Remarkable heterogeneity in meso- and bathypelagic bacterioplankton assemblage composition

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Abstract

We investigated meso- to bathypelagic (500–3,000 m) bacterioplankton assemblage composition at 19 locations in the North Atlantic Ocean beneath the offshore Amazon River plume, in the North Pacific Ocean near the Hawaiian archipelago, at the San Pedro Ocean Time Series (SPOTS) station off southern California, and in the Coral Sea off eastern Australia with a sensitive high-throughput fingerprinting approach, automated rRNA intergenic spacer analysis (ARISA), to examine variation between bacterial assemblages at different stations. Temperature and salinity were used to identify distinct water masses within gyres. ARISA fingerprints each contained 15–117 operational taxonomic units (OTUs) per assemblage; however, the OTU composition of fingerprints varied among stations, even within the same water mass. Fingerprints from 500 m at the SPOTS station over 4 yr shared on average a Sorensen Index (presence/absence similarity) of 0.68 ± 0.01 and a Whittaker Index (proportional representation similarity) of 0.68 ± 0.01, whereas at more oceanic stations at 500 m, fingerprints shared a Sorensen Index of 0.48 ± 0.01 and a Whittaker Index of 0.38 ± 0.01. At deeper depths (1,000 and 2,000 m), fingerprints were equally variable, sharing Sorensen Indices of 0.42 ± 0.02 and 0.50 ± 0.02 and Whittaker indices of 0.33 ± 0.01 and 0.34 ± 0.03, at 1,000 m and 3,000 m, respectively. Mesopelagic, moderately productive waters were more stable than those at less productive, open-ocean gyre stations, suggesting that variability in bacterioplankton communities at depth is influenced by organic matter supply and patchiness.

Bacterioplankton in the world's oceans are ecologically critical, processing typically 50% of primary production (Fuhrman and Azam 1982; Azam et al. 1983), comprising up to 70% of biomass in surface waters (Fuhrman et al. 1989), and mediating many global-scale nutrient transformations. Despite this, the richness and diversity of planktonic prokaryotic assemblages in the oceans have only recently been studied because of the advent of molecular techniques (Giovannoni et al. 1990; Fuhrman et al. 1992, 1993), which circumvent classical culture biases associated with >99% of bacterial taxa. These studies, and recent advances in molecular approaches to addressing

assemblage composition, have revealed an astonishingly high diversity of marine bacterioplankton (Curtis et al. 2002; Venter et al. 2004); however, the distribution of assemblages across a number of environments has not been previously studied, partially because of the extraordinary costs of large-scale sequencing projects. The development of less costly, high-sensitivity and -throughput fingerprinting approaches (Muyzer et al. 1993; Avaniss-Aghajani et al. 1994; Fisher and Triplett 1999) now allows researchers to examine the distribution of entire assemblages across a wider range of samples. Most studies of bacterioplankton assemblages to date have focused upon a handful of samples (Giovannoni et al. 1990; Delong 1992; Fuhrman et al. 1992) or one or two depth intervals (Venter et al. 2004); consequently, spatial variability across habitats has not been investigated extensively. Studies of bacterioplankton diversity via 16S rDNA clone libraries were among the first to reveal novel archaebacterial lineages (Delong 1992; Fuhrman et al. 1992), and demonstrated the predominance of the *Chloroflexi*-like SAR-202 cluster at depth (Morris et al. 2004). Despite these previous studies, there is almost no information on the spatial variability of meso- (500 m) or bathypelagic (1,000–3,000 m) bacterioplankton assemblages.

