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Relationship between bacterial community structure, light, and carbon cycling in the eastern subarctic North Pacific

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Abstract

Biogeochemical controls on the community structure of heterotrophic marine bacteria are not well understood, and these organisms play a critical role in the global carbon cycle. Through terminal restriction fragment length polymorphism (T-RFLP) analyses of bacterial 16S rRNA genes along a zonal transect in the eastern subarctic North Pacific, variations in community structure were compared to commonly obtained chemical and biological oceanographic measurements. It was found that heterotrophic bacterial community structure was strongly, but independently, related to both phytoplanktonic production and light. Based on computer-simulated T-RFLP analyses of database 16S rRNA gene sequences, it appeared that the relationship between bacterial community structure and light observed along the transect was driven by the distribution of α-proteobacteria with phototrophic capabilities. In addition, experiments were conducted at sea to measure the growth of heterotrophic bacteria in response to amendments of amino acids, protein, N-acetyl glucosamine, and chitin. Variation in the response of heterotrophic bacteria in the experiments did not correlate significantly with variation in community structure along the transect, which suggests that the ability of the heterotrophic bacterial community to use specific components of the dissolved organic carbon reservoir was not related to its phylogenetic structure as detected by T-RFLP. However, a possible relationship between community structure and the cycling of chitin is discussed. Overall, the results stress the importance of both heterotrophic and phototrophic metabolisms when considering environmental controls on the structure of bacterial communities in the sea.

The primary heterotrophic agents in the sea are planktonic bacteria (del Giorgio et al. 1997). Through respiration, heterotrophic bacteria check the accumulation of organic matter, regenerate nutrients for photosynthetic production, and thereby modulate the net flux of carbon dioxide into the sea. Molecular methods targeting 16S rRNA genes have revealed many details of the phylogenetic diversity of these organisms (e.g., Rappé et al. 1997), and direct connections between overall bacterial community structure and environmental parameters commonly measured by oceanographers are emerging. The integration of genetically derived community information with biological and chemical oceanographic data is critical to test whether community structure affects the function of heterotrophic bacteria within marine carbon and nutrient cycles.

Since growth by heterotrophic bacteria in the sea is generally correlated to growth by phytoplankton, it is commonly thought that the community structure of heterotrophic bacteria must also be related to phytoplanktonic growth. This view has been supported by studies showing that the abundance of specific bacterial groups correlates with chlorophyll *a* (Chl *a*) concentrations. For example, González et al. (2000) observed that the abundance of the *Roseobacter* clade of marine bacteria was correlated with Chl *a* in vertical profiles through an algae bloom in the North Atlantic, while in a horizontal survey of coastal North Pacific surface waters Suzuki et al. (2001) detected corresponding peaks in the abundance of the SAR86 clade and Chl *a*. Other work has shown that specific bacterial phyla specialize in the respiration of specific dissolved organic carbon (DOC) molecules produced by plankton (Ouverney and Fuhrman 1999; Cottrell and Kirchman 2000; Zubkov et al. 2001), which suggests complex relationships between the abundance of specific groups of heterotrophic bacteria and specific types of algae and zooplankton.

In contrast, solar irradiance alone, independent of its role in supporting phytoplanktonic production, may have a significant impact on the structure of the putatively heterotrophic bacterial community. It has been shown that members of the γ-proteobacterial SAR86 clade possess the ability to use light directly for ATP production (Béjà et al. 2000), while α-proteobacteria of the *Erythrobacter* genus (Kolber et al. 2001; Koblíek et al. 2003) and *Roseobacter* clade (Béjà et al. 2002; Allgaier et al. 2003) have been shown to engage in photosynthesis supported by bacteriochlorophyll *a* (BacChl *a*). These bacterial phototrophic strategies may be widespread in the sea (Kolber et al. 2000; Béjà et al. 2001; Goericke 2002); however, it is unclear what role phototrophy plays in the microbial loop or in shaping the structure of the heterotrophic bacterial community.

