Differing Growth Responses of Major Phylogenetic Groups of Marine Bacteria to Natural Phytoplankton Blooms in the Western North Pacific Ocean^{▽†}

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Growth and productivity of phytoplankton substantially change organic matter characteristics, which affect bacterial abundance, productivity, and community structure in aquatic ecosystems. We analyzed bacterial community structures and measured activities inside and outside phytoplankton blooms in the western North Pacific Ocean by using bromodeoxyuridine immunocytochemistry and fluorescence *in situ* hybridization (BIC-FISH). *Roseobacter/Rhodobacter*, SAR11, *Betaproteobacteria*, *Alteromonas*, SAR86, and *Bacteroidetes* responded differently to changes in organic matter supply. *Roseobacter/Rhodobacter* bacteria remained widespread, active, and proliferating despite large fluctuations in organic matter and chlorophyll *a* (Chl-*a*) concentrations. The relative contribution of *Bacteroidetes* to total bacterial production was consistently high. Furthermore, we documented the unexpectedly large contribution of *Alteromonas* to total bacterial production in the bloom. Bacterial abundance, productivity, and growth potential (the proportion of growing cells in a population) were significantly correlated with Chl-*a* and particulate organic carbon concentrations. Canonical correspondence analysis showed that organic matter supply was critical for determining bacterial community structures. The growth potential of each bacterial group as a function of Chl-*a* concentration showed a bell-shaped distribution, indicating an optimal organic matter concentration to promote growth. The growth of *Alteromonas* and *Betaproteobacteria* was especially strongly correlated with organic matter supply. These data elucidate the distinctive ecological role of major bacterial taxa in organic matter cycling during open ocean phytoplankton blooms.

The major ecological function of heterotrophic bacteria in interactions with phytoplankton is mineralization of organic matter for recycling of nutrients and secondary production, which is channeled mainly to the higher trophic levels of aquatic food webs (10, 66). Growth of phytoplankton leads to major changes in organic matter quantity and quality, which results in changes to bacterial community structure, abundance, and productivity (3). Previous studies have shown that bacterial abundance, production, and community structure change markedly during naturally occurring and experimentally induced phytoplankton blooms (16, 56, 61). These studies pointed to several key phylogenetic groups as actively responding to the blooms and utilizing organic matter derived from phytoplankton. *Bacteroidetes* and *Alpha*- and *Gammaproteobacteria* were reportedly important during the blooms. Their relative contributions to total bacterial abundance and its vari-

ability have been studied intensively by using fluorescence *in situ* hybridization (FISH). However, as abundance is determined by both growth and mortality, changes in bacterial abundance do not always indicate changes in growth. Mainly because of some methodological limitations, little is known about the relative contributions of these key groups to total bacterial production or its variability during phytoplankton blooms.

Several methods that enable linking the classification of bacterial populations with their growth are available. Microautoradiography (MAR) was combined with FISH to assess phylo-type-specific substrate uptake at the single-cell level (35), and several radiolabeled substrates (e.g., thymidine, leucine, and glucose) can be used as substrates for measuring the growth of cells (1, 11, 18, 32). One of the advantages of this method is the possible application of several organic materials (e.g., thymidine, leucine, and dimethylsulfoniopropionate [DMSP]) as tracers. However, MAR-FISH requires the use of radioisotopes and involves the cumbersome quantification of silver grains.

In this study, we used the single-cell-based method that combines bromodeoxyuridine immunocytochemistry and fluorescence *in situ* hybridization (BIC-FISH) (70, 71). Bromodeoxyuridine (BrdU), a halogenated nucleoside, serves as a thymidine analog and has been used as a tracer of *de novo* DNA synthesis in marine bacterial assemblages. BrdU incor-

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TABLE 1. Station names, latitudes, longitudes, environmental factors, and dates of sampling during SOLAS/BLOCKS cruise of R/V Tansei-maru

Station name	Latitude	Longitude	Water temp (°C)	Salinity (PSU)	Water mass	POC ($\mu\text{g liter}^{-1}$)	PON ($\mu\text{g liter}^{-1}$)	C-N ratio (vol/vol)	POC-Chl- <i>a</i> ratio (wt/wt)	DOC ($\mu\text{g liter}^{-1}$)	DMS (nmol liter $^{-1}$)	DMSPp (nmol liter $^{-1}$)	Date
1L	38°60'N	142°45'E	7.2	33.7	TW	146	34	5.1	117	ND ^a	0.7	14	19 April 2007
2L	40°40'N	143°33'E	8.1	33.9	TW	104	24	5.0	82	ND	0.7	25	19 April 2007
3H	41°12'N	143°19'E	3.7	33.3	OW	209	55	4.4	79	796	4.9	28	19 April 2007
5H	42°38'N	145°51'E	4.2	33.3	OW	384	73	6.1	90	854	3.1	35	20 April 2007
6H	42°36'N	145°29'E	4.1	33.3	OW	384	80	5.6	47	817	7.7	64	23 April 2007
7H	42°11'N	143°46'E	1.7	33.0	CO	492	94	6.1	50	986	9.0	8	24 April 2007
8L	42°06'N	144°13'E	2.3	33.2	CO	96	26	4.3	136	700	1.7	12	25 April 2007
9L	40°57'N	144°20'E	8.0	33.9	TW	109	25	5.1	87	821	2.3	31	25 April 2007

^a ND, no data.

poration and antibody detection techniques have been used for identifying *de novo* DNA synthesis in marine bacteria (26, 45, 53, 67, 72, 73). The single-cell-based BrdU technique can reveal the relative contribution of each phylogenetic group to total bacterial abundance and production, as well as its growth potential (26, 53, 71).

The purpose of this study was to examine the phylotype-specific growth responses of marine bacteria, in terms of abundance, productivity, and growth, to natural phytoplankton blooms formed in the open ocean and to determine the taxa and phylogenetic groups contributing to total bacterial productivity and the factors that control their growth responses. To our knowledge, this study is the first to quantitatively assess the phylotype-specific productivity of marine bacteria during natural spring blooms in the open ocean.

MATERIALS AND METHODS

Study sites and sample collection. Surface seawater samples were collected at 5-m depth in 12-liter Niskin bottles (General Oceanics, Miami, FL) from eight stations in the western North Pacific (WNP) ocean during the SOLAS/BLOCKS (Surface Ocean & Lower Atmosphere Study/Bloom Caused by Kosa Study) cruise of R/V *Tansei-maru* (16 to 30 April 2007) (Table 1). About 24 liters of seawater was prefiltered through 200- μm nylon mesh to remove zooplankton and transferred to 12-liter dark bottles that had been rinsed with ultrapure water and then autoclaved before use. Twelve-liter seawater samples were incubated with BrdU (20 nmol liter $^{-1}$ final concentration; Sigma-Aldrich, St. Louis, MO) at *in situ* temperature for 10 h. At the end of the incubation, 100-ml samples were fixed with paraformaldehyde (2% by volume final concentration) and stored at 4°C for 2 h. Samples were then filtered onto 0.2- μm -pore-size polycarbonate membrane filters (25 mm, type GTTP; Millipore, Cork, Ireland), which were stored at -80°C until further analysis.

Environmental factors. For determination of chlorophyll *a* (Chl-*a*) concentrations, duplicate seawater samples were filtered (<14 kPa) through Whatman GF/F filters and then the chlorophyll was extracted from the filter in the dark with *N,N*-dimethylformamide at 4°C for 24 h (69, 76). To determine the size distribution of Chl-*a*, seawater samples were filtered through stacked 10- μm -, 2- μm -, and 0.2- μm -pore-size Whatman polycarbonate membrane filters, and the chlorophyll was extracted as described above. The Chl-*a* concentration was determined fluorometrically (29).

