Planktonic Bacterial Population Dynamics with Environmental Changes in Coastal Areas of Suruga Bay

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We studied planktonic bacterial population dynamics in response to the changing environment in a coastal system during an observation period of over 5 years using fluorescence in situ hybridization. To estimate the environmental constraint on the bacterial community, we focused on temperature, salinity, abundance of photoplankton (chlorophyll a), and dissolved organic carbon (DOC). The total number of bacteria (TDC) amounted to $3.0 \times 10^5$ to $5.0 \times 10^6$ cells mL$^{-1}$, with $1.0 \times 10^5$ to $1.0 \times 10^6$ cells mL$^{-1}$ for Bacteria, accounting for 11.8 to 74.8% of TDC, and $1.0 \times 10^4$ to $1.0 \times 10^5$ cells mL$^{-1}$ for Gammaproteobacteria, 1.0 to 20.8% of TDC. The abundance of Archaea, which contributed from 0.1 to 12% to TDC, ranged from $2.0 \times 10^3$ to $3.0 \times 10^4$ cells mL$^{-1}$. We found a positive relationship between environmental parameters such as temperature, salinity, chlorophyll a, and DOC and the abundance of total bacteria and Bacteria. The number of Gammaproteobacteria correlated with temperature, salinity, and chlorophyll a, but not with DOC. We suggest that increasing the temperature under eutrophic conditions will lead to high bacterial abundance and probably a change in the bacterial community.

Key words: temperature, salinity, Gammaproteobacteria, fluorescence in situ hybridization, coastal environment

Molecular analyses of natural bacterial assemblages have provided a knowledge of bacterial community structure directly and precisely without cultivation bias. Bacterial diversity in various environments and the predominance of a certain bacterial population in a certain marine milieu was shown by gene analysis⁵. One preceding study revealed a difference in bacterial community constituents between attached aggregates and free-living bacterial assemblages⁷. Other findings, such as the abundance of Alphaproteobacteria in estuarine and coastal environments¹¹ and the existence of Archaea in coastal waters¹³, have been revealed by the molecular analysis of prokaryotic microbial communities. The environmental control of bacterial assemblages⁴,²⁵ and the relationship between the aquatic environment and the distribution of functional bacterial groups⁵,¹³ were also shown by molecular analyses of bacterial communities¹⁵,¹⁸. Among the methods of molecular analysis, the fluorescence in situ hybridization (FISH) technique, developed by DeLong et al.⁶, provides quantitative information about bacterial community structure and the abundance of individual organisms in populations. Direct quantitative comparisons of bacterial community structures have thus become possible¹². Population shifts in response to environmental changes, such as nutrient enrichment, have also been revealed by this technique⁷. In a coastal environment, a trend in the distribution of individual bacterial groups was explained by salinity in a study in which FISH was used with probes to identify Alphaproteobacteria, Betaproteobacteria, and Cytophaga-like groups¹⁷. We investigated the
environmental regulation of bacterial abundance, focusing particularly on temperature, salinity, abundance of phytoplankton (chlorophyll $a$), and dissolved organic carbon (DOC). To consider the influence of the flow of water into the estuary from the urban area, the Gammaproteobacterial population was chased as an indicator of the enterobacterial group$^{8,10,11,26}$. The influence of the urban environment on marine microbial communities was considered in relation to salinity.

Materials and Methods

Study site and sample collection

Observation of the natural environment and water sampling were carried out from December 2001 to August 2005 at two sampling sites in Suruga Bay. Sampling was always carried out from 9:00 a.m. to 12:00. Site 1 (35°00'56"N, 138°30'58"E) was inside Shimizu Port (Fig. 1), 1.5 km northeast of the estuary of Tomoe River which was 30–70 m in width and 1–2 m deep. It was expected that the environment of Site 1 would be affected by human activity in the surrounding urban area through the inflow of river water. Site 2 (35°01'18"N, 138°31'45"E) was outside Shimizu Port and was located 1 km northeast of Site 1 (Fig. 1). This site may represent the coastal environment of Suruga Bay with less influence from urban activity.

Surface water at each site was expected to be affected directly by climate changes, such as sunshine and rainfall, whereas the water at a depth of 10 m was expected to be less influenced by these factors, except at times of a typhoon or storm. However, the water current beneath the surface might be complex in these coastal environments. Since the Secchi depth ranged from 3 to 4 m, we supposed that primary production was restricted to above about 8 m of the water body.

