A latitudinal diversity gradient in planktonic marine bacteria

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For two centuries, biologists have documented a gradient of animal and plant biodiversity from the tropics to the poles but have been unable to agree whether it is controlled primarily by productivity, temperature, or historical factors. Recent reports that find latitudinal diversity gradients to be reduced or absent in some unicellular organisms and attribute this to their high abundance and dispersal capabilities would suggest that bacteria, the smallest and most abundant organisms, should exhibit no latitudinal pattern of diversity. We used amplified ribosomal intergenic spacer analysis (ARISA) whole-assemblage genetic fingerprinting to quantify species richness in 103 near-surface samples of marine bacterial plankton, taken from tropical to polar in both hemispheres. We found a significant latitudinal gradient in richness. The data can help to evaluate hypotheses about the cause of the gradient. The correlations of richness with latitude and temperature were similarly strong, whereas correlations with parameters relating to productivity (chlorophyll, annual primary productivity, bacterial abundance) and other variables (salinity and distance to shore) were much weaker. Despite the high abundance and potentially high dispersal of bacteria, they exhibit geographic patterns of species diversity that are similar to those seen in other organisms. The latitudinal gradient in marine bacteria supports the hypothesis that the kinetics of metabolism, setting the pace for life, has strong influence on diversity.

biogeography | latitude | biodiversity | kinetics | ARISA

In 1807, Alexander von Humboldt wrote "The nearer we approach the tropics, the greater the increase in the variety of structure, grace of form, and mixture of colors, as also in perpetual youth and vigor of organic life." The increase in numbers of animal and plant species from the poles toward the equator is one of the most pervasive patterns of life on earth. Although known at least since the early 1800s, this pattern still lacks a consensus explanation (1–6). And although well documented in large, multicellular animals and plants, this pattern is reported to be relatively weak or absent in morphospecies of unicellular organisms (4). The lack of apparent geographic pattern has been attributed to high abundances, frequent and long-distance dispersal, and low extinction rates (7). This argument would suggest that bacteria, even smaller, more abundant, and more readily dispersed than protists (8), would also show little or no latitudinal gradient of diversity.

Geographic patterns of diversity in bacteria may contribute to understanding the pervasiveness of the patterns and the underlying mechanistic processes in other organisms. Current explanations for geographic gradients of diversity can be divided into three major classes: historical, ecological, and evolutionary (5). The high abundances and dispersal potentials of bacteria may minimize the legacies of historical tectonic and climatic events on contemporary patterns of diversity, leaving ecological and evolutionary factors as primary causes (this is especially true for near-surface planktonic marine bacteria, the objects of this study). Numerous ecological and evolutionary hypotheses have been proposed for the gradient (5, 6), and current theory and data consistently support two ecological mechanisms. First, diversity increases with increasing productivity, because higher rates of resource supply can potentially support larger numbers and more specialized kinds of organisms (e.g., refs. 9–12). This could be termed "the larger pie can be divided into more pieces" hypothesis. Second, diversity increases with increasing environmental temperature because of the kinetics of biological processes, including rates of reproduction, dispersal, species interaction, mutation, adaptive evolution, and speciation (2, 13, 14). This could be termed "the Red Queen runs faster when she is hot" hypothesis.

These two mechanisms are by no means mutually exclusive. Their relative contributions can be assessed by analyzing patterns of diversity and relationships with environmental variables across different environments and taxa of organisms. So, for example, the role of productivity can be assessed by comparisons between terrestrial environments, where productivity is generally correlated with temperature, and marine and freshwater environments, where productivity is controlled primarily by nutrient supply. Many studies have documented a latitudinal gradient in diversity of marine organisms, including both fish and invertebrates, benthic and planktonic forms (e.g., 15–18). The fact that many of the species-rich tropical marine environments are high in temperature but relatively low in productivity suggests that kinetic mechanisms play a primary role.

Here, we quantified variation in diversity of pelagic marine bacteria in relation to latitude and environmental variables. We analyzed >100 samples from 57 locations around the world, carefully standardizing to ensure a consistent habitat, near-surface waters, a uniform protocol to collect and process samples, and a carefully controlled methodology to precisely quantify diversity. The 103 samples were collected from open ocean and coastal locations (Fig. 1), from all seasons over several years [supporting information (SI) Dataset S1]. Data associated with each sample, or at least a substantial subset thereof, included latitude and longitude, temperature, chlorophyll concentration, salinity at the depth of the sample, and direct bacterial counts. Estimated annual average sea surface temperature and annual average primary production rate were determined by using satellite-based data and standard algorithms.