Samples for analysis of particulate organic carbon (POC) and particulate organic nitrogen (PON) were filtered onto precombusted (450°C, 5 h) Whatman GF/F filters and then measured using a CHN analyzer (Flash EA-1112; Thermo Finnigan, CA). Dissolved organic carbon (DOC) was analyzed in filtrates from samples filtered through precombusted GF/F filters by using high-temperature combustion (51) in a Shimadzu TOC-5000A total organic carbon analyzer (Shimadzu Co., Kyoto, Japan).

Measurement of dimethylsulfide (DMS) and particulate dimethylsulfoniopropionate (DMSPp) concentrations was performed on board within a day of sample collection by a purge and trap system (46).

Bacterial abundances. For enumeration of total bacterial abundance, 1 to 5 ml of a paraformaldehyde-fixed sample was stained with 4',6-diamidino-2-phenylindole (DAPI) (final concentration, 2 $\mu\text{g ml}^{-1}$) in the dark and filtered onto a 0.2- μm -pore-size polycarbonate black membrane filter (25 mm, type GTBP; Millipore, Cork, Ireland) at $\leq 27\text{-kPa}$ vacuum. All filters were mounted on slides and observed under the oil immersion objective of an Olympus BX-51 epifluorescence microscope (Olympus Optical, Tokyo, Japan). At least 3,000 DAPI-stained cells were counted per sample.

Bacterial secondary production. Bacterial secondary production was measured by BrdU incorporation (67), with a few modifications to the procedure (25). The samples were collected from the dark bottle after incubation with BrdU, and the cells that had incorporated BrdU were enumerated. For calculating the bacterial carbon production, we used 20 fg C per bacterium as a cell-to-carbon conversion factor (34).

BIC-FISH. For the BIC-FISH assay, seawater samples were filtered through a poly-L-lysine-coated membrane filter to collect bacterial cells. The cells on membrane filters were dehydrated with serial treatment in 70%, 90%, and 100% ethanol each for 1 min. To quench endogenous peroxidase in the samples, the filters were treated with 3% H₂O₂ in phosphate-buffered saline (PBS; 145 mmol liter $^{-1}$ NaCl, 1.4 mmol liter $^{-1}$ NaH₂PO₄, 8 mmol liter $^{-1}$ Na₂HPO₄; pH 7.6) for 10 min at room temperature and washed with 50 ml PBS for 10 min. The filters were treated with 10 mmol liter $^{-1}$ HCl for 5 min at room temperature, which was then replaced with pepsin (0.5 mg ml $^{-1}$ in 10 mmol liter $^{-1}$ HCl) for 2 h at 37°C, washed with 50 ml PBS for 10 min, and then treated with lysozyme (10 mg ml $^{-1}$ in TE buffer [10 mmol liter $^{-1}$ Tris-HCl, 1 mmol liter $^{-1}$ EDTA; pH 8.0]) for 15 min at room temperature. Thereafter, the filters were washed with 50 ml ultrapure water for 5 min, dehydrated with 95% ethanol, and dried.

Filters containing bacterial cells were cut into small pieces for hybridization with horseradish peroxidase (HRP)-labeled oligonucleotide probes. The HRP-labeled probe was added at a final DNA concentration of 0.28 ng μl^{-1} to 300 μl of hybridization buffer. The hybridization solution contained 900 mmol liter $^{-1}$ NaCl, 20 mmol liter $^{-1}$ Tris-HCl (pH 7.5), 10% (wt/vol) dextran sulfate, 0.05% (vol/vol) Triton X-100, 1% (vol/vol) blocking reagent, and the concentration of formamide (FA) determined by the online database probeBase (<http://www.microbial-ecology.net/probebase/>). We used FISH probes that target bacteria affiliated with *Bacteria* (Eub338; FA, 20%) (2), *Alphaproteobacteria* (Alf968; FA, 20%) (47), *Betaproteobacteria* (Bet42a; FA, 35%) (37), *Gammaproteobacteria* (Gam42a; FA, 35%) (37), *Bacteroidetes* (Cf319a; FA, 35%) (38), *Roseobacter/Rhodobacter* (GRb; FA, 30%) (20), SAR86 (SAR86-1249; FA, 50%) (15), SAR11 (SAR11 mixed probe; FA, 15%) (59), and *Alteromonas* (Alt1413; FA, 40%) (15), along with a negative control (Non338; FA, 20%) (74). Hybridization was performed at 42°C for 12 to 15 h. Probes Bet42a and Gam42a were used with competitor oligonucleotides (37). Thereafter, filter pieces with probes targeting bacteria were washed in 50 ml prewarmed washing buffer (20 mmol liter $^{-1}$ Tris-HCl [pH 7.4], 5 mmol liter $^{-1}$ EDTA [except for SAR11 mixed probes], 0.01% sodium dodecyl sulfate, and 225 mmol liter $^{-1}$ NaCl for Eub338, Non338, and Alf968 probes; 80 mmol liter $^{-1}$ NaCl for Bet42a, Gam42a, and Cf319a probes; 112 mmol liter $^{-1}$ NaCl for GRb probe; 28 mmol liter $^{-1}$ NaCl for SAR86-1249 probe; 318 mmol liter $^{-1}$ NaCl for SAR11 mixed probe; or 56 mmol liter $^{-1}$ NaCl for Alt1413 probe) at 46°C for 15 to 20 min and then washed with 50 ml PBST (1 $\times$ PBS, 0.05% [vol/vol] Triton X-100) buffer. Subsequently, the filter pieces were transferred to amplification buffer (10% [wt/vol] dextran sulfate, 2 mol liter $^{-1}$ NaCl, 0.1% [vol/vol] blocking reagent, and 0.0015% [vol/vol]

H₂O₂ in PBS) containing 4% (by volume) fluorescein isothiocyanate (FITC)-labeled tyramide and incubated at 46°C for 45 min.

After amplification, filter pieces were washed in 50 ml PBST for 15 min and rinsed with ultrapure water. After the catalyzed reporter deposition (CARD)-FISH step, probe-derived HRP was quenched with 10 mmol liter⁻¹ HCl for 10 min. Filter pieces were then washed with 50 ml PBS and ultrapure water. Intracellular DNA was denatured by treatment with nucleases (1:100 in incubation buffer) for double-stranded DNA for 2 h at 37°C and washed with 50 ml PBS for 10 min. Thereafter, anti-BrdU monoclonal antibodies conjugated with peroxidase were diluted 1:50 in TNB buffer (100 mmol liter⁻¹ Tris-HCl [pH 7.5], 150 mmol liter⁻¹ NaCl, and 0.5% [vol/vol] blocking reagent), applied to samples for 120 min at 37°C, and washed with 50 ml PBS. The antibody signal was amplified by incubating the filters with biotin-labeled tyramide diluted 1:25 in amplification buffer for 45 min at 46°C. The filter pieces were then washed with 50 ml PBST buffer for 10 min.

The filter pieces were treated with Texas Red-labeled streptavidin in TNB buffer (1:100) for 30 min at room temperature. Thereafter, filter pieces were washed with 50 ml PBST buffer, rinsed with ultrapure water, and dehydrated in 95% ethanol. Finally, the filter pieces were air dried and counterstained with a DAPI mix (5.5 parts [by volume] Citifluor [Citifluor Ltd., London, United Kingdom], 1 part Vectashield [Vector Labs, Burlingame, CA], 0.5 parts PBS with DAPI at a final concentration of 1 µg ml⁻¹). The slide was examined using an Olympus BX-51 epifluorescence microscope (Olympus Optical, Tokyo, Japan) equipped with an ORCA-ER-1394 charge-coupled-device (CCD) camera (Hamamatsu Photonics, Hamamatsu, Japan).

Image analysis. Epifluorescence microscopic images were stored as TIFF files and analyzed by using the image analysis software Image Pro-Plus 6.0 (Media Cybernetics, Silver Spring, MD). For BrdU-positive cells or FISH analysis, the image threshold was determined at a gray value that did not detect the negative control. Negative-control images were obtained from the 0-h-incubated samples for BrdU and from the Non338 probe images for FISH. Exposure times for BrdU and FISH images were optimized using samples with the negative-control probe to restrict background counts to <1% of the DAPI-stained cells. Bacterial cell volumes were determined from DAPI fluorescence by using a biovolume algorithm described previously (64), after edge detection by the Marr-Hildreth method (39).

