3, temperature <3°C); Tw: Tsugaru warm water (salinity >33.8, temperature >6°C)

The chl *a* concentration, a common indicator of total phytoplankton was highest in March (10 m depth) at 5.9 µg l^{-1} (Fig. 4A). When the spring phytoplankton bloom period (March and April data) was excluded, the mean chl *a* concentration was 0.7 ± 0.1 µg l^{-1} (0 to 30 m layer), 0.5 ± 0.2 µg l^{-1} (40 to 60 m layer), and 0.4 ± 0.2 µg l^{-1} (70 to 90 m layer). In this study, bacterial abundance was higher in the euphotic layer, and especially in March and September (>7×10^5 cells ml^{-1}) (Fig. 4B). Mean bacterial abundance in the 0 to 30 m, 40 to 60 m and 70 to 90 m layers was 6.3 ± 0.7 × 10^5,

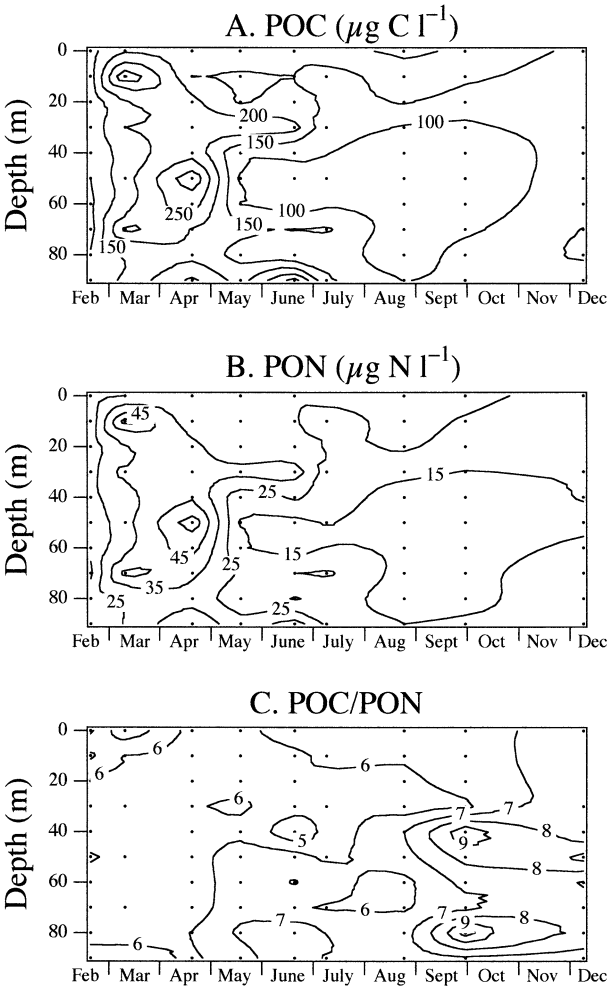

Fig. 3. Distribution of (A) particulate organic carbon (POC), (B) particulate organic nitrogen (PON) and (C) POC:PON ratio at Stn 30, Funka Bay