We used rapid and high-resolution whole-assemblage genetic fingerprinting to characterize bacterial genotype diversity. The version we used, ARISA, involves PCR amplification of the highly variable intergenic spacer region between the 16S and 23S rRNA genes, followed by separation and detection of the different-length

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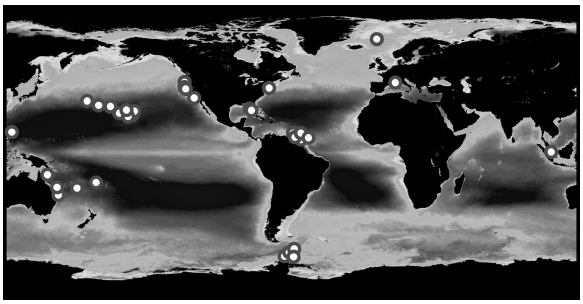

Fig. 1. Approximate sample locations superimposed on a SeaWiFS satellite image of average ocean color, with darker colors representing lower chlorophyll concentrations.

products (19). ARISA is highly repeatable and differentiates "operational taxonomic units" (OTUs) that differ by ≈98% or less in 16S rRNA sequence similarity, which is typically considered near the "species" level of taxonomic resolution (20). To our knowledge, ARISA detects members of all known major groups of surface-dwelling marine planktonic bacteria, and potential artifacts from factors like multiple gene copies are expected to be small in this environment (21). Here, we quantify bacterial diversity in terms of richness: the number of detectably different 16S-23S rRNA spacer sequence lengths or OTUs, in a standardized sample. These methods have allowed us to detect patterns of diversity.

Results

The data, displayed as bivariate plots (Fig. 2) or as summary statistics (Table 1), reveal several patterns. Richness as a function of environmental variables exhibited considerable variation. Coefficients of correlation (r) were modest, even when regressions were highly significant because of the large sample size. So there was considerable unexplained variation. In part because of this variation, the data points in bivariate plots were often not well fit by linear regressions. Instead, the values were often distributed within what appeared to be "constraint envelopes" of roughly-triangular shapes (Fig. 2).

Despite the variation, strong patterns were evident. Bacterial richness was strongly and inversely correlated with latitude, whether we used all of the data ($r = -0.388$; $P = 0.00005$) or treated the average of the multiple samples from the San Pedro Ocean Time Series (SPOT) that were all collected at the same location over a period of years as a single data point ($r = -0.422$, $P = 0.0011$) (Table 1). Richness values for the SPOT samples were normally distributed about the mean (Fig. S1), supporting the use of parametric statistical analysis and suggesting that some of the variation among the single samples from other locations likely reflected such within-site temporal variation.

As indicated in Table 1, bacterial richness was also strongly and positively correlated with water temperature at the time of sampling ($r = 0.337$; $P = 0.0005$), and even more strongly with average annual sea-surface temperature ($r = 0.449$; $P = 0.0004$, with SPOT data averaged). Because richness was about equally well correlated with temperature and latitude, it is impossible to say which environmental parameter best accounts for the variation in richness. The approximately-triangular constraint envelopes suggest that samples from all high-latitude areas had low richness (≈40–60 OTUs), but only samples from warm tropical waters had high richness (>100 OTUs).

In contrast to its strong correlations with latitude and temperature, richness was not as well correlated with most other variables, especially those indexing productivity. These included chlorophyll concentration at time of sampling ($r = 0.039$; $P = 0.74$), annual primary productivity ($r = 0.189$; $P = 0.16$), distance from shore ($r = 0.129$; $P = 0.34$), and bacterial abundance at the time of sampling ($r = -0.167$; $P = 0.20$). Richness was somewhat correlated with salinity at the time of sampling ($r = 0.275$, $P = 0.005$). This relation was strongly influenced by one outlier, an estuarine sample from Long Island Sound with exceptionally low salinity and richness; removing it yielded $r = 0.225$, $P = 0.023$. So the relationship between richness and salinity was relatively weak within the oceanic range of salinity.

There were some other notable significant correlations among environmental variables other than richness (Table 1): Chlorophyll was negatively correlated with temperature and salinity. Annual average primary productivity was negatively correlated with latitude and salinity, but positively correlated with temperature and chlorophyll (note that these two latter parameters were included in the calculation of productivity).