Statistical analysis. Nonparametric Spearman's correlation analysis was employed to determine correlations between environmental and biological factors. A canonical correspondence analysis (CCA) and a permutation test (R version 2.10.0 software and R package Vegan) (52, 60) were used to test for significant effects of environmental variables on variations in the bacterial community structure. The best model was selected by the function "ordisep," which uses permutation *P* values (999 permutations). One-way analyses of variance (ANOVA) with a *post hoc* Tukey-Kramer honestly significant difference (HSD) test and nonlinear regression analyses between phylotype-specific growth potential and Chl-*a* concentration were performed using JMP 8.0 (SAS Institute, Cary, NC).

RESULTS

Environmental characteristics. During the cruise, areas of higher phytoplankton abundance in the WNP were located by using satellite ocean color images and by onboard measuring of surface concentrations of Chl-*a* and nutrients. We then selected four sampling stations with high Chl-*a* concentrations (3H, 5H, 6H, and 7H) and four with low Chl-*a* concentrations (1L, 2L, 8L, and 9L) (Fig. 1A). The size-fractionated Chl-*a* data (Fig. 1B) show that approximately 80% of total Chl-*a* was in the >10-µm size fraction in stations with high Chl-*a* concentrations.

Typically, the water mass at stations 1L, 2L, and 9L is representative of the Tsugaru Warm current (TW), whereas that at stations 8L and 7H represents the coastal Oyashio water (CO). Stations 3H, 5H, and 6H were within the offshore tongue of Oyashio water (OW). Summaries of POC, PON, carbon-nitrogen (C-N) ratio, POC-Chl-*a* ratio, and DOC, DMS, and DMSP concentrations at each station are shown in Table 1. In this study, we defined the stations with Chl-*a* concentrations that were >2.5 µg liter⁻¹ as "high" Chl-*a* sta-

FIG. 1. Chl-*a* concentration (A) and size distribution (B) at sampling stations in the WNP.

tions and the others as "low" Chl-*a* stations. Our results for bacterial abundance, bacterial production, proportions of BrdU-positive cells, and abundance of BrdU-positive cells at each station are presented in Fig. 2. The highest bacterial production rates and proportions of BrdU-positive cells were at stations 3H and 5H (1.9 and 2.3 µg C liter⁻¹ day⁻¹, respectively, and 37% and 34% of DAPI-stained cells, respectively) (ANOVA, *P* < 0.001; Tukey-Kramer HSD test, *P* < 0.001). The largest numbers of total bacteria and BrdU-positive cells were at station 5H (7.8×10^5 and 2.6×10^5 cells ml⁻¹, respectively) (ANOVA, *P* < 0.001; Tukey-Kramer HSD test, *P* < 0.001).

Proportions of bacterial phylotypes within total and BrdU-positive cells. The *Alphaproteobacteria* groups SAR11 and *Roseobacter/Rhodobacter* accounted for 72% to 100% of total alphaproteobacterial cells. Typically, SAR11 was dominant at all stations except for stations 5H and 6H and accounted for 22% to 52% of DAPI-stained cells (ANOVA, *P* < 0.001; Tukey-Kramer HSD test, *P* < 0.001) (Table 2). The abundance of this group at stations 5H and 6H, classified as the OW water mass, was lower than at the other stations (ANOVA, *P* < 0.001; Tukey-Kramer HSD test, *P* < 0.001). The abundance of *Roseobacter/Rhodobacter* at station 6H, classified as OW, was higher than at the other stations except for station 5H (ANOVA, *P* < 0.001; Tukey-Kramer HSD test, *P* < 0.05); they made up a smaller proportion than SAR11, accounting for 1.5% to 5.5% of DAPI-stained cells.

Betaproteobacteria were small components at all stations and accounted for 0.7% to 2.6% of DAPI-stained cells.

SAR86 and *Alteromonas* within the *Gammaproteobacteria* subclass accounted for 29% to 94% of total gammaproteobacterial cells. SAR86 was the dominant *Gammaproteobacteria* member at all stations except for station 7H (ANOVA, *P* < 0.001; Tukey-Kramer HSD test, *P* < 0.001); it accounted for 24% to 78% of the gammaproteobacterial cells. The propor-

FIG. 2. Bacterial abundance (A), bacterial production (B), proportion of total bacterial cells that were BrdU positive (C), and number of BrdU-positive cells (D) at each sampling station. Values are means from 5 to 10 counting fields; error bars indicate standard deviations (SD).

tion and abundance of this group were higher in the OW than in the other regions (ANOVA, $P < 0.001$; Tukey-Kramer HSD test, $P < 0.001$). At station 7H, *Alteromonas* was the most dominant, accounting for 62% of total gammaproteobacterial cells (ANOVA, $P < 0.001$; Tukey-Kramer HSD test, $P < 0.001$). Members of *Bacteroidetes* contributed from 15% to 31% of DAPI-stained cells, with the highest abundance at station 6H (ANOVA, $P < 0.001$; Tukey-Kramer HSD test, $P < 0.001$).

Although FISH results showed the *Roseobacter/Rhodobacter* group to be numerically less abundant than other groups in the

Alphaproteobacteria (2.3% to 14% of alphaproteobacterial cells [Table 2]), their contribution to BrdU-positive cells was large, accounting for 25% to 70% of BrdU-positive alphaproteobacterial cells. The proportion of *Roseobacter/Rhodobacter* within BrdU-positive cells at station 6H was the highest except for station 5H (ANOVA, $P < 0.001$; Tukey-Kramer HSD test, $P < 0.05$). *Betaproteobacteria* accounted for 0.2% to 2.3% of BrdU-positive cells.

The BrdU-positive proportions of *Gammaproteobacteria* varied between stations. The two taxa *Alteromonas* and SAR86 accounted for about 50% to 100% of BrdU-positive gamma-proteobacterial cells at the high Chl-*a* stations (Table 2). *Alteromonas* was most abundant at station 7H, where it accounted for 18% of total BrdU-positive cells (ANOVA, $P < 0.001$; Tukey-Kramer HSD test, $P < 0.001$). The abundance of SAR86 was higher (15% and 13% of total BrdU-positive cells, respectively) at stations 3H and 5H, classified as OW, than at the other stations (ANOVA, $P < 0.001$; Tukey-Kramer HSD test, $P < 0.001$). The *Bacteroidetes* members were the most abundant at all stations except for stations 2L and 7H, ranging from 23% to 41% of BrdU-positive cells.

Proportion of actively growing cells within each phylogenetic group. The proportion of BrdU-positive cells within FISH-positive cells of each phylogenetic group indicates the proportion of the population that is actively growing (Fig. 3). These proportions were higher at stations within the OW than at stations in the other regions. In particular, the proportions of actively growing *Bacteroidetes* and *Alteromonas* at stations 3H and 5H, classified as OW, were conspicuously high and accounted for 25% to 69% and 36% to 63%, respectively, of the cells detected by the specific FISH probes (ANOVA, $P < 0.001$; Tukey-Kramer HSD test, $P < 0.001$). The proportion of BrdU-positive *Roseobacter/Rhodobacter* cells among the FISH-positive cells was higher than that of the other phylotypes at all stations except for station 1L (ANOVA, $P < 0.001$; Tukey-Kramer HSD test, $P < 0.05$); they accounted for 33% to 87% of the specific probe-positive cells. In contrast, the proportion of BrdU-positive SAR11 cells was always low (1.4% to 14%), even at stations with high Chl-*a*.