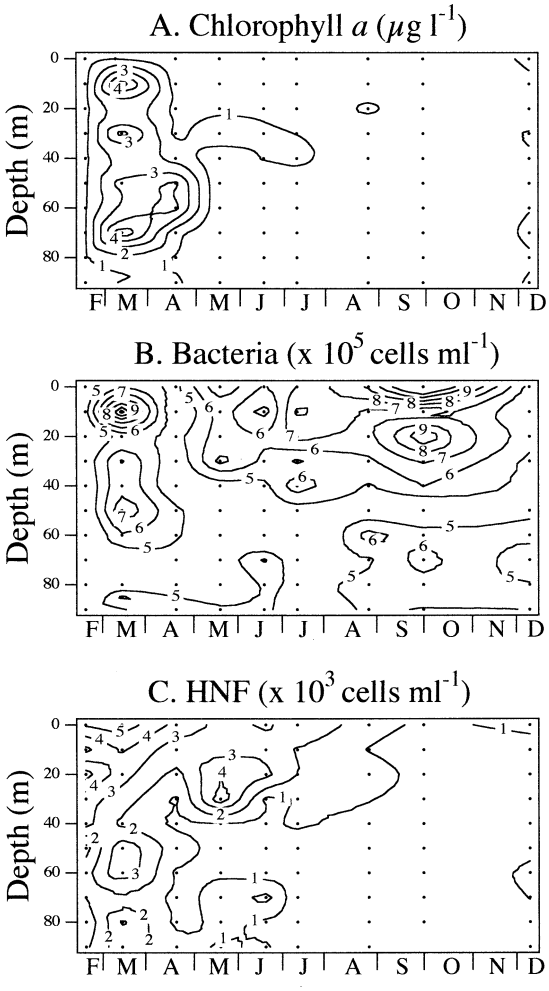

Fig. 4. Distribution of (A) chlorophyll *a*, (B) bacterial abundance, and (C) heterotrophic nanoflagellate (HNF) abundance at Stn 30, Funka Bay

$4.9 \pm 0.5 \times 10^5$, and $4.8 \pm 0.3 \times 10^5$ cells ml^{-1}, respectively. HNF abundance ranged from less than 0.5×10^3 to 5.6×10^3 cells ml^{-1}; the average was $1.6 \pm 0.3 \times 10^3$ cells ml^{-1} (Fig. 4C). HNF abundance decreased gradually throughout the water column towards winter.

In this study, the TdR incorporation rate ranged from 0.2 to 31.8 pM h^{-1} (Fig. 5), and bacterial production estimated from TdR incorporation ranged from 0.1 to 22.9 μg C l^{-1} d^{-1}. Bacterial production was higher in the euphotic layer, and was highest in March and lowest in December. Integrated bacterial production for the whole water column ranged from 0.04 to 0.79 g C m^{-2} d^{-1}. Bacterial growth rate calculated ranged from 0.02 to 2.57 d^{-1}; the average was 0.62 ± 0.54 d^{-1}. This meant that the mean turnover time or the reciprocal of growth rate for bacteria was 1.6 d.

The production rate of attached bacteria was also estimated (Table 1); they contributed 54 ± 12% to the total bacterial production. This is similar to the results of Kirchman & Mitchell (1982), who found that in some aquatic systems more than 40% substrate incorporation rate could be attributed to attached bacteria. The percentage attached bacterial production contributed to total bacterial production ranged from 30 to 70, 20 to 90 and 40 to 90% for the 10, 50 and 90 m depth samples, respectively. The HNF potential grazing rate in August was 100 cells HNF^{-1} h^{-1} at 10 m depth and 16 cells HNF^{-1} h^{-1} at 90 m depth, whereas in December the grazing rate at 10 m was lower (33 cells HNF^{-1} h^{-1}) and was undetectable at 90 m depth (Table 2). The HNF potential grazing rates obtained in this study were within the range reported by Weisse & Scheffel-Möser (1991), who used the size-fractionation method. Quantitatively, the use of this potential grazing rate is doubtful, as uncertainty due to the assumptions made remains. However, the comparison of these rates in