Discussion

Planktonic marine bacteria can be added to the long and rapidly increasing list of taxa that exhibit a latitudinal gradient of increasing diversity from the poles toward the equator. The exact nature of the latitudinal gradient in bacteria is still somewhat uncertain, because of the substantial unexplained variation. The apparently triangular relationships suggest a limit, but not tight control, on richness related to latitude. Nevertheless, our data clearly show that many samples from warm tropical waters had richness >100 OTUs, whereas all samples from cold, high-latitude waters had only about half that richness. This matches the general richness pattern previously observed for nine clone libraries, including two polar ones, reported by Pommier *et al.* (22).

Because our method does not detect genotypes that individually constitute <0.1% of the individuals in a sample, our estimates of richness are conservative. In addition, they are highly likely to underestimate the magnitude of the latitudinal gradient, because the samples from warm tropical waters with high observed genotype richness almost certainly contained a greater proportion of undetected rare genotypes. This is indicated by the ranked species abundance distributions for samples for which we had data on the frequency of genotypes (exemplified in Fig. 3). The higher richness samples from warm tropical sites were much flatter (contained proportionately more rare genotypes) than low richness samples from colder waters. This suggests that our results are conservative, because our detection methodology with a 0.1% cutoff differentially undersampled the total richness of warm tropical waters.

Our data and analyses support the preliminary report by Pommier *et al* (22) of a latitudinal gradient in marine bacterial diversity, but our conclusions are based on a much larger number of samples and a different but carefully standardized methodology, and we include an analysis of the environmental correlates of diversity. Our results are also consistent with an increasing number of studies showing local and regional differentiation and adaptation in microbes (reviewed in refs. 23 and 24). Note that this microbial diversity is revealed primarily by molecular genetic studies, such as ours. Our results contrast with a recent study of soil bacteria by Fierer and Jackson (25), who detected no latitudinal pattern and found that richness was strongly correlated with factors such as pH, soil type, and ecosystem type. We also note that a recent all-habitat metaanalysis reported salinity as the most important variable influencing bacterial community composition (26), but those authors compared terrestrial and aquatic environments ranging from essentially salt-free to hypersaline, a huge range of habitat types including many "extreme" environments. In contrast to both these studies, our samples were from relatively uniform marine surface waters where most chemical and physical environmental variables, including salinity, exhibited only modest variation, allowing us to detect a strong relationship with latitude and temperature despite considerable still unexplained variation in richness.

The patterns we observe directly challenge the suggestion that the lack of geographic patterns of morphospecies diversity, reported for protists, can be extrapolated to genotype diversity