Cell volume. The mean cell volumes of *Betaproteobacteria* were largest (ANOVA, $P < 0.001$; Tukey-Kramer HSD test, $P < 0.05$) at stations 3H, 5H, and 6H, classified as the OW water mass (Fig. 4). Those of *Alteromonas* were largest at stations 3H, 5H, and 7H (ANOVA, $P < 0.001$; Tukey-Kramer HSD test, $P < 0.001$). The average cell volumes of SAR11 and SAR86 were $0.08 \pm 0.03 \mu\text{m}^3$ and $0.03 \pm 0.02 \mu\text{m}^3$, respectively, which were smaller than those of the other subgroups (ANOVA, $P < 0.001$; Tukey-Kramer HSD test, $P < 0.001$).

Correlation analysis. Spearman's correlation analysis of environmental factors and the number of FISH-positive cells indicates that the abundance of *Alteromonas* was significantly correlated with Chl-*a* and POC concentrations (Table 3). Correlation analysis of environmental factors and the number of cells in the major bacterial taxa indicates that the numbers of BrdU-positive *Roseobacter/Rhodobacter* and *Alteromonas* cells were significantly correlated with POC concentrations. Correlation analysis of environmental factors and the proportion of BrdU-positive cells within FISH-positive cells shows a significant correlation between *Alteromonas* and Chl-*a* and POC concentrations.

TABLE 2. Percentages of major marine bacterial taxa within DAPI-stained and BrdU-positive cells at each station

Parameter tested	Station characteristic and name	Water mass	Percentage (mean $\pm$ SD) of:									
			Eubacteria	Alphaproteobacteria	Roseobacter/ Rhodobacter	SAR11	Betaproteobacteria	Gammaproteobacteria	Alteromonas	SAR86	Bacteroidetes	
Abundance ^a	Low Chl- <i>a</i>	TW	84 $\pm$ 5	53 $\pm$ 2	2.1 $\pm$ 0.4	40 $\pm$ 3	0.7 $\pm$ 0.3	18 $\pm$ 2	1.5 $\pm$ 0.4	6.2 $\pm$ 1.3	25 $\pm$ 3	
		TW	83 $\pm$ 9	46 $\pm$ 8	2.8 $\pm$ 0.7	50 $\pm$ 5	2.6 $\pm$ 0.8	18 $\pm$ 3	2.2 $\pm$ 0.9	9.1 $\pm$ 1.8	21 $\pm$ 2	
		CO	84 $\pm$ 2	55 $\pm$ 3	1.8 $\pm$ 0.5	45 $\pm$ 2	1.7 $\pm$ 0.5	19 $\pm$ 1	ND ^c	11 $\pm$ 1	18 $\pm$ 1	
	9L	TW	80 $\pm$ 3	64 $\pm$ 3	1.5 $\pm$ 0.4	52 $\pm$ 1	1.7 $\pm$ 0.8	16 $\pm$ 5	ND	4.4 $\pm$ 1.1	15 $\pm$ 3	
	High Chl- <i>a</i>	OW	82 $\pm$ 10	44 $\pm$ 7	3.4 $\pm$ 0.4	36 $\pm$ 2	1.3 $\pm$ 0.4	18 $\pm$ 1	2.9 $\pm$ 0.5	14 $\pm$ 1	20 $\pm$ 2	
		5H	80 $\pm$ 5	38 $\pm$ 6	5.5 $\pm$ 0.4	22 $\pm$ 2	1.9 $\pm$ 0.3	29 $\pm$ 3	5.9 $\pm$ 0.7	14 $\pm$ 1	21 $\pm$ 2	
		6H	OW	78 $\pm$ 10	39 $\pm$ 3	4.7 $\pm$ 1.1	26 $\pm$ 3	2.0 $\pm$ 0.7	32 $\pm$ 1	5.0 $\pm$ 0.5	16 $\pm$ 3	31 $\pm$ 1
		7H	CO	76 $\pm$ 3	47 $\pm$ 3	3.0 $\pm$ 0.8	49 $\pm$ 6	0.8 $\pm$ 0.3	18 $\pm$ 1	11 $\pm$ 3	4.3 $\pm$ 0.8	19 $\pm$ 2
	Productivity ^b	Low Chl- <i>a</i>	TW	97 $\pm$ 4	19 $\pm$ 2	5.8 $\pm$ 2.4	16 $\pm$ 2	0.2 $\pm$ 0.2	11 $\pm$ 2	1.9 $\pm$ 1.0	7.0 $\pm$ 2.1	41 $\pm$ 4
			2L	TW	90 $\pm$ 10	17 $\pm$ 6	11 $\pm$ 5	17 $\pm$ 8	1.4 $\pm$ 1.7	28 $\pm$ 8	1.9 $\pm$ 1.9	5.2 $\pm$ 2.6
8L			CO	83 $\pm$ 5	23 $\pm$ 8	9.1 $\pm$ 3.2	8.8 $\pm$ 4	1.0 $\pm$ 1.1	16 $\pm$ 3	ND	5.7 $\pm$ 5.6	32 $\pm$ 3
9L		TW	77 $\pm$ 6	20 $\pm$ 5	5.5 $\pm$ 1.5	6.2 $\pm$ 2	0.3 $\pm$ 1.0	12 $\pm$ 5	ND	2.0 $\pm$ 1.3	23 $\pm$ 4	
High Chl- <i>a</i>		OW	90 $\pm$ 7	26 $\pm$ 3	6.4 $\pm$ 0.9	16 $\pm$ 4	0.7 $\pm$ 0.5	12 $\pm$ 1	4.4 $\pm$ 1.5	15 $\pm$ 2	30 $\pm$ 3	
		5H	OW	82 $\pm$ 7	27 $\pm$ 2	13 $\pm$ 2.1	6.3 $\pm$ 1	2.3 $\pm$ 1.1	30 $\pm$ 3	9.4 $\pm$ 1.6	13 $\pm$ 2	38 $\pm$ 3
		6H	OW	94 $\pm$ 3	25 $\pm$ 5	17 $\pm$ 6	4.6 $\pm$ 2	2.2 $\pm$ 1.1	29 $\pm$ 3	9.1 $\pm$ 0.8	5.3 $\pm$ 1.4	41 $\pm$ 3
		7H	CO	77 $\pm$ 10	26 $\pm$ 7	9.8 $\pm$ 3.1	8.7 $\pm$ 2	0.6 $\pm$ 0.8	24 $\pm$ 3	18 $\pm$ 5	0.6 $\pm$ 0.6	24 $\pm$ 3

^a Percentage within DAPI-stained cells.^b Percentage within BrdU-positive cells.^c ND, not detected.

FIG. 3. Proportion of BrdU-positive cells within each taxon of marine bacteria as determined by FISH probes at each station. ND, not detectable.

Interrelationships between bacterial community structure and environmental factors. The CCA indicates that resource supplies (POC, PON, and Chl-*a* concentrations) have a significant effect on bacterial community structures ($P < 0.05$) and that POC concentration is the predominant factor for determining the bacterial community structure of both total and BrdU-positive fractions (Fig. 5). As POC, PON, and Chl-*a* concentrations were highly correlated, only a single variable (POC concentration) was selected in the best model. The POC concentration explained 35.4% and 38.0% of the variation in the community structures of the total and actively growing fractions, respectively ($P < 0.05$). The CCA plot also implies that the presence of *Alteromonas* (results of FISH) and its contribution to bacterial production (results of BIC-FISH) were strongly associated with high POC concentrations.

DISCUSSION

The WNP is one of the most productive areas in the world ocean and is composed of six major water masses—Tsugaru Warm current, Oyashio water, Kuroshio water, cold lower layer water, surface-layer water, and Coastal Oyashio water—each of which has different physical characteristics (28). This area is characterized by the occurrence of dense patches of

spring diatom blooms (31), which provide for rich fishery grounds and make this an important region for CO₂ fixation and sequestration because of an active biological pump (30).