We hypothesized that bacterial community structure was

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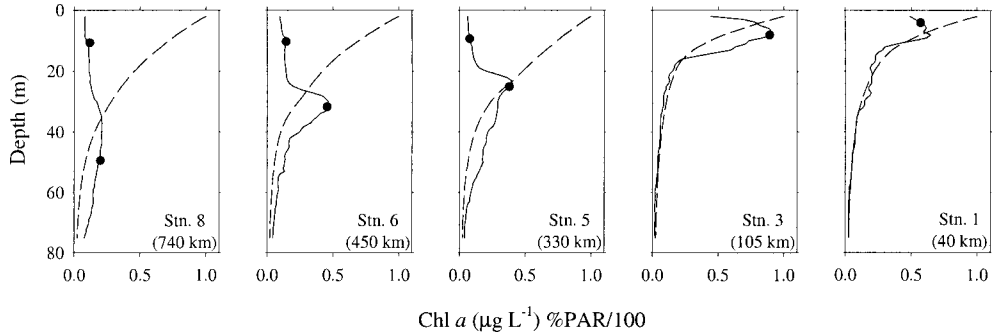

Fig. 1. Profiles of Chl *a* (solid) and %PAR (dashed) with depth. Circles in the Chl *a* profiles indicate depths where samples were taken for this study.

related to light and other environmental conditions in the sea and that differences in community structure were related to the ability of heterotrophic bacteria to respond to specific DOC molecules. To test these hypotheses, we studied water samples collected at five stations in a >700-km transect off the coast of Washington State. This transect bracketed the range of eastern subarctic North Pacific chemical and biological conditions (high-nitrate, low-chlorophyll to nearshore upwelling) and spanned nearly 2 orders of magnitude in $[NO_3^-]$, $[PO_4^{3-}]$, bacterial production, and Chl *a* fluorescence. We examined the phylogenetic structure of bacterial communities along our transect using terminal restriction fragment length polymorphism (T-RFLP) analyses of 16S rRNA genes (Moeseneder et al. 1999; González et al. 2000) to determine the number and ecological distribution of operationally defined taxonomic units (OTUs). We applied multidimensional scaling (MDS) to the T-RFLP results to determine the relationships between the bacterial communities we sampled. We then used this information to identify connections between bacterial community structure, chemical and physical gradients, and the growth response of heterotrophic bacteria in short-term experiments amended with defined components of the dissolved organic carbon (DOC) reservoir. Our results suggest that bacterial community structure was independently related to both phytoplanktonic production and light along the transect we studied. The growth response of the total bacterial community to the amendments was generally unrelated to community structure as defined by T-RFLP.

Methods

Our cruise was conducted along 47°N from 124°W to 134°W in July 2001 during a period of strong seasonal upwelling. Samples were taken at the surface (5–10 m) at all stations and at the deep chlorophyll maximum (DCM) at Stas. 5, 6, and 8 (Fig. 1). Transmissivity, Chl *a*, $[O_2]$, and temperature were determined aboard the R/V *Thomas G. Thompson* with Seabird instruments mounted on a Niskin bottle rosette. Light at the depths we sampled is expressed as a percentage of the photosynthetically available radiation (%PAR) at the sea surface and was calculated from a standard model that incorporates light attenuation as a function of water depth and depth-integrated Chl *a* (Mobley 1994). Nutrients were determined onshore from frozen samples, except for samples at Stas. 6 and 8, which were estimated from temperature using data from this transect and our extensive database at the University of Washington.

T-RFLP is a technique used to rapidly distinguish bacterial communities based on the size of the terminal fragments of 16S rRNA genes following restriction endonuclease digestion. We applied the protocol described by Moeseneder et al. (1999). Seawater was filtered through 0.2-μm pore size cellulose nitrate membranes (Whatman), and DNA was extracted from the membranes using standard lysozyme and proteinase K digestions followed by a phenol/chloroform extraction and ethanol precipitation. 16S rRNA genes were amplified with FAM-labeled 27F and nonlabeled 1492R primers (Lane 1991). PCR products were purified using spin columns (Qiagen), cut in three separate digests using four-cutter restriction enzymes (*Hin*6I, *Rsa*I, and *Bsh*1236I; Fermentas), and analyzed on a Mega-BACE DNA sequencer (Amersham Biosciences) in genotyping mode with internal Et-ROX labeled DNA size standards. OTUs were identified as peaks composing >1.0% of the total peak area for 16S rRNA positions ca. 60 through ca. 930. Positions were scored for presence or absence of an OTU and thus were converted to one 870-character binary sequence for each digest. The sequences from each digest were then combined for each sample, and a distance matrix was created (Moeseneder et al. 1999). This matrix was solved in two dimensions by MDS (B. Crump pers. comm.) using Statistica software (Statsoft) to provide a mathematical representation of the distances between the T-RFLP data for each sample, a process that identified clusters of samples. We then applied UPGMA cluster analysis with bootstrap resampling using Phylip software (J. Felsenstein, University of Washington, Seattle) to provide confidence estimates for the clusters identified by MDS.