Water samples were collected from the surface and a depth of 10 m at both sites. The water at the surface was collected with a sterilized Pyrex bottle at each sampling site. The water at 10 m was collected with a 10-liter Niskin sampler (model 5026-D, Rigosa, Tokyo, Japan) and immediately subsampled into a sterilized Pyrex bottle for microbial analyses, measurement of chlorophyll $a$, and chemical analyses. All samples were transported to the laboratory on ice within a few hours of collection.

Sample processing

In the laboratory, a subsample was fixed with neutralized formaldehyde (pH 7.4, final concentration 5%) for a total direct count of bacteria (TDC). A subsample for FISH analysis was fixed with paraformaldehyde (pH 7.4, final concentration 3%) and kept at 4°C for up to 24 h. The fixed sample was then filtered gently on a 0.2-µm Nuclepore filter (25 mm diameter). Cells trapped on the filter were rinsed three times with PBS and dehydrated with 1 mL of 50, 80, and 100% ethanol for 3 min each, and the filter was air-dried. Filters were stored at −20°C until hybridization.

Measurement of environmental parameters

Water temperature, salinity, pH, conductivity, and dissolved oxygen (DO) were measured at the site using a water quality checker (model U-10, Horiba, Tokyo, Japan) for surface samples and 10 m deep samples from Sites 1 and 2. Chlorophyll $a$ was measured by the UNESCO method$^{28}$. DOC was analyzed with a total organic carbon analyzer (TOC-5000, Shimadzu, Kyoto, Japan) for the samples collected between 6 August 2002 and 1 December 2004. For

![Fig. 1. Sampling site: Site 1 (35°00'56"N, 138°30'58"E); Site 2 (35°01'18"N, 138°31'45"E).](image-url)
the samples collected on 15 April and 2 August 2005, DOC was analyzed with a total organic carbon analyzer (TOC-VCSH, Shimadzu). Concentrations of nutrients (NO$_3^-$, NO$_2^-$, NH$_4^+$, and PO$_4^{3-}$) were analyzed with a TRAACS 2000 analyzer (Bran+Luebbe, Norderstedt, Germany) for the samples collected between 6 August 2002 and 2 August 2005. Ammonia and phosphate concentrations were analyzed with the indophenol method$^{29}$ and the molybdenum blue method$^{22}$, respectively, for the samples collected on 1 December 2001.

**Total direct bacterial count**

Fixed samples were stained with 4',6-diamidino-2-phenylindole (DAPI; final concentration, 0.01 µg mL$^{-1}$)$^{27}$ and more than 1000 bacterial cells in 20 objective fields were counted by epifluorescence microscopy (BX50-FLA, Olympus, Tokyo, Japan).

**Whole-cell in situ hybridization**

The 16S rRNA-targeted oligonucleotide probes employed in this study are listed in Table 1. They comprised a domain-specific probe for *Bacteria* (EUB338)$^1$ and *Archaea* (ARCH915)$^{30}$, and probes specific for the phylogenetic group Gammaproteobacteria (GAM42a)$^{20}$. These probes were labeled with rhodamine (Nihon Bioservice, Saitama, Japan). In addition, we employed a non-labeled specific probe for Betaproteobacteria (BET42a)$^{20}$ as a competitor to GAM42a, because there was only one base pair mismatch between these probes.

As a negative control, we used NON338$^{32}$ and NON-GAM (5’-TTT GCT ACA CCC TTC CG-3’), complementary to probes EUB338 and GAM42a, to confirm the accuracy of our FISH procedure for *Bacteria* and Gammaproteobacteria.

Hybridization stringency was adjusted by varying the concentration of formamide in the hybridization buffer and NaCl in the washing solution$^{1,6}$. Hybridizations were performed at 46°C for 90 min on filters placed on slides coated with gelatin, hybridization buffer containing 0.9 M NaCl, 20 mM Tris-HCl (pH 7.4), 0.01% SDS, formamide (20% for EUB338 and NON338, or 35% for ARCH915, GAM42a, and NON-GAM), and 5 ng µL$^{-1}$ of the respective labeled probe. Each filter was washed at 48°C for 15 min in prewarmed washing buffer containing NaCl (0.225 M for EUB338 and NON338, 0.070 M for ARCH915, and 0.080 M for GAM42a and NON-GAM), 20 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.01% SDS, then rinsed with distilled water and air-dried. The preparations, which were counterstained with 0.1 µg mL$^{-1}$ DAPI on glass slides for 5 min, were observed under a universal epifluorescence microscopic system (BX50-FLA, Olympus) with a 3-CCD camera (model C5810, Hamamatsu Photonics, Shizuoka, Japan) and a digital camera (model DP-70, Olympus) with an image analysis system (SP500F and DP controller, Olympus).