Primary production and phytoplankton community structure have been measured at various locations and seasons in the WNP (49, 63). In this study, the Chl-*a* size fraction data show the dominance of large phytoplankton at the high Chl-*a* stations. Diatoms are reportedly the dominant components of the >10- μ m size fraction during spring blooms in the WNP (50), and high-performance liquid chromatography (HPLC) analysis of photosynthetic pigments collected during this cruise showed a high concentration of fucoxanthin, a diatom marker pigment, at all high Chl-*a* stations (Y.-J. Eum and K. Suzuki, unpublished data). We concluded that the high Chl-*a* stations we observed in this study were characterized by patches of diatoms, as in previous studies.

A potential limitation of techniques using BrdU is that not all microorganisms can incorporate or take up BrdU (73). However, recent studies have shown that many wild-type bacteria isolated from lake water and seawater (in total, 61 out of 66 bacterial isolates) can incorporate BrdU (26, 27, 53). These results suggest that BrdU techniques have the potential to be broadly applicable to almost all major phylotypes of bacteria in pelagic marine assemblages.

FIG. 4. Cell volumes of marine bacterial taxa at each station. ND, not detectable. Values are means from 50 to 100 cells; error bars indicate SD.

The use of constrained ordination techniques (e.g., CCA) sheds light on the patterns linking bacterial community structure with contextual environmental factors (58). In this study, POC was the only environmental factor that statistically correlated with the variability of community structures (Fig. 5). Variation in POC explained 35% and 38% of the variation in total and BrdU-positive communities, respectively. The residual 60% to 65% of variance remains unexplained. It is possible that unrecorded environmental factors, such as the availability of specific chemical substrates or inorganic nutrients (bottom-up effects) or grazing pressure and viral lysis (top-down effects), might have affected bacterial activities and community structures.

Phylotype growth potential, defined as the proportion of BrdU-positive cells within each group of FISH-positive cells, shows that there were certain optimum concentrations of organic resources that facilitated growth (Fig. 6). Bell-shaped curves provided good fits to the change of growth potentials, although the shapes of the curves differ among phylotypes (e.g., broad versus sharp peaks). In particular, the fitted curves for *Alteromonas* and *Betaproteobacteria* were statistically significant ($P < 0.05$, $n = 8$), suggesting strong regulation of growth by organic matter supply.

The high contribution of *Bacteroidetes* to total bacterial abundance (the percentage of FISH-positive cells within DAPI-stained cells) and production (percentage of FISH-pos-

itive cells within BrdU-positive cells) (Table 2) suggests that this group is important in the processes utilizing organic matter in this region. However, there was no significant linear relationship between growth potential and environmental factors such as Chl-*a*, POC, and DMS concentrations or water temperature (Table 3). The growth potential was highest at stations 3H and 5H (Fig. 3E), which had relatively high POC–Chl-*a* ratios (presumably postbloom stations) among the high Chl-*a* stations (Table 1). Some *Bacteroidetes* members are commonly associated with particulate organic matter produced by phytoplankton (14, 16, 56, 61), and these are known to be able to degrade high-molecular-weight (HMW) organic matter (11). It is therefore reasonable to propose that their adaptive advantage of utilizing HMW organic matter leads to their importance as a key bacterial subgroup during postbloom periods.

Roseobacter/Rhodobacter was a highly proliferating group compared with the other phylogenetic groups (Fig. 3F and 6). The growth potential was always high under varied temperatures and Chl-*a*, POC, and DMS concentrations. Metagenomic analysis of these subgroups revealed that they have versatile mechanisms for energy and carbon acquisition (48). The results of this study support the hypothesis that the *Roseobacter* group is an “ecological generalist” that sustains basic bacterial production in the ocean (8, 43, 45). Such characteristics of *Roseobacter/Rhodobacter* have previously been reported in eutrophic marine environments (70). This suggests that *Roseobacter/Rhodobacter* bacteria maintain their constant productivity under various environmental conditions because of their nutritional versatility in the use of phytoplankton-derived organic matter as carbon and energy sources. Also, in spite of observed high growth potentials, the pattern of abundance of *Roseobacter/Rhodobacter* bacteria was contradictory (Fig. 7A). A previous study using MAR-FISH showed that this group was underrepresented in abundance compared to their potential for *in situ* uptake of substrates throughout the year (1). It was implied that their contribution to total bacterial production was higher than that expected from their *in situ* abundance and also that this group might be susceptible to protozoan grazing (54) and viral lysis (75).

An analysis of the whole-genome sequences of *Roseobacter* isolates revealed some gene homologues for transport of DMSP, a precursor of DMS (43, 48). A previous report showed that members of *Roseobacter* might exert major control on DMS production (78). However, in this study there was no significant positive correlation between productivity or growth potential of *Roseobacter/Rhodobacter* and DMS or DMSP concentrations (Table 3). MAR-FISH studies have also revealed that this group can assimilate DMSP in natural seawater, but the contribution to DMSP turnover was not always high (36). This group could contribute to the DMS flux, but not to a large degree, in this region.

SAR11 was abundantly represented in the total bacterial communities but underrepresented in the BrdU-positive cells (Fig. 7B). Previous studies on size-selective ingestion (grazing) by bacterivorous protozoa revealed that protozoa selectively graze larger-sized bacteria (23). One possible explanation for the SAR11 abundance is its advantage in escaping from protozoan grazing due to the fact that its cell size is much smaller than that of the other phylotypes (Fig. 4B) (5, 77). The SAR11

TABLE 3. Results from Spearman’s correlation analysis of relationships between environmental factors and numbers of FISH-positive cells, numbers of BrdU-positive cells, and percentages of BrdU-positive cells within FISH-positive cells

Group and factor	<i>r</i> value ^a					
	<i>Roseobacter/Rhodobacter</i>	SAR11	<i>Betaproteobacteria</i>	<i>Alteromonas</i>	SAR86	<i>Bacteroidetes</i>
No. of FISH-positive cells						
Temp	0.02	0.67	0.64	−0.24	0.10	0.38
Salinity	−0.04	0.75*	0.55	−0.34	0.04	0.31
Chl- <i>a</i>	0.68	−0.36	0.00	0.92**	0.18	0.26
POC	0.57	−0.38	−0.19	0.86**	0.07	0.36
PON	0.45	−0.64	−0.36	0.76*	0.10	0.24
C-N ratio	0.42	−0.17	0.21	0.76*	0.00	0.43
POC-Chl- <i>a</i> ratio	−0.47	0.14	0.02	−0.57	−0.05	−0.02
DMS	0.43	−0.48	−0.14	0.67	0.07	0.10
DMSPp	0.57	−0.14	−0.74*	0.31	0.64	0.62
No. of BrdU-positive cells						
Temp	−0.21	0.21	−0.12	−0.31	0.05	0.10
Salinity	−0.23	0.22	−0.01	−0.38	0.01	0.10
Chl- <i>a</i>	0.70	−0.37	0.36	0.84**	0.00	0.34
POC	0.79*	−0.48	0.43	0.86**	0.10	0.50
PON	0.71*	−0.48	0.40	0.79*	0.17	0.40
C-N ratio	0.59	−0.60	0.38	0.69	0.00	0.43
POC-Chl- <i>a</i> ratio	−0.43	0.33	−0.02	−0.45	0.24	−0.10
DMS	0.60	−0.05	−0.02	0.62	−0.12	0.19
DMSPp	0.52	0.12	0.40	0.21	0.48	0.57
Proportion of BrdU-positive cells within phylotype						
Temp	−0.52	−0.17	−0.47	−0.24	0.17	0.05
Salinity	−0.54	−0.15	−0.56	−0.23	0.23	0.02
Chl- <i>a</i>	0.44	0.36	0.68	0.71*	−0.13	0.18
POC	0.52	0.48	0.62	0.74*	0.05	0.40
PON	0.57	0.48	0.71*	0.67	0.02	0.36
C-N ratio	0.50	0.10	0.40	0.40	−0.02	0.38
POC-Chl- <i>a</i> ratio	−0.10	−0.17	−0.38	−0.52	0.21	0.12
DMS	0.64	0.14	0.64	0.56	−0.10	0.17
DMSPp	0.07	0.17	0.40	0.38	0.52	0.31

^a Level of significance: *, $P < 0.05$; **, $P < 0.01$.

contribution to total bacterial production and subsequent carbon transfer to bacteriivores might be less than expected from the large abundance.