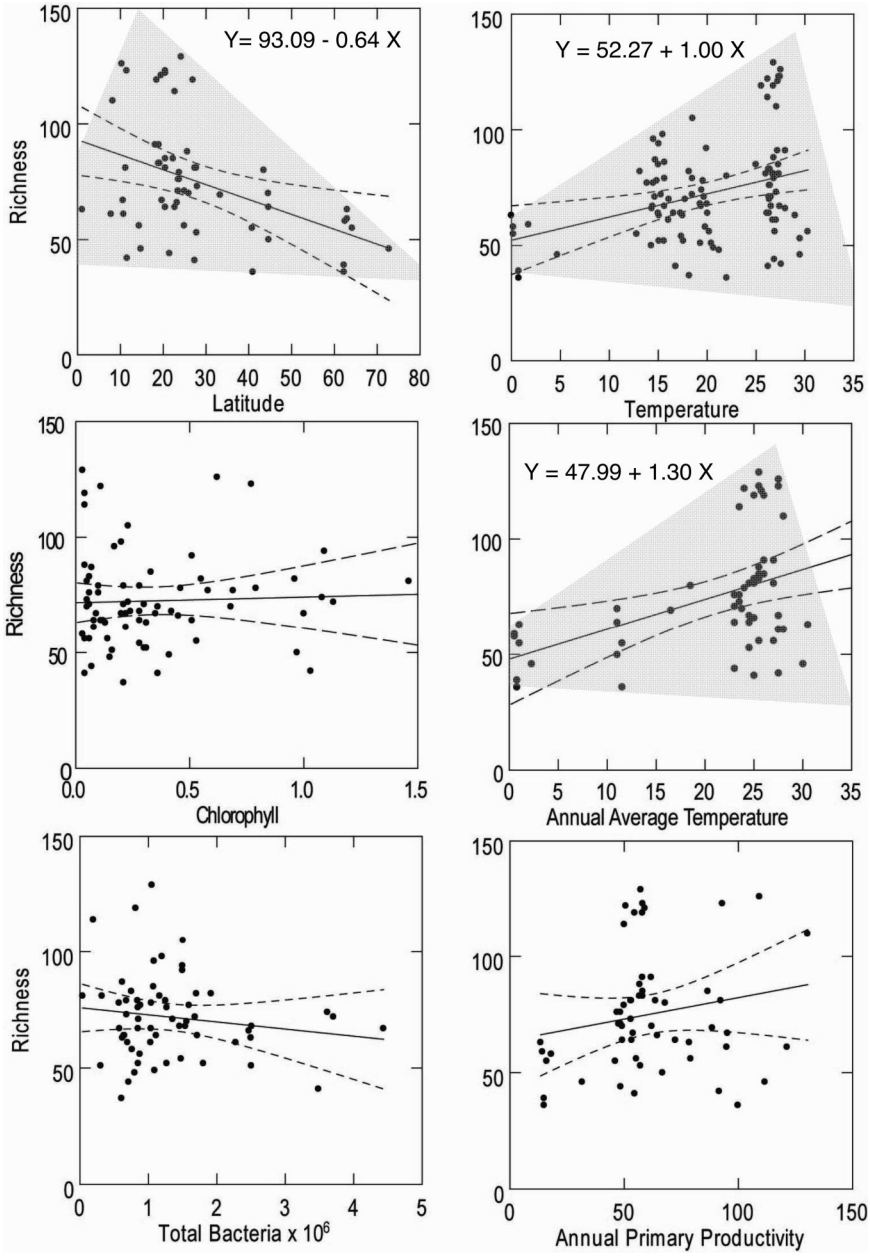

Fig. 2. Scatterplots of richness vs. latitude, temperature, annual average temperature, chlorophyll, bacterial abundance, and annual productivity. Linear regression lines and their 95% confidence limits (dashed) are shown. The three plots with significant correlations (to latitude and temperature) include shaded gray polygons to illustrate how the variation appears to fall within hypothesized constraint envelopes.

and to bacteria. A metaanalysis shows that the latitudinal gradient weakens as eukaryotic organism size decreases, with essentially no gradient expected if one extrapolates to bacterial sizes (4). These sorts of observations, plus reports of some cosmopolitan protists and bacteria, have led to the hypothesis that small size, high abundance, and high rates of dispersal prevent local and regional differentiation of unicellular organisms (7). But our documentation of a latitudinal gradient in bacteria suggests otherwise. We note that one group of marine protists, planktonic foraminiferans, have long been known to have a global latitudinal gradient of diversity (16), with 90% of the variability explained by temperature (27). Interestingly, this study reported a subtropical diversity maximum, as also occurs for some zooplankton. Although our data are too scattered to make firm conclusions, our results also hint at a possible maximum off the equator; when we fit a third-order polynomial to the data, richness peaks near 15° latitude ($r^2 = 0.22$).

Genetic studies such as ours have been challenged as not relevant to biogeography. Fenchel (28) suggests that genetic variation of the sort measured in this study could represent within-species variation and therefore be essentially ecologically neutral. We have evidence, however, indicating that the genetic variation we measure is ecologically relevant. The different bacterial taxa defined by ARISA "behave" like different species; OTUs identified by ARISA at our San Pedro Channel study site are seasonally variable, annually repeatable, and highly predictable from environmental parameters, with different OTUs associated with different parameters (29). This indicates that the genetic variation we observe is not neutral, the different bacterial OTUs occupy different niches, and the patterns with latitude and

Table 1. Pearson correlation coefficients among all parameters

Characteristic	Richness $n = 103$ (57)‡	Latitude†	Temperature	Annual average temperature†	Salinity	Chlorophyll	Total bacteria	Annual average primary productivity†
Latitude† $n = (57)$‡	−**0.422***							
Temperature $n = 103$ (57)‡	**0.337****	−**0.925*****						
Annual average temperature† $n = (57)$‡	**0.449****	−**0.965*****	**0.973*****					
Salinity $n = 103$ (57)‡	**0.275***	0.237	**0.377*****	**0.355***				
Chlorophyll $n = 74$ (30)‡	0.039	0.022	−**0.340***	−0.232	−**0.504*****			
Total bacteria $n = 60$ (16)‡	−0.167	−0.402	−**0.262**	−0.091	−**0.335***	**0.317**		
Annual average primary productivity† $n = (57)$‡	0.189	−**0.691*****	**0.598*****	**0.629*****	−0.147	**0.586**	**0.775****	
Distance from shore $n = (57)$‡	0.129	−0.129	0.237	0.241	−**0.236**	−0.207	−0.240	0.007

Bold data represent $P < 0.05$; *, $P < 0.01$; **, $P < 0.001$; ***, $P < 0.0001$.