**Statistical analysis**

We analyzed the relationship between bacterial numbers (TDC and the numbers of *Bacteria*, *Archaea*, and Gammaproteobacteria) and the environmental parameters using the Pearson product-moment correlation coefficient ($p < 0.05$ or 0.01). Statistical analysis was performed with Microsoft Office Excel 2003 SP2.

**Results**

*In situ* observations and sampling for chemical and microbial analyses were carried out nine times between 1 December 2001 and 2 August 2005 at Sites 1 and 2 (Fig. 1). Environmental fluctuation throughout the observation period is summarized in Fig. 2.

**Environmental parameters**

**Temperature** (Fig. 2a)

The observed water temperature ranged from 12.5 to 28.9°C, with no significant difference between the sites.

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Table 1. Probe sequences and target site

<table>
<thead>
<tr>
<th>Probe name</th>
<th>Target organisms</th>
<th>Sequence (5'-3')</th>
<th>Target site* rRNA Position</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUB338</td>
<td>Bacteria</td>
<td>GCT GCC TCC CGT AGG AGT</td>
<td>16S, 338–355</td>
<td>1</td>
</tr>
<tr>
<td>BET42a</td>
<td>Betaproteobacteria</td>
<td>GCC TTC CCA CTT CGT TT</td>
<td>23S, 1027–1043</td>
<td>20</td>
</tr>
<tr>
<td>GAM42a</td>
<td>Gammaproteobacteria</td>
<td>GCC TTC CCA CAT CGT TT</td>
<td>23S, 1027–1043</td>
<td>20</td>
</tr>
<tr>
<td>ARCH915</td>
<td>Archaea</td>
<td>GTG CTC CCC CGC CAA TTC CT</td>
<td>16S, 915–934</td>
<td>30</td>
</tr>
<tr>
<td>NON338</td>
<td>negative control of EUB338</td>
<td>ACT CCT ACG GGA GCC AGC</td>
<td>16S, 338–355</td>
<td>32</td>
</tr>
<tr>
<td>NON-GAM</td>
<td>negative control of GAM42a</td>
<td>TTT GCT ACA CCC TTC CG</td>
<td>23S, 1027–1043</td>
<td>this study</td>
</tr>
</tbody>
</table>

* Escherichia coli numbering$^3$. 

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inside and outside the port. Water temperature was relatively high at the surface in summer, and was high at a depth of 10 m in winter. The water temperature inside and outside Shimizu port related with that measured in the Kuroshio Current, which passes roughly 100–200 km from the shore, the difference being approximately 0.2–8.0°C.

Salinity (Fig. 2b)

Salinity fluctuated greatly at the surface both inside and outside the port irrespective of season, ranging from 25.4 to 31.2, whereas at 10 m deep, it fluctuated within a relatively small range, from 29.7 to 33.0. Except for the unusually low salinity observed on 6 August 2002 at the surface at Site 2, the pattern of fluctuation in salinity was similar inside and outside the port. This, together with the high salinity values obtained, indicates that the deep water was not strongly affected by the freshwater supply from the river, even inside the port.

pH (Fig. 2c)

The pH values observed did not fluctuate greatly except for the surface water at Site 1. The median pH value was
over 8.0, although low values were measured at the surface at Site 1 on 8 November 2002 and 30 August 2003.

**NO$_3^-$ (Fig. 2d)**

High concentrations of NO$_3^-$ were observed at the surface at Site 1, ranging mostly from 0.49 to 17.91 µM. At the three other sampling points, the concentration of NO$_3^-$ was below 10 µM. The highest concentration of NO$_3^-$ was measured at the surface inside the bay on 8 November 2002. However, no significant relation was found between NO$_3^-$ and chlorophyll $a$, as was also true for other nutrients (data not shown).