The growth potential of SAR11 bacteria was consistently low

under the entire range of Chl-*a* concentrations (Fig. 6). In general, bacteria regulate the catabolism of organic substrates to attain the correct intracellular stoichiometry with respect to nutrients such as nitrogen and phosphorus (13). Also, “*Candi-*

FIG. 5. Canonical correspondence analysis of total (FISH-positive) bacterial community structure (A) and BrdU-positive community structure (BIC-FISH) (B) at each sampling station, showing the positions of dominant phylotypes in the two-dimensional space of the plot. The arrow indicates the direction of increasing value of the POC variable, and the length of the arrow indicates the degree of correlation of the variable with the community data. “T” indicates total community data; “B” indicates BrdU-positive community data only. Ros, *Roseobacter/Rhodobacter*; Bet, *Betaproteobacteria*; Alt, *Alteromonas*; Bactero, *Bacteroidetes*.

FIG. 6. Relationship between phylotype-specific growth potentials and Chl-*a* concentrations. The bell-shaped relationship was statistically significant for *Alteromonas* and *Betaproteobacteria* ($R^2 = 0.75$, $P < 0.05$, $n = 8$, and $R^2 = 0.72$, $P < 0.05$, $n = 8$, respectively). Nonlinear regression equations were as follows: for *Alteromonas*, $y = -2.79x^2 + 31.6x - 20.4$; for *Betaproteobacteria*, $y = -1.45x^2 + 16.3x - 11.1$.

datus *Pelagibacter ubique*,” a representative strain of this group, is known to have proteorhodopsin (PR), which is a light-driven proton pump that enhances ATP production (4, 22, 40). Quantitative PCR analysis revealed that the PR gene of SAR11 group bacteria is strongly regulated by light and dark conditions (33); therefore, the growth of this subgroup might benefit from the higher light levels at the ocean’s surface in addition to the nature of organic substrates there.

We detected an *Alteromonas* bloom at high Chl-*a* stations (Table 2). This group apparently responded well to the organic matter derived from phytoplankton. The *Alteromonas* probe used in this study (Alt1413) detects *Alteromonadaceae* and *Cobwelliaceae*, which have been cultured from algal samples (7) and have also been recovered as a dominant component of the total biomass in mesocosm experiments (57, 62). These bacteria are well known as an easily culturable and widely distributed group of *Gammaproteobacteria* (19, 55) but are believed to be numerically minor in seawater environments (15). Our study demonstrates that they can increase their abundance enough to account for a major part of bacterial production in response to an increase of organic matter from phytoplankton. In a recent study, transcriptome analysis of heterotrophic bacteria revealed that *Alteromonas* is one of the “ecological specialists” that grow rapidly with the expression of metabolic genes (e.g., TonB-associated transporter, nitrogen assimilation, fatty acid catabolism, and tricarboxylic acid [TCA] cycle enzyme genes) associated with the utilization of HMW dissolved organic matter in response to resource supply (42). The drastic change in their growth potential observed in this study might have been caused by upregulation of these genes under optimum substrate concentrations.

Betaproteobacteria are well known as a dominant and highly active group in freshwater systems but also known to be a minor group in seawater environments (12, 21). This group was also minor in this study, although their growth potential was high and they had a large cell volume at some high Chl-*a* stations (Fig. 3C and 4E). Although far less is known about marine *Betaproteobacteria*, the abundance of the OM43 clade,

FIG. 7. Relationship between the proportions of FISH-positive bacterial cells and BrdU-positive bacterial cells.

affiliated with uncultured *Betaproteobacteria*, reportedly increased in response to a diatom bloom along the Oregon coast (44). It would be interesting to further investigate the growth of marine *Betaproteobacteria* using substrates derived from diatoms.

Bacterial growth potentials were lower under high Chl-*a* concentrations (Fig. 6). One possible reason could be the allelopathic interaction between phytoplankton and bacteria. Chemicals produced by phytoplankton reportedly inhibit the growth of competing organisms, thus indirectly preventing them from consuming common resources, such as nutrients (9, 68). Fistarol et al. (17) reported that compounds excreted from *Prymnesium parvum* (*Haptophyta*) inhibited leucine incorporation by heterotrophic bacteria. In contrast, it was reported that a *Flexibacteriaceae* strain completely inhibited the growth of the diatom *Thalassiosira rotula* (24). Also, Mayali et al. (41) reported that a *Roseobacter* strain promoted the breakdown of a dinoflagellate bloom. Phytoplankton proliferation seems to stimulate the excretion of extracellular DOC whereby bacterial growth requires the additional uptake of nutrients (6). Ironically, too much stimulation of bacterial growth during a phytoplankton bloom leads to rapid depletion of inorganic nutrients from bacterial consumption, overwhelming remineralization, which prevents further growth of phytoplankton and facilitates termination of the bloom. Therefore, the bell-shaped pattern of bacterial growth potential as a function of phytoplank-

ton abundance may be advantageous for sustaining a mutualistic relationship in marine environments.

Specific binding of FISH probes is known to be affected by hybridization temperature. In this study, we performed the hybridization at 42°C rather than at 46°C as used by Wallner et al. (74) for optimizing the BIC-FISH technique. Such lowering of hybridization temperature might lead to overestimation of target organisms owing to nonspecific hybridization reactions. However, the differences in the percentages of FISH-positive cells from each probe between hybridization at 42°C and 46°C on samples from two stations in our study were at most two percentage points and usually one percentage point or less (see Table S1 in the supplemental material). This suggests that hybridization at 42°C did not lead to significant changes in estimates of FISH probe-positive cells.

The factor used to convert cell volume to carbon is critical for accurate calculations of bacterial production. In this study, the bacterial production was calculated by using a fixed conversion factor of 20 fg C cell⁻¹ (34). This factor was originally determined for planktonic bacterial cells of ~0.05 µm³. In this study, cell volumes of different bacterial phylotypes varied substantially (Fig. 4). The larger cells contribute more to total bacterial production and biomass than the smaller ones (65). By measuring the cell volumes of actively growing cells using the BIC-FISH method and by using multiple conversion factors for each phylotype, it would be possible to obtain more realistic estimates of production by each bacterial phylotype.

In conclusion, this study revealed distinct growth responses of marine bacteria belonging to the subgroups *Roseobacter*/*Rhodobacter*, *Betaproteobacteria*, *Bacteroidetes*, *Alteromonas*, SAR11, and SAR86 to natural phytoplankton blooms in the mesotrophic open ocean. Organic matter supply was a significant factor for determining bacterial community structures in this region. In particular, the growth of *Alteromonas* and *Betaproteobacteria* was strongly correlated with the organic matter supply, showing that some optimal concentrations of organic matter maximized their growth potential. Our data and previous studies suggest that the *Roseobacter*/*Rhodobacter* group of bacteria may respond as generalists and the *Alteromonas* group as specialists for using organic matter. The *Roseobacter*/*Rhodobacter* group was always active regardless of large fluctuations in organic matter (Chl-*a* and POC) concentrations, whereas *Alteromonas* was highly active and became abundant only in the presence of phytoplankton blooms. The BIC-FISH method should be useful for quantifying the abundance and activity of major phylogenetic groups of bacteria and for monitoring their dynamics in natural environments.