†For calculating correlations to these parameters that had a single value for the San Pedro Ocean Time Series, the overall average value for the time series was used once (rather than counting all 46 time points separately). All other correlations used values for all individual dates, to correlate parameters as they varied between sampling dates and locations.

‡The number of samples listed in parentheses is the number when the average value for the San Pedro Ocean Time Series was used just once (and individual values for each date were not used) for the correlations described by †.

temperature reflect underlying geographic distributions of species. It remains an open question whether a similar genetic evaluation of protists would yield a similar result.

The nearly equally strong correlations of marine bacterial diversity with latitude and temperature raise questions about whether the latitudinal gradient simply reflects the effects of solar energy flux on sea surface temperature. We note that conventional measures of productivity (based on chlorophyll a-based photosynthesis) may not fully account for the abilities of microorganisms to use solar energy. Recent reports describe the significance of other solar energy-capturing processes in marine bacteria: newly discovered and abundant proteorhodopsin-containing bacteria (30–32), surprisingly common bacteriochlorophyll-containing cells (33–35), and microorganisms that take up photochemically generated labile organic substrates (reviewed in ref. 36). Because these organisms can contribute to the patterns of diversity reported here, at least some of the latitudinal gradient might reflect processes directly related to light availability.

The environmental correlates of pelagic marine bacterial richness offer potentially valuable insights into fundamental mechanisms that underlie geographic patterns of diversity. Our data and analyses support the hypothesis that bacterial diversity in a given habitat is largely generated and maintained by effects of temperature on the kinetics of metabolism. The metabolic rate, which increases exponentially with increasing temperature, sets the pace of life and hence the rates of nearly all biological activities. At the moment it is still uncertain just how warm temperatures contribute to the generation and maintenance of diversity (but see refs. 13, 14, 37, and 38) and just how the possible mechanisms may operate in bacteria

Our study and that of Pommier *et al.* (22) on bacteria are consistent with patterns of diversity in other marine organisms that are strongly related to temperature and at most weakly related to productivity (e.g., ref. 27). These studies should not be taken as evidence that variation in productivity does not contribute to geographic patterns of diversity, especially in terrestrial environments but also in marine and freshwater ones; previous studies have found a variety of relationships between productivity and aquatic microbial diversity, including positive, negative, U- and hump-shaped, as reviewed by Smith (39). But our results do make a strong case for fundamental kinetic controls on the generation and maintenance of diversity, controls that operate in a generally similar way across a wide variety of environments and taxonomic groups.

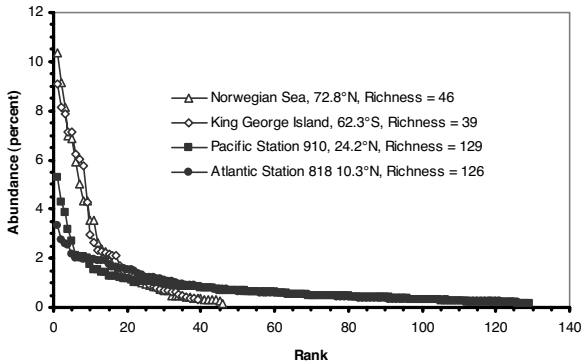

Fig. 3. Rank abundance curve of bacterial OTU from two low-latitude (Atlantic and Pacific), and two high-latitude (Arctic and Antarctic) locations.

Methods

Sample Collection and Auxiliary Data. Samples were collected from within 10 m of the sea surface at various locations (Dataset S1) by Niskin bottle or acid-washed plastic bucket. Seawater (4- to 20-liter volumes, exceptions were the Norwegian Sea sample at 100 liters and a Maxwell Bay sample at 60 liters) was filtered through Gelman A/E glass fiber filters (nominal pore size 1.2 μm) to remove eukaryotic cells (containing plastids which complicate the interpretation of ARISA fingerprints). The A/E filtered seawater, containing the free-living bacterioplankton that have been shown to compose ≈85% of the total bacteria (40), was then filtered through a 0.2-μm Durapore filter (Millipore) to collect the bacteria. Filters were frozen at −80°C before analysis at the University of Southern California.