To specifically investigate the impact of phototrophic bacteria on the overall community structure as determined by T-RFLP, we conducted computer-simulated restriction digests with 16S rRNA sequences obtained from GenBank and identified OTUs potentially derived from bacteria with demonstrated phototrophic capabilities. This approach is in lieu of the construction of a clone library from the same location (e.g., González et al. 2000) that would have yielded more definitive assignments. The sequences in the simulation in-

cluded those from γ-proteobacteria in the SAR86 cluster (GenBank accession AF001651 and AF001650), as well as α-proteobacteria of the *Erythrobacter* genus (AF310724, AB012062) and the *Roseobacter* clade (U78912, AB018689, AJ534215). Terminal restriction fragments from these phototrophic organisms were screened for specificity against sequences from representatives of other major marine clusters of γ- and α-proteobacteria (Rappé et al. 1997): SAR83 cluster (U70680), SAR116 cluster (U70678), SAR11 cluster (U70684), *Teridinibacter* subgroup (U70696), *Oceanospirillum* ass. (U70699), *Pseudomonas* subgroup (U70697), and the *Colwellia* ass. (U70695). Some of these sequences were not complete for the region of the 27F forward primer, and the size of the terminal restriction fragment for these sequences was estimated by filling in the gap with a sequence from a close relative (González et al. 2000).

Seawater samples, from the same depth and locations analyzed by T-RFLP, were incubated at in situ temperature and amended with either amino acids, protein, N-acetyl glucosamine (NAG), or chitin. These DOC amendment incubations were conducted by placing unfiltered seawater in triplicate, clean, 20-ml, glass vials that were immediately amended to 50 μmol C L^{-1}. This DOC concentration was chosen because it was the lowest concentration that yielded a measurable response with our technique at nearshore stations (Van Mooy 2003). These experiments were kept in the dark at in situ temperature, and after 1.5 h a 0.5-h ^{3}H-thymidine incorporation measurement was conducted using a standard method (Bell 1993). All amendments were >95% pure (Sigma Chemical). The protein amendment was bovine serum albumin (BSA), and the amino acids were mixed individually to compose the same distribution as BSA. Chitin was mildly hydrolyzed as described previously (Kirchman and White 1999).

Results

Surface water [NO_3^-] concentrations ranged from 18.4 to <0.05 μmol L^{-1}, and bacterial ^{3}H-thymidine incorporation rates ranged from 30.8 to 0.2 nmol L^{-1} h^{-1}; both parameters decreased offshore (data not shown). Salinity varied by only 0.1 in the surface waters of the transect, which indicates that input from rivers was not a source of variation in our data (data not shown). Seasonal upwelling had weakened nearshore water column stratification and supplied abundant dissolved nutrients to the surface layer, which supported phytoplankton growth as indicated by the surface maximum in Chl *a* (Stas. 1 and 3; Fig. 1). Farther offshore (Stas. 5, 6, and 8), the water column was stratified and surface waters were depleted of dissolved nutrients; phytoplankton were thus constrained to the lower, nutrient-rich region of the euphotic zone below the mixed layer, as indicated by the typical North Pacific deep chlorophyll maximum (DCM).