**NO$_2^-$ (Fig. 2e)**

Values were not high, ranging between 0.17 and 1.27 µM. However, there was a tendency for NO$_2^-$ concentrations to be higher at Site 1 than at Site 2. The highest concentration was observed in surface water at Site 1 on 14 June 2003.

**NH$_4^+$ (Fig. 2f)**

High concentrations of NH$_4^+$ were sometimes observed at the surface inside the port, where the range was from below the detection limit to 11.33 µM. The highest concentration was observed in the surface water inside the port on 8 November 2002. The NH$_4^+$ concentration was low, ranging from below the detection limit to 1.58 µM, throughout from 14 June 2003 to 1 December 2004.

**Total inorganic N (Fig. 2g)**

The total inorganic N concentration, the sum of NO$_3^-$, NO$_2^-$, and NH$_4^+$, was mostly high in the surface water at Site 1. The highest concentration was observed at the surface inside the port on 8 November 2002, when pH was significantly low suggesting a strong influence of inflowing water. The fluctuation of total inorganic N seemed to be governed by that of NO$_3^-$.

**PO$_4^{3-}$ (Fig. 2h)**

When the two observation sites were compared, a relatively high concentration of PO$_4^{3-}$ was found at Site 1 but the measured concentration was low, being 0.01–1.05 µM throughout the observation period. The highest concentration was observed in the surface water at Site 1 on 1 December 2001. The concentration of PO$_4^{3-}$ showed a tendency to be high when water temperature was lower than 20°C (data not shown).

**Chlorophyll $a$ (Fig. 2i)**

The chlorophyll $a$ concentration fluctuated widely at the surface, ranging from 0.27 to 16.31 mg Chl. $a$ m$^{-3}$, while at a depth of 10 m it ranged from 0.29 to 7.23 mg Chl. $a$ m$^{-3}$. High concentrations were found at the surface in winter as well as in summer. When we compared the concentration of chlorophyll $a$ between the surface and at 10 m, higher concentrations were sometimes observed outside the port, as occurred on 28 February 2004, when relatively high values were observed at both sites.

**Dissolved oxygen (Fig. 2j)**

The DO concentration did not fluctuate widely, but relatively high values were observed in surface water. The concentration was highest on 28 February 2004 (9.03–11.42 mg L$^{-1}$), which corresponded to the high chlorophyll $a$ concentration mentioned above.

**Dissolved organic carbon (Fig. 2k)**

Excepting the high concentrations measured on 6 August 2002 (164.7–206.6 µM), samples showed relatively stable values, ranging between 61.7 and 121.5 µM. No significant relation between the concentrations of DOC and chlorophyll $a$, a major source of DOC, was found at the study sites.

**Characteristics of observation sites**

**Horizontal comparison**

Compared with values outside the bay (Site 2), the concentrations of nutrients and chlorophyll $a$ inside the bay (Site 1) were high, especially at the surface, but salinity was low. Temperature, salinity, and the DO concentration showed similar patterns of fluctuation at the two sites.

**Vertical comparison**

Surface water showed considerable fluctuations in temperature and salinity, and high concentrations were found for nutrients, chlorophyll $a$, and DO. On the other hand, we observed less fluctuation in each parameter at a depth of 10 m than at the surface.

**Total direct count of bacteria**

TDC did not fluctuate markedly, with the range between 3.0 x 10$^6$ and 5.0 x 10$^6$ cells mL$^{-1}$ throughout the observation period at all stations (Fig. 3), though in two of three cases in August (6 August 2002 and 2 August 2005), abundance was high (Fig. 4a). TDC at the surface tended to be higher than that at depth for both Sites 1 and 2. Figure 4a shows that
Percentage of cells hybridized with probes

About 14.7–76.7% of all bacteria were hybridized with gene probes, targeted to *Bacteria* or *Archaea* (Fig. 4b). A high proportion of hybridization was found for samples from surface water at Site 2, with eight out of nine samples showing hybridization with more than 40% of TDC, compared to only two samples from 10 m deep at Site 2. At Site 1, four of the surface samples showed hybridization with more than 40% of TDC, compared to only two samples at 10 m. It is remarkable that bacteria sampled in summer did not necessarily show a high proportion of hybridization, and vice versa for winter samples. A high proportion of hybridization was found for surface water at Site 1 on 28 February 2004. In general, bacteria from surface samples showed a higher proportion of hybridization than samples taken at a depth of 10 m for both Sites 1 and 2 (Fig. 4b). A low proportion of hybridization was observed for all samples on 30 August 2003, which seemed to be related to a low phytoplankton biomass (Fig. 2i), though the reverse was not necessarily true as for the case of 15 April 2005.