Temperature and salinity were measured by conductivity–temperature–depth sensors on research cruises (88 of the 103 samples, listed on Dataset S1), or otherwise estimated from seasonal historical data from the sample location, e.g., from the NOAA World Ocean Atlas (www.nodc.noaa.gov/OC5/WOA05/pr_woa05.html). Chlorophyll was measured by fluorometry (41), but when not directly measured it was not estimated from historical data, because of its inherently higher temporal and spatial variability compared to temperature and salinity.

Annual average productivity was calculated from annual mean temperature, chlorophyll, irradiance, and mixed layer depth values, which were obtained directly from images at the NASA MODIS ocean color website (http://oceancolor.gsfc.nasa.gov/). Productivity was calculated by using equation 10 of Behrenfeld and Falkowski (42) and by using temperature-optimal photosynthesis values calculated with the exponential equation of Eppley (43).

DNA Extraction and Amplification. We prefiltered all samples to prevent the inadvertent inclusion of protists, whose plastids can show up as apparent ''or-

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ganisms'' in 16S rRNA-based molecular diversity surveys unless they are removed first (44). We used fixed DNA concentrations for both the PCR and detection steps, reducing potential variance because of different quantities of bacterial DNA. DNA was extracted from frozen filters by hot SDS lysis, followed by phenol–chloroform purification of nucleic acids (45), and DNA was stored frozen at −80°C in TE buffer or dry. ARISA (19, 46) was conducted on 10 ng of DNA as measured by PICO green fluorescence (45). A standard amount of template genomic DNA was used in each PCR, with the intention of analyzing the same amount of bacteria from each sample. PCRs (50 μl) contained 1× PCR buffer, 2.5 mM $MgCl_2$, 250 μM of each deoxynucleotide, 200 nM each of universal primer 16S–1392F (5′-G[C/T]ACACACCGCCCGT-3′) and bacterial primer 23s–125R labeled with a 5′ TET (5′-GGGTT[C/G/T]CCCCATTC(A/G)G-3′), 2.5 units of *Taq* polymerase (Promega), and BSA (A-7030, 40 ng/μl final concentration; Sigma) (45). These primers target specifically bacteria, hence archaea are not included in our analysis, and we know of no significant group of marine bacteria in surface waters whose DNA these primers fail to amplify (21). Thermocycling was preceded by a 3-min heating step at 94°C, followed by 30 cycles of denaturing at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 45 s, with a final extension step of 7 min at 72°C. Amplification products were cleaned by using Clean & Concentrator-5 (Zymo Research), and DNA in purified products was measured by PICO green fluorescence. Purified products were then diluted to 5 ng/μl so that we could load a standardized amount in the fragment analysis and prevent differences arising from different amounts of loaded DNA. Products were then run for 5.5 h on an ABI 377XL automated sequencer operating as a fragment analyzer (47), with a custom-made ROX-labeled 1,500-bp standard (Bioventures). The sequencer electropherograms were then analyzed by using ABI Genescan software.

Outputs from the ABI Genescan software were transferred to Microsoft Excel for subsequent analysis. Peaks less than five times the baseline fluorescence intensity were discarded because they were judged as not clearly distinguishable from instrument noise (46). With this criterion, the practical detection limit for one OTU is ≈0.09% of the total amplified DNA (46). The richness of fingerprints was then calculated by summing the total number of remaining peaks, each containing >0.09% of total amplified DNA. The fragment sizes range from 400 to 1,200 bp, so the maximum possible number of detectable different taxa would be several hundred; the maximum we report is 129, so the profiles were never near saturation. Regarding possible overestimation of richness, previous work has indicated that with marine bacterioplankton, it is extremely unlikely that ARISA overestimates the number of OTUs from diverse multiple operon copies within one organism, because slowly growing marine bacteria are expected to have one or a few copies of the gene, and in all known cases so far these are identical to each other in length (21).

Correlation and Other Statistical Analyses. Pearson's correlation coefficient, the 95% confidence interval on regressions, and *P* values were calculated with Systat ver.11 with pairwise deletions (and double-checked with listwise deletions), through standard and built-in functions. A null hypothesis of zero correlation was used.

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