Our T-RFLP measurements yielded a total of 180 unique OTUs with an average of 54 ± 6 OTUs in each sample. MDS was applied to determine the relative phylogenetic relatedness of the communities by sample (Fig. 2). These results showed community variation along two major dimensions that separated the bacterial communities into three

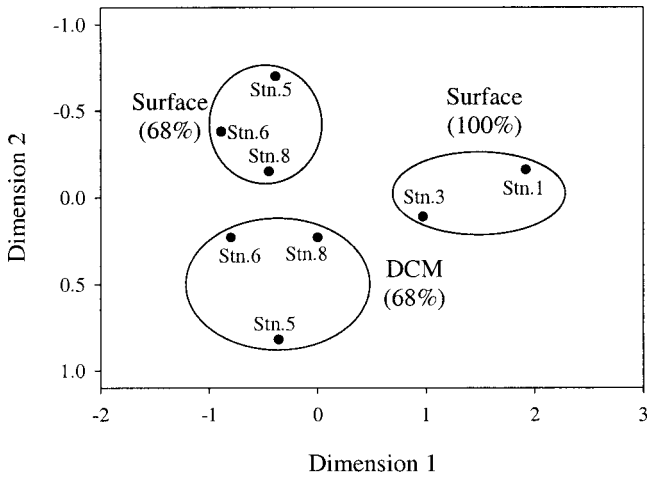

Fig. 2. Multidimensional scaling plot of the bacterial communities from North Pacific stations based on T-RFLP of 16S rRNA genes, which shows that samples from similar oceanographic settings were relatively similar to one another (circled clusters). The clusters were supported by UPGMA analysis, and the percentage of 100 bootstrap resamplings is indicated in parentheses. Like a treeplot, which is essentially a one-dimensional visualization of a distance matrix, this plot is a two-dimensional visualization of the distance matrix. The coordinates for each sample in this plot are values that describe the samples' position within the overall community variation we observed on our transect. The stress of the plot is 0.045, which is well below the commonly held threshold of 0.15 for acceptability.

clusters: surface communities at nearshore Stas. 1 and 3; surface communities at offshore Stas. 5, 6, and 8; and DCM communities at offshore Stas. 5, 6, and 8. These clusters are statistically robust as indicated by UPGMA bootstrap resampling percentages (Fig. 2).

We performed regression analyses with dimensions 1 and 2 from the T-RFLP/MDS analyses and the environmental parameters we collected on the cruise (Web Appendix 1: http://www.aslo.org/lo/toc/vol_xx/issue_x/xxxxa1.pdf). Dimension 1 was significantly related to O_2 supersaturation (Fig. 3), a general proxy for net photosynthetic production, as well as Chl *a*, ^{3}H-thymidine incorporation, [NO_3], [PO_4], turbidity, and distance from shore. All of these parameters generally covary with phytoplanktonic production. In contrast to dimension 1, dimension 2 covaried with only one environmental variable, %PAR (Fig. 4); %PAR did not covary with other variables associated with photosynthetic production because of stratification-induced nutrient depletion in the offshore stations. Plastid genes (from cyanobacteria or eukaryotic phytoplankton) did not contribute significantly to the T-RFLP–derived community data along this transect (Van Mooy 2003), so the correlation of %PAR with bacterial community structure variation along dimension 2 (Fig. 4) is a signal only of the putatively heterotrophic bacteria. Ultraviolet radiation was not measured, but recent work has suggested ultraviolet radiation has little impact on bacterial community structure (Winter et al. 2001).

The computer-simulated restriction digests offered suggestive evidence for the presence of phototrophic bacteria on our transect. The simulation indicated that each restriction

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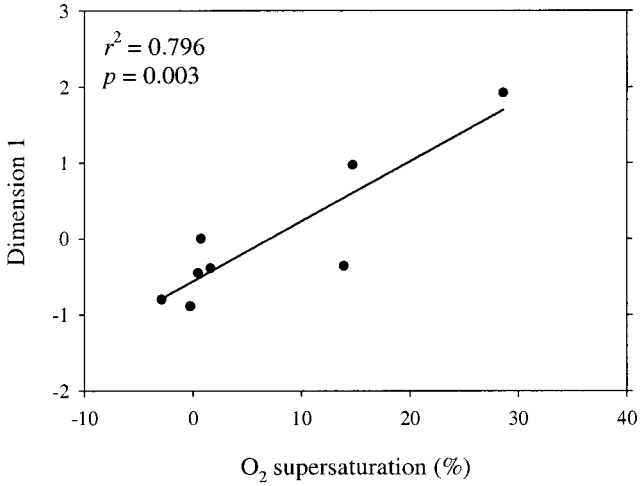

Fig. 3. Type I linear regression of dimension 1 on O_2 supersaturation (%).