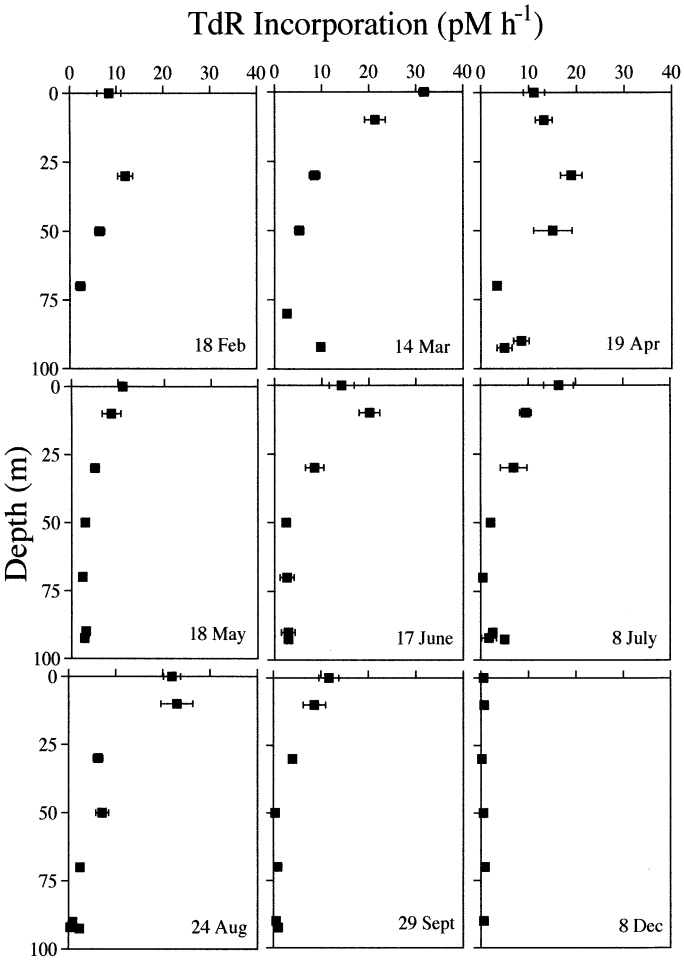

Fig. 5. Mean radio-labelled thymidine (TdR) incorporation rate at Stn 30, Funka Bay on each sampling occasion. Error bars (±SD) are shown, except where they were smaller than the symbols

August and December did suggest how grazing could affect bacterial abundance. When HNF grazing rate was high (100 cells HNF^{-1} h^{-1}), bacterial growth rate calculated from TdR incorporation method was also high (1.6 d^{-1}).

DISCUSSION

In Funka Bay, the spring phytoplankton bloom occurs every March or April, and chl *a* concentration at its peak is usually above 10 µg l^{-1} (Odate 1992, Kudo & Matsunaga 1999). In this study, the highest chl *a* concentration observed was 5.9 µg l^{-1}, and the peak in spring phytoplankton bloom could have occurred between the March and April samplings. After the bloom, chl *a* concentration remained below 1.3 µg l^{-1} throughout the rest of the sampling period. The POC and PON concentrations in this study were similar to those previously reported by Yanada & Maita (1978). In the euphotic layer, integrated chl *a* was closely correlated with POC ($r = 0.87$, $n = 9$, $p < 0.01$), and the POC concentration increase in March could be attributable to the increase in phytoplankton.

HNF abundance was within the range reported for various coastal areas (Fukami et al. 1996, Ferrier-Pagès & Gattuso 1998), and both bacterial abundance and bacterial production were similar to the range detected in the open ocean in the subarctic Pacific (Simon et al. 1992), in the Ise Bay, Japan (Naganuma 1997), and the Seto Inland Sea, Japan (Naganuma & Miura 1997). To discuss the possible mechanisms controlling bacterial abundance, we separated our data into 2 periods: (1) Early period, February until May, which covered both the spring bloom and post-spring bloom: there was a rapid change in chl *a* concentrations, and the monthly sampling strategy missed the peak in spring phytoplankton bloom; in view of this, sampling could at best provide 'snap shots' of the system at each date. (2) Late period, June until December, which covered the summer and early winter season: this period was relatively stable with respect to chl *a* concentrations,

Table 1. Contribution of attached bacteria to total bacterial production in Funka Bay. Attached bacterial production was calculated as the difference between the measured bacterial production in both the total and <1 µm fraction

Sample depth (m)	Bacterial production (mg C m^{-3} d^{-1})		% attached bacterial production contributed to total bacterial production
	Total	Attached bacteria	
19 Apr 1999			
10	9.48	5.41	57.1
90	6.06	4.65	76.7
18 May 1999			
10	6.16	3.06	49.7
50	2.08	0.48	23.1
17 Jun 1999			
10	14.47	8.67	59.9
50	1.74	0.61	35.1
90	2.05	1.38	67.3
8 Jul 1999			
10	6.79	1.08	15.9
50	1.47	0.79	53.7
90	1.81	0.68	37.6
24 Aug 1999			
10	16.55	4.97	36.1
50	5.11	4.34	84.9
90	0.60	0.56	93.3
29 Sep 1999			
10	6.19	1.59	25.7
8 Dec 1999			
10	0.47	0.32	68.1
50	0.37	0.32	86.5
90	0.46	0.21	45.7