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REFERENCES

- Alonso-Sáez, L., and J. M. Gasol. 2007. Seasonal variation in the contribution of different bacterial groups to the uptake of low-molecular-weight compounds in NW Mediterranean coastal waters. *Appl. Environ. Microbiol.* **73**:3528–3535.
- Amann, R. L., et al. 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Appl. Environ. Microbiol.* **56**:1919–1925.
- Azam, F. 1998. Microbial control of oceanic carbon flux: the plot thickens. *Science* **280**:694–696.
- Bejà, O., et al. 2000. Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. *Science* **289**:1902–1906.
- Boenigk, J., P. Stadler, A. Wiedlroither, and M. W. Hahn. 2004. Strain-specific differences in the grazing sensitivity of closely related ultramicrobacteria affiliated with the Polynucleobacter cluster. *Appl. Environ. Microbiol.* **70**:5787–5793.
- Bratbak, G., and T. F. Thingstad. 1985. Phytoplankton-bacteria interaction: an apparent paradox? Analysis of a model system with both competition and commensalism. *Mar. Ecol. Prog. Ser.* **25**:23–30.
- Brinkmeyer, R., M. Rappé, S. Gallacher, and L. Medlin. 2000. Development of clade (*Roseobacter* and *Alteromonas*)- and taxon-specific oligonucleotide probes to study interactions between toxic dinoflagellates and their associated bacteria. *Eur. J. Phycol.* **35**:315–329.
- Buchan, A., J. M. González, and M. A. Moran. 2005. Overview of the marine *Roseobacter* lineage. *Appl. Environ. Microbiol.* **71**:5665–5677.
- Cole, J. J. 1982. Interactions between bacteria and algae in aquatic ecosystems. *Annu. Rev. Ecol. Syst.* **13**:291–314.
- Cole, J. J., S. Findlay, and M. L. Pace. 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Mar. Ecol. Prog. Ser.* **43**:1–10.
- Cottrell, M. T., and D. L. Kirchman. 2000. Natural assemblages of marine proteobacteria and members of the Cytophaga-Flavobacter cluster consuming low- and high-molecular-weight dissolved organic matter. *Appl. Environ. Microbiol.* **66**:1692–1697.
- Cottrell, M. T., and D. L. Kirchman. 2004. Single-cell analysis of bacterial growth, cell size, and community structure in the Delaware estuary. *Aquat. Microb. Ecol.* **34**:139–149.
- del Giorgio, P., and J. J. Cole. 1998. Bacterial growth efficiency in natural aquatic systems. *Annu. Rev. Ecol. Syst.* **29**:503–541.
- DeLong, E. F., D. G. Franks, and A. L. Alldredge. 1993. Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. *Limnol. Oceanogr.* **38**:924–934.
- Eilers, H., J. Pernthaler, F. O. Glöckner, and R. Amann. 2000. Culturability and in situ abundance of pelagic bacteria from the North Sea. *Appl. Environ. Microbiol.* **66**:3044–3051.
- Fandino, L. B., L. Riemann, G. F. Steward, R. A. Long, and F. Azam. 2001. Variations in bacterial community structure during a dinoflagellate bloom analyzed by DGGE and 16S rDNA sequencing. *Aquat. Microb. Ecol.* **23**:119–130.
- Fistrol, G. O., C. Legrand, and E. Graneli. 2003. Allelopathic effect of *Prymnesium parvum* on a natural plankton community. *Mar. Ecol. Prog. Ser.* **255**:115–125.
- Fuhrman, J. A., and F. Azam. 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. *Mar. Biol.* **66**:109–120.
- García-Martínez, J., S. G. Acinas, R. Massana, and F. Rodríguez-Valera. 2002. Prevalence and microdiversity of *Alteromonas macleodii*-like microorganisms in different oceanic regions. *Environ. Microbiol.* **4**:42–50.
- Giuliano, L., M. De Domenico, E. De Domenico, M. G. Höfle, and M. M. Yakimov. 1999. Identification of culturable oligotrophic bacteria within naturally occurring bacterioplankton communities of the Ligurian Sea by 16S rRNA sequencing and probing. *Microb. Ecol.* **37**:77–85.
- Glöckner, F. O., B. M. Fuchs, and R. Amann. 1999. Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. *Appl. Environ. Microbiol.* **65**:3721–3726.
- Gómez-Consarnau, L., et al. 2007. Light stimulates growth of proteorhodopsin-containing marine Flavobacteria. *Nature* **445**:210–213.
- González, J. M., E. B. Sherr, and B. F. Sherr. 1990. Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. *Appl. Environ. Microbiol.* **56**:583–589.
- Grossart, H.-P., and M. Simon. 2007. Interactions of planktonic algae and bacteria: effects on algal growth and organic matter dynamics. *Aquat. Microb. Ecol.* **47**:163–176.
- Hamasaki, K. 2006. Comparison of bromodeoxyuridine immunoassay with tritiated thymidine radioassay for measuring bacterial productivity in oceanic waters. *J. Oceanogr.* **62**:793–799.
- Hamasaki, K., R. Long, and F. Azam. 2004. Individual cell growth rates of marine bacteria, measured by bromodeoxyuridine incorporation. *Aquat. Microb. Ecol.* **35**:217–227.
- Hamasaki, K., A. Taniguchi, Y. Tada, R. A. Long, and F. Azam. 2007. Actively growing bacteria in the Inland Sea of Japan identified by combined