The composition of bacterioplankton communities in the ocean is believed to be influenced by resource availability (Torsvik et al. 2002) and selective loss factors (e.g., bacterivory and viral lysis [Fuhrman 1992; Thingstad and Lignell 1997]). Microbial growth and decay are very slow under cold and high-pressure deep-sea conditions (Jannasch et al. 1970; Cho and Azam 1988; Ducklow and Carlson 1992). This has been dramatically demonstrated by strong preservation of food materials after the accidental sinkings of the HMS Titanic (Holden 1985) and the deep-
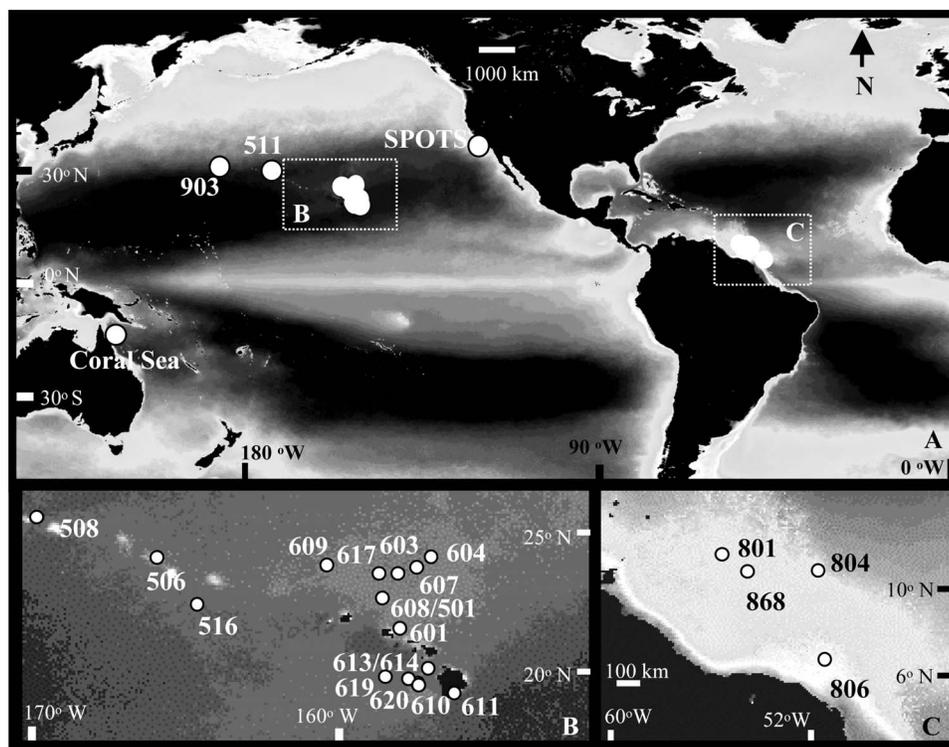
Fig. 1. (A) Sampling locations at the SPOTS station, the tropical western Atlantic, and the North Pacific gyre. The background to this map is a global mean surface chlorophyll *a* SeaWIFS image from 1997–2002 (National Aeronautics and Space Administration). Bacterioplankton assemblages were collected in the San Pedro Basin as part of Microbial Observatories project from 2000 to 2004; (B) in the tropical western Atlantic in April/May 2003; and (C) in the North Pacific in July 2002, September/October 2002, and July/August 2003.

sea submersible Alvin (Jannasch et al. 1970). Surface waters of the ocean experience variable irradiance and temperature with latitude and season, and with resource inputs from mixing, recycling, aeolian dust, and terrestrial sources. In contrast, meso- and bathypelagic environments (>500-m depths) experience remarkably consistent physicochemical environments within each ocean basin, experiencing permanently cold, dark, inorganic nutrient–rich, organic nutrient–poor, and poorly mixed conditions through most of the world. This environment receives ephemeral and often seasonal inputs of resources in the form of particulate and dissolved organic carbon from overlying waters, with inputs varying over large scales (Nagata et al. 2000; Hansell and Ducklow 2003), particularly latitudinally within oceanic basins (Carlson et al. 1994; Hansell and Carlson 1998; Bendtsen et al. 2002). Deep-water bacterioplankton have very slow assemblage turnover times (0.2–30 yr; Nagata et al. 2000; Bendtsen et al. 2002; Hansell and Ducklow 2003). Thus it is a reasonable hypothesis that bacterioplankton assemblages are homogeneous over large portions of the bathypelagic ocean, because resource availability, physical conditions, and selective loss factors are similar across a region, perhaps even to some extent globally.

The aim of this study was to examine variability of bacterioplankton in meso- to bathypelagic waters in an attempt to understand factors that may be affecting their assemblages. Bacterioplankton were examined from 500 m at the San Pedro Oceanic Time Series (SPOTS) station on the Southern California Bight monthly for 4 yr to determine the temporal scale of mesopelagic bacterioplankton change. These data were compared to automated rRNA intergenic spacer analysis (ARISA) fingerprints from a further 18 stations in the North Atlantic Ocean, North Pacific Ocean, and Coral Sea, collected to examine spatial variability in meso-bacterioplankton assemblage composition. Furthermore, samples from 1,000 m were collected at 16 stations, and from 3,000 m at 6 stations, to compare assemblage variation in deeper to shallower waters.

Materials and methods

To understand the temporal patterns of bathypelagic bacterioplankton assemblages, we investigated time-dependent variation in assemblage composition at SPOTS (33.33°N, 118.40°W), which is within a small 900-m-deep basin (dimensions ca. 20 × 30 km, sill depth ca. 740 m) and experiences seasonally varying surface water productivity and transient hypoxic conditions at and below 500 m. We also investigated meso- and bathypelagic assemblage composition at 12 stations in the North Pacific gyre, 1 station in the Coral Sea, and 4 stations in the western Tropical Atlantic (Fig. 1) to determine spatial distribution of meso- and bathypelagic bacterioplankton assemblages.