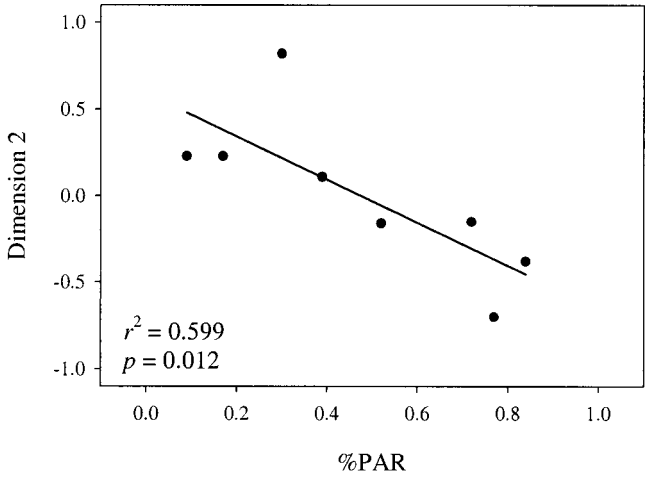

Fig. 4. Type I linear regression of dimension 2 on %PAR.

enzyme we employed would yield a unique terminal restriction fragment (i.e., OTU), for the SAR86 cluster: *Hin*6I a fragment of 365 base pairs (bp), *Rsa*I a fragment of 644 bp, and *Bsh*1236I a fragment of 108 bp. Every sample we analyzed by T-RFLP contained these OTUs. However, because the SAR86 OTU was present throughout our transect, it did not affect any presence/absence signal and, hence, did not significantly contribute to variation in T-RFLP/MDS results. In contrast, the simulations revealed a variety of OTUs for the *Erythrobacter* genus and *Roseobacter* clade, and these OTUs were present in some samples but not in others. Both groups yielded fragments in the range of 96–99 bp and 420–423 bp with the *Bsh*1236I and *Rsa*I restriction endonucleases, respectively. *Hin*6I yielded fragments in the range of 337–341 bp for some *Roseobacter* sequences, while other *Roseobacter* and *Erythrobacter* OTUs were <80 bp and were not reliably detected by the Mega-BACE sequencer. Nearly all of the α-proteobacterial OTUs identified by the computer simulation were found in at least one of the environmental samples. Further, the number of OTUs from the *Erythrobacter* genus and *Roseobacter* clade was negatively correlated with %PAR ($p = 0.006$; data not shown) and positively correlated the MDS dimension 2 ($p = 0.008$; data not shown). Thus, the correlation between dimension 2 and %PAR is driven, at least in part, by the distribution of α-proteobacteria with potential phototrophic capabilities.

The heterotrophic bacterial community expressed a wide range of responses to amendments of specific biomolecules in DOC, as indicated by ^{3}H-thymidine incorporation rates. Data from each DOC amendment experiment were normalized to the no-addition control and are expressed as response factors (Fig. 5). All of the amendments generally stimulated growth at the nearshore surface stations, but additions of some compounds inhibited growth in some samples, particularly those from the DCM. Regressions of these data against dimensions 1 and 2 from the T-RFLP analyses revealed no statically significant ($p < 0.05$) correlations. However, the correlation between dimension 2 and chitin response factor, though perhaps not statistically significant ($p = 0.087$), is suggestive of a possible relationship (Fig. 6). In addition, the growth response to chitin was significant in six of the eight locations tested (Fig. 5).