Table 2. Estimated heterotrophic nanoflagellate (HNF) potential grazing rate in August and December. nd: not detectable

Depth (m)	Bacterial growth rate (h^{-1}) <10 µm fraction	<0.7 µm fraction	HNF abundance in <10 µm fraction ($\times 10^3$ cells ml^{-1})	HNF potential grazing rate (cells HNF^{-1} h^{-1})
24 Aug 1999				
10	0.029	0.055	0.30	100
90	0.003	0.012	0.30	16
8 Dec 1999				
10	0.023	0.059	0.40	33
90	0.019	0.013	0.30	nd

which remained at less than 1.3 µg l^{-1}, and except for rare storm events that upwelled nutrients from below the thermocline, we were able to evaluate the whole period.

From large-scale comparisons, Cole et al. (1988) found that bacterial abundance correlated with chl *a* concentration, and concluded that in all aquatic systems bacterial production is sustained ultimately by organic matter from primary producers. Our results were consistent with this general model. In this study, bacterial abundance correlated with both chl *a* (Fig. 6A, early period: r = 0.36, n = 35, $p < 0.05$; late period: r = 0.26, n = 49, $p < 0.05$) and POC concentrations for both periods (Fig. 6B, early period: r = 0.55, n = 35, $p < 0.001$; late period: r = 0.34, n = 42, $p < 0.05$). The dependence of bacterial production upon POM was also reflected by the high percentage of total bacterial production contributed by the >1 µm fraction in this study. However, dissolved organic matter (DOM) is also important, especially since high DOM concentration in Funka Bay (during the spring phytoplankton bloom and in the summer according to Yoshida et al. 1984) coincided with the high bacterial growth rates in this study.

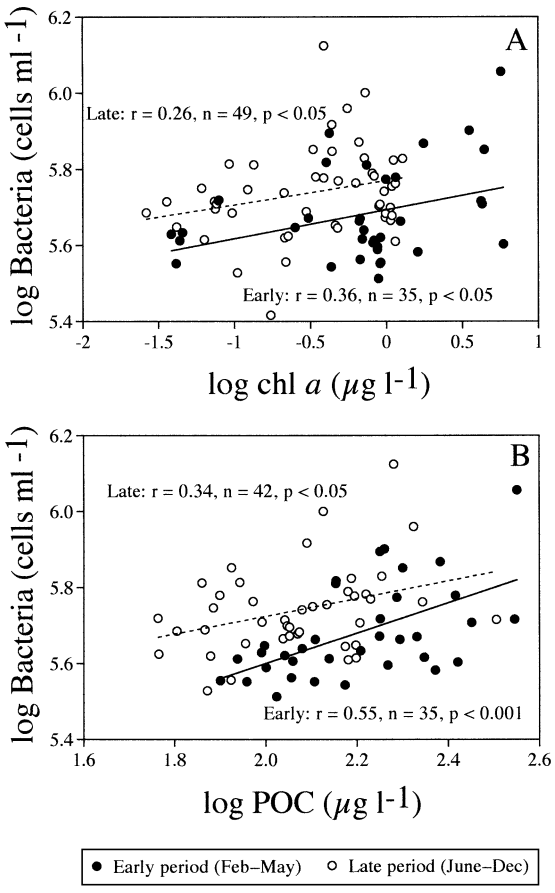

Fig. 6. Correlation analyses for both early (spring bloom and post-spring bloom) and late (summer and early winter) periods for (A) log bacterial abundance and log chlorophyll *a* (chl *a*) concentration, and (B) log bacterial abundance and log particulate organic carbon (POC) concentration