**Bacteria**

The number of *Bacteria* ranged from $1.0 \times 10^5$ to $1.0 \times 10^6$ cells mL$^{-1}$ throughout the observation period (Fig. 3). *Bacteria*, identified by FISH, constituted 11.8–76.1% of TDC. The proportion of *Bacteria* in surface water was generally higher than that at a depth of 10 m. At 10 m, Site 2 showed a higher number of *Bacteria* than Site 1 (Fig. 4c).

A remarkably large number of *Bacteria* was found on 6 August 2002 in the surface water at Site 2 ($2.6 \times 10^6$ cells mL$^{-1}$) but the proportion with respect to TDC did not exceed 50%.

The proportion of *Bacteria* to TDC was highest on 14 June 2003 except for surface water at Site 1. In the subsequent observation carried out on 30 August 2003, both the number of cells and the proportion of *Bacteria* to TDC were lowest, when a remarkably low chlorophyll *a* concentration was observed even in the surface water at Site 1 (Fig. 2i). It is worth noting that the percentage of the sum of *Bacteria* and *Archaea* in surface water at Site 2 exceeded that at Site 1 in five out of nine observations.

**Gammaproteobacteria**

The number of *Gammaproteobacteria* ranged from $1.0 \times 10^4$ to $1.0 \times 10^5$ cells mL$^{-1}$ throughout the observation period for all stations. The number in surface water showed a tendency to be higher inside the port (Site 1) than at other sampling points (Fig. 3). The number reached ca. $3.0 \times 10^5$ cells mL$^{-1}$ in surface water at Site 1 on 1 December 2001 and 2 August 2005. The proportion of *Gammaproteobacteria* as a percentage of TDC detected by FISH ranged from 1.0 to 11.7%, except in surface water at Site 1 on 1 December 2003, where it was 20.8%. The proportion of *Gammaproteobacteria* as a percentage of *Bacteria* ranged from 1.9 to 30.4%. The highest proportion was found on 30 August 2003, when the smallest proportion of *Bacteria* relative to TDC was observed (see above). The proportion of *Gammaproteobacteria* relative to TDC and also to *Bacteria* (data not shown) at the surface showed a tendency to be higher at Site 1 than at any other sampling points.

**Archaea**

The number of *Archaea* ranged between $2.0 \times 10^1$ and $3.0 \times 10^4$ cells mL$^{-1}$, which was smaller than that of *Bacteria* by two orders of magnitude (Fig. 3). The number of *Archaea* was very low, but we detected *Archaea* in all samples examined. The proportion of *Archaea* to TDC detected by FISH ranged from 0.1 to 3.2% except for surface water at Site 2 on 1 December 2001, when *Archaea* constituted 12.0% of TDC. *Archaea* were present in greater abundance compared with all other samples on 2 August 2005, when the proportion of *Archaea* ranged from 2.1 to 2.8% of TDC (Fig. 4e).
Although the densities of Bacteria ($9.75 \times 10^4$ to $2.29 \times 10^5$ cells mL$^{-1}$, 11.8–23.8% of TDC) and Gammaproteobacteria ($2.68 \times 10^4$ to $4.70 \times 10^4$ cells mL$^{-1}$, 3.6–4.7% of TDC) were very low on 30 August 2003, the density of Archaea was not extremely low ($4.85 \times 10^3$ to $1.88 \times 10^4$ cells mL$^{-1}$, 0.7–2.3% of TDC). On 1 December 2004, the number of Archaea observed was very low compared with other samples. Neither the number nor the proportion of Archaea showed seasonal fluctuation.

**Discussion**

In the studied area, the number of bacteria decreased in the order TDC, Bacteria, Gammaproteobacteria, and Archaea. The proportion of Bacteria found in this study ranged from 11.8 to 76.1% of TDC, with a mean of 40%, which is within the range found in Chesapeake Bay$^{20}$. However, this was lower than values reported for the Baltic Sea$^{12}$, the German Bight$^9$, surface waters of coastal California$^5$, and the Delaware Estuary$^{17}$. These studies did not describe the environmental conditions, thus it is difficult
to explain the differences in these estimates. One reason may be differences in the methods employed. Since nearshore samples, in particular, contained abundant particles, we employed criteria to exclude very tiny cells and cell-like particles with low fluorescence in order to differentiate target cells clearly from non-bacterial particles in FISH.