- bromodeoxyuridine immunocapture and denaturing gradient gel electrophoresis. *Appl. Environ. Microbiol.* **73**:2787–2798.
28. Hanawa, K., and H. Mitsudera. 1987. Variation of water system distribution in the Sanriku coastal area. *J. Oceanogr.* **42**:435–446.
 29. Holm-Hansen, O., C. J. Lorenzen, R. W. Holmes, and J. D. H. Strickland. 1965. Fluorometric determination of chlorophyll. *J. Cons. Perm. Int. Explor. Mer.* **30**:3–15.
 30. Honda, M. C., M. Kusakabe, S. Nakabayashi, S. J. Manganini, and S. Honjo. 1997. Change in pCO₂ through biological activity in the marginal seas of the western North Pacific: the efficiency of the biological pump estimated by a sediment trap experiment. *J. Oceanogr.* **53**:645–662.
 31. Kasai, H., H. Saito, and A. Tsuda. 1998. Estimation of standing stock of chlorophyll *a* and primary production from remote sensed ocean color in the Oyashio region, the western subarctic Pacific, during the spring bloom in 1997. *J. Oceanogr.* **54**:527–537.
 32. Kirchman, D., E. K. Nees, and R. Hodson. 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. *Appl. Environ. Microbiol.* **49**:599–607.
 33. Lami, R., M. T. Cottrell, B. J. Campbell, and D. L. Kirchman. 2009. Light dependent growth and proteorhodopsin expression by *Flavobacter* and SAR11 in experiments with Delaware coastal waters. *Environ. Microbiol.* **11**:3201–3209.
 34. Lee, S., and J. A. Fuhrman. 1987. Relationships between biovolume and biomass of naturally derived marine bacterioplankton. *Appl. Environ. Microbiol.* **53**:1298–1303.
 35. Lee, N., et al. 1999. Combination of fluorescent in situ hybridization and microautoradiography—a new tool for structure-function analyses in microbial ecology. *Appl. Environ. Microbiol.* **65**:1289–1297.
 36. Malmstrom, R. R., R. P. Kiene, and D. L. Kirchman. 2004. Identification and enumeration of bacteria assimilating dimethylsulfoniopropionate (DMSP) in the North Atlantic and Gulf of Mexico. *Limnol. Oceanogr.* **49**:597–606.
 37. Manz, W., R. Amann, W. Ludwig, M. Wagner, and K. H. Schleifer. 1992. Phylogenetic oligodeoxynucleotide probes for the major subclasses of proteobacteria: problems and solutions. *Syst. Appl. Microbiol.* **15**:593–600.
 38. Manz, W., R. Amann, W. Ludwig, M. Wagner, and K. H. Schleifer. 1996. Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum *Cytophaga-Flavobacter-Bacteroides* in the natural environment. *Microbiology* **142**:1097–1106.
 39. Marr, D., and M. Hildreth. 1980. Theory of edge detection. *Proc. R. Soc. Lond. B.* **207**:187–217.
 40. Martinez, A., A. S. Bradley, J. R. Waldbauer, R. E. Summons, and E. F. DeLong. 2007. Proteorhodopsin photosystem gene expression enables photophosphorylation in a heterologous host. *Proc. Natl. Acad. Sci. U. S. A.* **104**:5590–5595.
 41. Mayali, X., P. J. S. Franks, and F. Azam. 2008. Cultivation and ecosystem role of a marine Roseobacter clade-affiliated cluster bacterium. *Appl. Environ. Microbiol.* **74**:2595–2603.
 42. McCarren, J., et al. 2010. Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. *Proc. Natl. Acad. Sci. U. S. A.* **107**:16420–16427.
 43. Moran, M. A., et al. 2004. Genome sequence of *Silicibacter pomeroyi* reveals adaptations to the marine environment. *Nature* **432**:910–913.
 44. Morris, R. M., K. Longnecker, and S. J. Giovannoni. 2006. Pirellula and OM43 are among the dominant lineages identified in an Oregon coast diatom bloom. *Environ. Microbiol.* **8**:1361–1370.
 45. Mou, X., R. E. Hodson, and M. A. Moran. 2007. Bacterioplankton assemblages transforming dissolved organic compounds in coastal seawater. *Environ. Microbiol.* **9**:2025–2037.
 46. Nagao, I., et al. 2009. Responses of DMS in the seawater and atmosphere to iron enrichment in the subarctic western North Pacific (SEEDS-II). *Deep Sea Res. II* **56**:2899–2917.
 47. Neef, A. 1997. Anwendung der in situ-Einzelzell-Identifizierung von Bakterien zur Populationsanalyse in komplexen mikrobiellen Biozönosen. Ph.D. thesis. Technische Universität München, Munich, Germany.
 48. Newton, R. J., et al. 2010. Genome characteristics of a generalist marine bacterial lineage. *ISME J.* **4**:784–798.
 49. Odate, T., and K. Furuya. 1995. Primary production and community respiration in the subarctic water of the western North Pacific, p. 239–253. In H. Sakai and Y. Nozaki (ed.), *Biogeochemical processes and ocean flux in the western Pacific*. Terra Scientific Publishing, Tokyo, Japan.
 50. Odate, T., and Y. Maita. 1988/1989. Regional variation in the size composition of phytoplankton communities in the western North Pacific Ocean, spring 1985. *Biol. Oceanogr.* **6**:65–77.
 51. Ogawa, H., R. Fukuda, and I. Koike. 1999. Vertical distributions of dissolved organic carbon and nitrogen in the Southern Ocean. *Deep Sea Res. I* **46**:1809–1826.
 52. Oksanen, J., et al. 2010. Vegan: community ecology package. <http://vegan.r-forge.r-project.org/>.
 53. Pernthaler, A., J. Pernthaler, M. Schattner, and R. Amann. 2002. Identification of DNA-synthesizing bacterial cells in coastal North Sea plankton. *Appl. Environ. Microbiol.* **68**:5728–5736.
 54. Pernthaler, J. 2005. Predation on prokaryotes in the water column and its ecological implications. *Nat. Rev. Microbiol.* **3**:537–546.
 55. Pinhassi, J., and T. Berman. 2003. Differential growth response of colony-forming alpha- and gamma-Proteobacteria in dilution culture and nutrient addition experiments from Lake Kinneret (Israel), the Eastern Mediterranean Sea, and the Gulf of Eilat. *Appl. Environ. Microbiol.* **69**:199–211.
 56. Pinhassi, J., et al. 2004. Changes in bacterioplankton composition under different phytoplankton regimens. *Appl. Environ. Microbiol.* **70**:6753–6766.
 57. Pukall, R., et al. 1999. High sequence diversity of *Alteromonas macleodii*-related cloned and cellular 16S rDNAs from a Mediterranean seawater mesocosm experiment. *FEMS Microbiol. Ecol.* **28**:335–344.
 58. Ramette, A. 2007. Multivariate analyses in microbial ecology. *FEMS Microbiol. Ecol.* **62**:142–160.
 59. Rappé, M. S., S. A. Connon, K. L. Vergin, and S. J. Giovannoni. 2002. Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. *Nature* **418**:630–633.
 60. R Development Core Team. 2009. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
 61. Riemann, L., G. F. Steward, and F. Azam. 2000. Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. *Appl. Environ. Microbiol.* **66**:578–587.
 62. Schäfer, H., P. Servais, and G. Muyzer. 2000. Successional changes in the genetic diversity of a marine assemblage during confinement. *Arch. Microbiol.* **173**:138–145.
 63. Shiomoto, A. 2000. Chlorophyll-*a* and primary production during spring in the oceanic region of Oyashio Water, the north-western Pacific. *J. Mar. Biol. Assoc. U. K.* **80**:343–354.
 64. Sieracki, M. E., S. E. Reichenbach, and K. L. Webb. 1989. Evaluation of automated threshold selection methods for accurately sizing microscopic fluorescent cells by image analysis. *Appl. Environ. Microbiol.* **55**:2762–2772.
 65. Simon, M., and F. Azam. 1989. Protein content and protein synthesis rates of planktonic marine bacteria. *Mar. Ecol. Prog. Ser.* **51**:201–213.
 66. Smith, D. C., G. F. Steward, R. A. Long, and F. Azam. 1995. Bacterial mediation of carbon fluxes during a diatom bloom in a mesocosm. *Deep Sea Res. II* **42**:75–97.
 67. Steward, G. F., and F. Azam. 1999. Bromodeoxyuridine as an alternative to H-3-thymidine for measuring bacterial productivity in aquatic samples. *Aquat. Microb. Ecol.* **19**:57–66.
 68. Suikkanen, S., G. O. Fistarol, and E. Graneli. 2005. Effects of cyanobacterial allelochemicals on a natural plankton community. *Mar. Ecol. Prog. Ser.* **287**:1–9.
 69. Suzuki, R., and T. Ishimaru. 1990. An improved method for the determination of phytoplankton chlorophyll using N, N-dimethylformamide. *J. Oceanogr.* **46**:190–194.
 70. Tada, Y., A. Taniguchi, and K. Hamasaki. 2009. Phylotype-specific productivity of marine bacterial populations in eutrophic seawater, as revealed by bromodeoxyuridine immunocytochemistry combined with fluorescence in situ hybridization. *Microb. Environ.* **24**:315–321.
 71. Tada, Y., A. Taniguchi, and K. Hamasaki. 2010. Phylotype-specific growth rates of marine bacteria measured by bromodeoxyuridine immunocytochemistry and fluorescence in situ hybridization. *Aquat. Microb. Ecol.* **59**:229–238.
 72. Taniguchi, A., and K. Hamasaki. 2008. Community structures of actively growing bacteria shift along a north-south transect in the western North Pacific. *Environ. Microbiol.* **10**:1007–1017.
 73. Urbach, E., K. L. Vergin, and S. J. Giovannoni. 1999. Immunochemical detection and isolation of DNA from metabolically active bacteria. *Appl. Environ. Microbiol.* **65**:1207–1213.
 74. Wallner, G., R. Amann, and W. Beisker. 1993. Optimizing fluorescent in situ-hybridization with rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms. *Cytometry* **14**:136–143.
 75. Weinbauer, M. G., and F. Rassoulzadegan. 2004. Are viruses driving microbial diversification and diversity? *Environ. Microbiol.* **6**:1–11.
 76. Welschmeyer, N. A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. *Limnol. Oceanogr.* **39**:1985–1992.
 77. Yokokawa, T., and T. Nagata. 2005. Growth and grazing mortality rates of phylogenetic groups of bacterioplankton in coastal marine environments. *Appl. Environ. Microbiol.* **71**:6799–6807.
 78. Zubkov, M. V., et al. 2001. Linking the composition of bacterioplankton to rapid turnover of dissolved dimethylsulphoniopropionate in an algal bloom in the North Sea. *Environ. Microbiol.* **3**:304–311.