Bacterial abundance was relatively stable throughout the year (CV = 20%). This relative variability was low compared to that of both chl *a* (CV = 130%), and HNF (CV = 100%). Bacterial abundance was stable, even though the CV for bacterial production was high (CV = 100%). This suggests that grazing (top-down) is an important control mechanism in Funka Bay. One of the major bacterial predators is the HNF. In this study, the HNF seemed to be directly cropping bacterial production, as there were significant correlations between bacterial production and HNF abundance for both the early (r = 0.42, n = 20, $p < 0.05$), and late (r = 0.45, n = 28, $p < 0.01$) periods (Fig. 7A). Higher bacterial production from February until May supported higher HNF abundance, which regulated the bacterial abundance through grazing. Although lower bacterial production in the late period (June until December) supported lower HNF abundance, decreasing HNF abundance throughout the water column from summer to winter could also be attributable to grazing activities by micro- and net-zooplankton. Odate (1992) reported that the food requirements of these potential HNF predators are highest during this period.

Temperature has also been reported to be important, in affecting the bacterial growth rate, e.g., in Chesapeake Bay (Shiah & Ducklow 1994). In this study, bacterial growth rate (log h^{-1}) correlated with temperature (°C) only in the period from June until December (Fig. 7B: r = 0.51, n = 30, $p < 0.01$). As seawater temperatures increased in summer, bacterial growth rate increased nearly 3-fold for every 10°C rise in temperature (Q_{10} = 3.0). From February until May, changes in bacterial growth rates were independent of tempera-

ture (Fig. 7B: r = 0.11, n = 21, p > 0.25). In this period, the spring phytoplankton bloom occurred, and the changes in bacterial growth rates were probably connected with nutrients from the phytoplankton bloom. This is in agreement with the suggestion by Felip et al. (1996) that temperature has little effect on bacterial growth in resource-rich environments.

In this study, annual bacterial production was estimated at 140 g C m^{-2} yr^{-1}; this is high in relation to the annual primary production for Funka Bay (100 g C m^{-2} yr^{-1} for 1974 and 1975: Yanada & Maita 1978; 140 g C m^{-2} yr^{-1} for 1984 and 1985: Odate 1992; 170 g C m^{-2} yr^{-1} for 1999 and 2000: Kudo unpubl. data). This is higher than the annual mean ratio of bacterial to primary production of 20% for aquatic systems suggested by Ducklow (1999). To account for the bacterial respiration loss, we calculated bacterial carbon demand (BCD) according to the following equation: BCD = BP/BGE, where BGE is the bacterial growth efficiency. Assuming a BGE of 30% (del Giorgio & Cole 2000), the annual BCD would be 470 g C m^{-2} yr^{-1}, higher than annual primary production. This was true even after taking into account that 50% of primary production is released as DOM (Nagata 2000), giving a gross annual primary production of 340 g C m^{-2} yr^{-1}. Periods in which bacterial carbon demand was more than phytoplankton production have been previously reported for both estuarine and coastal ecosystems (Goosen et al. 1997, Amon & Benner 1998). Given the constraint that the amount of carbon available to bacteria from primary production was insufficient to balance the measured demand in this study, an additional source probably existed.

Possible sources of organic matter include terrigenous DOM (riverine input) (Opsahl & Benner 1997), and DOM from periods during which primary production is high relative to bacterial production ('uncoupling') (del Giorgio et al. 1997). Inflow of Tsugaru warm water into Funka Bay was first hypothesized by Yanada & Maita (1978) to bring in additional organic matter. As salinity increases during this period could be attributed to the inflow of Tsugaru warm water, correlation analyses were carried out between POC, phytoplankton biomass and salinity (Fig. 8). To avoid particulate organic matter from primary production in the euphotic layer from affecting the analyses, data for