Archaea contributed to only 0.1–3.0% of TDC in our study, which is similar to the percentage found in Chesapeake Bay.

When we analyzed the relationship between environmental parameters and bacterial abundance, temperature showed a positive relation with TDC \((p<0.01, \text{Fig. 5a})\) and Bacteria \((p<0.05, \text{Fig. 5b})\). Horizontal studies conducted in the Delaware Estuary using FISH found a good relationship between temperature and the abundance of Bacteria\(^2\). They also showed a good relation between temperature and leucine...
incorporation. Thus, temperature may directly govern bacterial activity, resulting in high abundance; however, the balance between growth and grazing loss needs to be considered carefully. In our study, a negative relationship with salinity was found for all bacterial groups analyzed: TDC \((p<0.01, \text{Fig. 5c})\), Bacteria \((p<0.01, \text{Fig. 5d})\), Gammaproteobacteria \((p<0.01, \text{Fig. 5e})\), and Archaea \((p<0.05, \text{Fig. 5f})\). Troussellier et al.\(^{31}\) also showed that the abundance of bacteria decreased with increasing salinity in a study conducted along an estuarine gradient in the Mediterranean Sea. Because we found a negative relation between the abundance of Gammaproteobacteria and salinity, and relatively low salinity was observed in surface water (Fig. 2b), we surmised that the abundance of Gammaproteobacteria was influenced by discharged river water either directly or indirectly. Understanding of the environmental control of the abundance of Archaea is still limited, particularly for coastal waters\(^{23}\). The relation between salinity and the abundance of Archaea needs detailed analysis with respect to genetic composition and whether a comparison can be made with marine archaean profiles\(^{23}\).

The abundance of phytoplankton, represented by chlorophyll \(a\), displayed a clearer relation with the number of Bacteria \((p<0.01, \text{Fig. 6b})\) and somewhat less of a relation with TDC and the number of Gammaproteobacteria \((p<0.05, \text{Fig. 6a and 6c})\). The possibility of controlling bacterial abundance by using phytoplankton biomass has long been suggested and recently it was experimentally elucidated by Horner-Devine et al.\(^{14}\). Furthermore, TDC and the number of Bacteria related strongly with DOC \((p<0.01, \text{Fig. 6d and 6e})\). DOC is known to be a key factor that drives bacterial activity and abundance\(^{16,24}\).
Measurements of salinity suggested that the freshwater discharge directly affected the surface water chemistry at Site 1, being located inside the Shimizu Port and surrounded completely by factories and domestic buildings, but the influence did not seem to reach a depth of 10 m at this site. The freshwater discharge containing a large amount of DOC (about 200 µM) led to a 10–60% higher concentration of DOC in surface water at Site 1 compared with the other sampling points.

Furthermore, it was suggested that the freshwater supplied a large amount of nutrients to Site 1. High concentrations of nutrients were also observed at the surface at Site 1 compared with the other sampling points. Nutrient concentrations there were 1- to 40-fold higher than at 10 m depth at Site 2, particularly (Fig. 2).

Relations between environmental parameters and bacteria, thus, provide us an overview of planktonic bacterial abundance in a coastal area being influenced by freshwater supply through a river. Bacterial abundance was influenced by differences in temperature, though this effect did not appear in archaeal abundance. Phytoplanктон was another regulator of bacterial abundance. The interesting relation between temperature and bacterial abundance, which appeared clearly in samples from a depth of 10 m at Site 1, where salinity did not change much (Fig. 2b) and bacterial abundance (except Archaea) related well with DOC, may suggest that increasing temperature accompanying eutrophication will lead to high bacterial abundance and probably a change in bacterial community structure. The importance of temperature as a constraint on marine bacterial communities may increase with the continuation of the greenhouse effect.

Another exciting finding concerns Archaea and their environmental control. Whether a negative relation between salinity and the abundance of Archaea can be explained by the supply of freshwater is uncertain, at least until information is available on the genetic constituents of Archaea in comparison with those at upstream locations.

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