Discussion

We sought to compare bacterial community structure to chemical, physical, and biological data collected on an offshore transect into the North Pacific. Dimension 1 of the T-RFLP community analysis was correlated strongly with those variables that typically covary with phytoplanktonic production (such as percentage O_2 supersaturation; Fig. 3), which generally supports the view that bacterial community structure was controlled by the same processes that controlled bacterial growth rate. Our data do not specifically

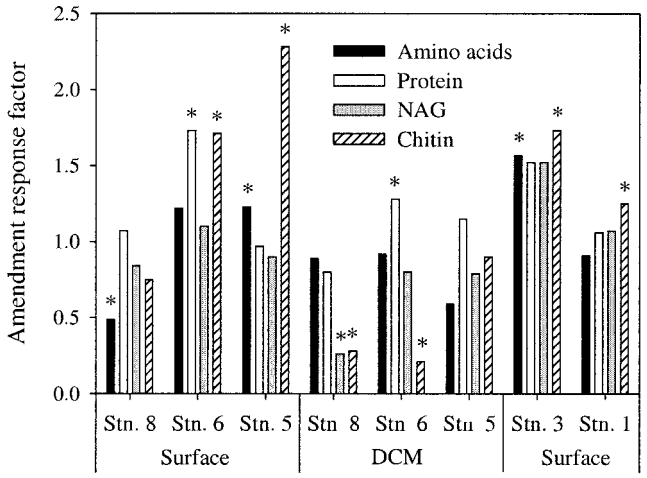

Fig. 5. Relative response of heterotrophic bacteria in the DOC amendment experiments as measured by ^{3}H-thymidine incorporation. The rates in each experiment were divided by the rate in the no-amendment control experiment. Thus, a value of 1 indicates no response to the amendment. Asterisks denote the responses that were significantly different (*t*-test; $p < 0.05$) from the no-amendment control experiments (i.e., those values that are significantly different from a value of 1).

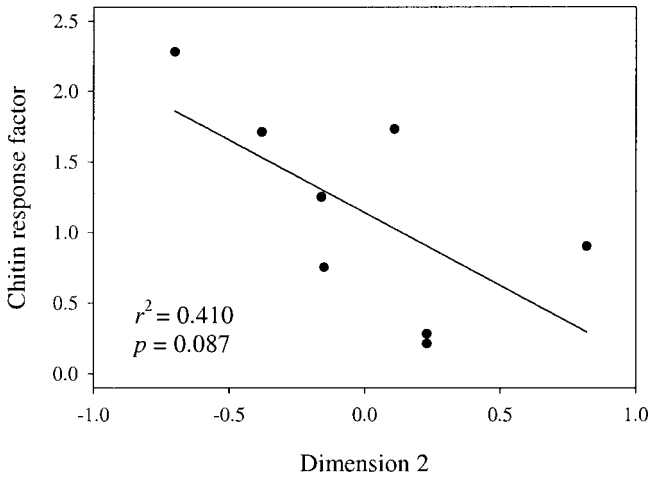

Fig. 6. Type I linear regression of chitin response factor on dimension 2.

constrain whether substrate availability or grazing controls were at play on our transect, but given that bacteria in the surface waters generally exhibited enhanced ^{3}H-thymidine incorporation in response to DOC amendments (Fig. 5), substrate control was likely at these locations. This result is in agreement with Kirchman (1990), who also observed growth stimulation by nitrogen-rich substrates in surface waters of the subarctic North Pacific. In contrast, the DCM populations were inhibited by the amendments; Button et al. (1993) have shown that some bacteria isolated from the subarctic North Pacific are intolerant to organic substrates at concentrations similar to those used in this study. So although it is equivocal whether community structure is controlled by substrate availability or grazing, the transect-wide correlation between dimension 1 and photosynthetic parameters suggests some common control that covaries with photosynthetic production.

The correlation between dimension 2 and %PAR suggests that light was acting as an additional independent and direct control on bacterial community structure (Fig. 4). Marine bacteria within the α-proteobacteria were previously thought to be strictly heterotrophic in oxic seawater (e.g., Schmidt et al. 1991). However, recent work has shown that many organisms within the *Erythrobacter* genus (Koblíek et al. 2003) and *Roseobacter* clade (Allgaier et al. 2003) are capable of BacChl *a*–mediated photosynthesis. Variation in the distribution of OTUs from these two groups was observed to correlate with %PAR in our transect off the Washington coast, and this suggests that the quantity of light may have been an important factor in determining the distribution of these two groups. Indeed, data from Kolber et al. (2001) show that the abundance of BacChl *a*–containing cells composed a variable fraction of the total bacterial community in a depth profile that was obtained off Washington in close proximity to Sta. 6; we conjecture that such variation could have been a reflection of phototrophic bacterial community structure variation as a function of %PAR. In contrast to the findings reported here, González et al. (2000) hypothesized that the abundance of the *Roseobacter* clade in the open North Atlantic was related to the ability of these organisms to use dissolved organic compounds produced by phytoplankton. But in the data reported here, there was no correlation between %PAR and indicators of production by phytoplankton, and hence it is not likely that there was a relationship between %PAR and DOC production. Further, Suzuki et al. (2001) reported no such correlation between their measurements of the abundance of *Roseobacter* clade cells and Chl *a* in the coastal North Pacific off California. Thus, it is quite possible that the *Roseobacter* clade may be controlled by the availability of light in some environmental settings, but by the availability of DOC in others.