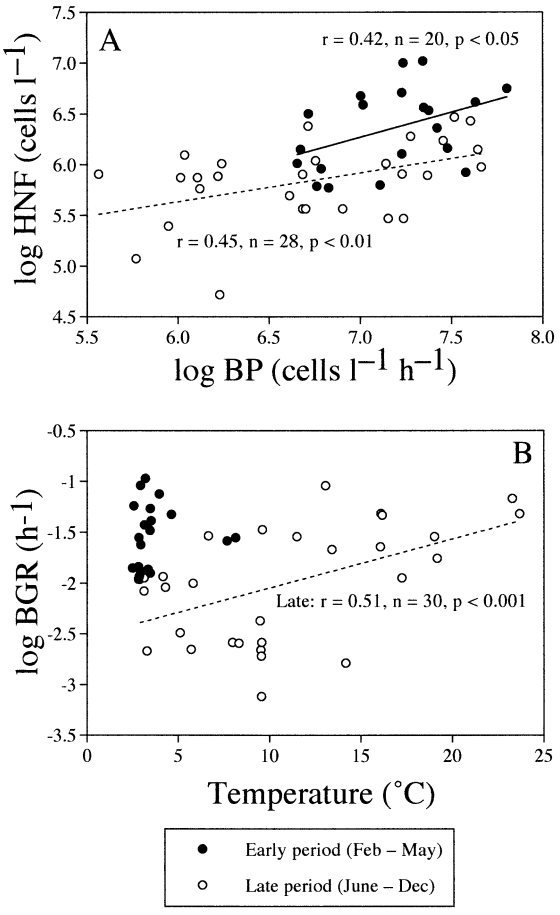

Fig. 7. Correlation analyses for both early and late periods for (A) log bacterial production (BP) and log heterotrophic nanoflagellate abundance (HNF), and (B) log bacterial growth rate (BGR) and seawater temperature

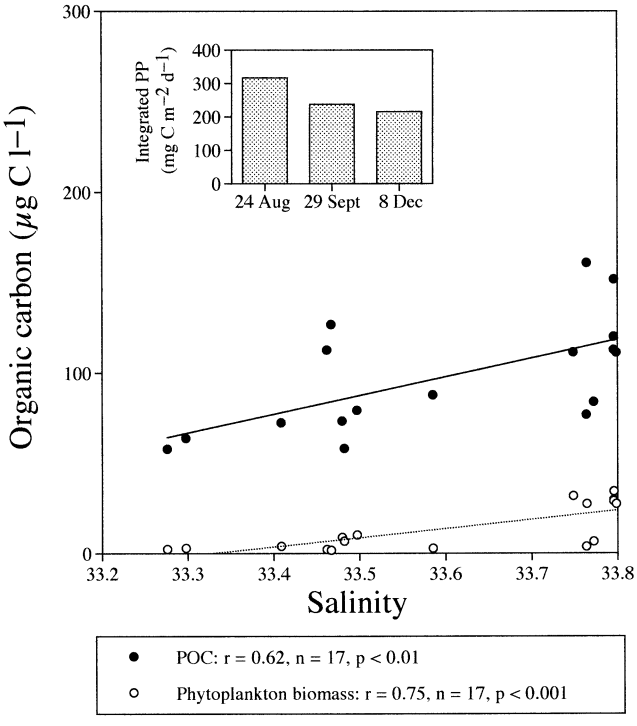

Fig. 8. Relationship between particulate organic carbon (POC) and salinity and between phytoplankton biomass and salinity. Data set from the 30 to 90 m layer from August until December. Inset: integrated primary production (PP) observed during same period (Kudo unpubl. data)

the correlation analyses were taken from below the euphotic layer from August until December. The results show that both POC and phytoplankton biomass correlated with salinity ($r = 0.62$, $p < 0.01$, $n = 17$ and $r = 0.75$, $p < 0.001$, $n = 17$, respectively). This source of organic matter (POC and phytoplankton biomass) could not be attributed to the primary production in the euphotic layer, as integrated primary production during this period did not increase (Kudo unpubl. data) (see inset in Fig. 8). In view of this, the inflow of Tsugaru warm water must have brought additional organic matter into Funka Bay. However, POC concentration differed between September and December samplings by about an average of 40 µg C l^{-1} (or only 4 g C m^{-2} in a 100 m water column). This is small compared to the estimated deficit of 130 g C m^{-2} yr^{-1}, and probably insignificant, as the Tsugaru warm water inflow also coincided with the winter season when bacterial production was low.

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