To test the second component of our hypothesis, that bacterial community structure was related to the ability of the planktonic bacteria to respond to DOC, we conducted amendment experiments with defined components of the DOC reservoir. Amino acids, protein, NAG, and chitin were chosen because they constitute the bulk of planktonic production in the sea (Keil and Kirchman 1999; Kirchman and White 1999) and are turned over rapidly by bacteria (Fuhrman 1987; Cottrell and Kirchman 2000). These experiments assessed the growth response of bacteria over a 2-h interval, an approach that varies significantly from the more commonly practiced incubation strategies where cell numbers and community structure are measured over the course of several days (e.g., Carlson et al. 2002). We believe that data from our experiments reflect the instantaneous demand by bacteria for the amendments, while findings from longer term incubations generally indicate the potential of the community as a whole to adapt to amendment conditions. We used an amendment concentration of 50 μmol C L^{-1}, which represented a marked enrichment over bulk seawater concentrations; however, environmental concentrations may approach these levels in patches in the sea (Azam and Ammerman 1984), such as in marine snow where bulk DOC concentrations may approach 10 mmol C L^{-1} (Alldredge 2000). Thus, we posit that data from our experiments are a reflection of the short-term ability of bacteria to respond to pulses of DOC as may be experienced in the environment and that this information is complementary to longer term experiments using less concentrated amendments.

The results of the amendment experiments suggest that there was no relationship between the structure of the heterotrophic bacterial community and the ability of the community to grow in response to specific biomolecules. Broadly defined groups of bacteria such as the α-proteobacteria or the *Cytophaga-Flavobacter* cluster have been shown to take up (Cottrell and Kirchman 2000), and grow in response to (Van Mooy 2003), the amendments applied in this study. Indeed the data presented here do not preclude the same responses to these amendments by these same groups. Instead, the data show only that the presence or absence of specific OTUs does not necessarily constrain the ability of the overall community to respond to defined components of DOC. This suggests that the anabolic capabilities of the communities on our transect were not correlated with the phylogenetic diversity identified by T-RFLP. A possible exception to this finding is the notable ($p = 0.087$) correlation between the growth response to chitin and community structure (Fig. 6). Cottrell and Kirchman showed that although only a few percent of marine bacteria were chitin degraders

(Cottrell et al. 1999), members of both the α-proteobacteria (Cottrell and Kirchman 2000; Cottrell et al. 2000) and the *Cytophaga-Flavobacter* (Cottrell and Kirchman 2000) cluster were particularly adept at the metabolism of chitin. Organisms within these groups apparently either mediate different steps in the overall metabolism of chitin or express different chitinase genes (Cottrell et al. 2000); thus, it is conceivable that the ability of the overall community to respond to chitin may be linked to the presence/absence of specific OTUs.

In conclusion, we observed that bacterial community structure was related to photosynthetic production and, independently, %PAR on our transect in the eastern subarctic North Pacific. It appears, based on computer-simulated restriction digests, that variation in the presence of phototrophic α-proteobacteria was responsible for correlation between bacterial community structure and %PAR. These observations lend support, albeit indirect, to the emerging notion that phototrophic strategies are critical for growth by many groups of marine bacteria that were, until recently, thought to be exclusively heterotrophic. The heterotrophic bacterial community exhibited a range of growth responses to amino acids, protein, NAG, and chitin; however, our methods failed to identify any strong relationships between these data and structure of the community. These results imply interplay between bacterial community structure, solar irradiance, and the metabolism of specific biomolecules that remains to be fully elucidated.

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