Sample collection—Sampling for meso- to bathypelagic bacterioplankton assemblages occurred in the North Pacific Ocean on board the R/V *Ka'Imikai-o-Kanaloa* in July 2002, the R/V *Kilo Moana* in September/October 2002, and the R/V *Roger Revelle* in July/August 2003; in the North Atlantic Ocean on board the R/V *Seward Johnson* in April/May 2003; and in the Coral Sea on board the R/V *Maurice Ewing* in November 1999. Samples from Southern California were taken as part of the USC Microbial Observatory from 2000 to 2004 on board the R/V *Sea Watch*. At all stations, conductivity, temperature, depth (CTD) telemetry data on temperature and salinity were collected; however, other physicochemical parameters collected were not consistently calibrated or collected between cruises, and are therefore not included in this study.

Water samples ranging from 10 to 40 liters at depths from 500 to 3,000 m were collected by Niskin Bottle rosette mounted on a CTD array. Samples were withdrawn from Niskin bottles directly into acid-washed and sample-rinsed low-density polyethylene cubitainers and kept out of sunlight until processing (which occurred within 6 h of sampling). Bacterioplankton assemblages for molecular analyses were in most cases collected by positive pressure filtration. Samples were collected by serially filtering seawater through 142-mm- or 47-mm-diameter, 1.2 μm (nominal pore size) A/E Glass Fiber Filters (Gelman) then onto 142-mm- or 47-mm-diameter, 0.22 μm Durapore (Millipore) filters in either a polycarbonate or stainless steel inline positive-pressure filtration manifold that was rinsed with deionized water between samples. A positive air pressure <1.4 bar was maintained on the positive-pressure manifold, and at the conclusion of filtration, vacuum (0.8 bar) was placed on the outflow to prevent backflow from disrupting the glass-fiber filter, which potentially could cause plastid contamination on the Durapore filter. Samples from the North Atlantic Ocean were filtered through 47-mm A/E filters, then onto 0.2-μm Sterivex capsule filters (Durapore internal material), using an inline manifold attached to a peristaltic pump. Between samples, the manifolds were rinsed with deionized water to reduce cross-contamination potential. In all cases, filters and Sterivexes containing bacterioplankton cells were placed in sterile Whirl-paks (Nasco®) and frozen at −80°C until analysis at the University of Southern California.

Bacterioplankton production—Bacterioplankton production was estimated by incorporation of ³H-leucine into protein as described previously (Kirchman et al. 1985; Simon and Azam 1989). Briefly, 10 mL seawater samples in sterile, sample-rinsed, polypropylene centrifuge tubes were inoculated with 8.6 μCi ³H-leucine (5 nmol L⁻¹ final concentration) and incubated 1 h at ambient temperature and at 50% attenuated irradiance in a flow-through outdoor incubator. One replicate was killed immediately before inoculation by addition of 5% formalin. After incubation, samples were filtered onto 25-mm-diameter 0.45-μm Millipore (Type HA) nitrocellulose filters to dryness. Small molecules were then extracted by incubation for 2 min with 2 mL ice-cold trichloroacetic acid (TCA). After extraction, TCA was filtered through membranes, the filter containing labeled protein washed repeatedly with TCA, then placed into 6 mL scintillation vials containing 5 mL Ultima Gold scintillation fluid. After incubating vials for 2 h at room temperature to allow clearing of filter membranes in scintillation fluid, radiolabel incorporation was measured in a Beckman-Coulter LS6500 scintillation counter. We used a conversion factor of 2×10^{15} cells mmol⁻¹ *leu* incorporated (Simon and Azam 1989) to estimate bacterial production.

Bacterial abundance—Bacterial abundance was determined by SYBR Green I staining and epifluorescence microscopy (Noble and Fuhrman 1998). Briefly, samples (50 mL) were fixed with 0.02-μm-filtered formaldehyde, to a final concentration of 2%, and kept at 4°C in the dark until processing, which occurred within 24 h of sampling. Samples were processed by first filtering aliquots (1–20 mL, depending on depth) onto 0.02 μm Anodisc Al$_2$O$_3$ filters, drying the filter on tissue paper, then staining on a 100-μL, 1 : 2500-diluted drop of SYBR Green I. After staining, the filters were redried on tissue paper and mounted on a glass slide with a solution of 50 : 50 : 0.01 glycerol : phosphate buffered saline : p-phenylenediamine as mountant. Slides were observed under blue light excitation at ×1,000 magnification on an Olympus BH-60 microscope. More than 200 bacterial cells were counted in 20 fields.

DNA extraction—Filters were extracted using protocols of Fuhrman et al. (1988). Briefly, 500 μL STE (100 mmol L⁻¹ NaCl, 10 mmol L⁻¹ Tris, 1 mmol L⁻¹ EDTA) : 10% sodium doecyl sulfate (SDS) (9 : 1) was added to the filters in 2.0 mL microcentrifuge tubes, or to the capsule filters directly, which were placed in a boiling water bath for 2 min to lyse bacterial cells. Sterivex capsule filters were capped with sterile leur lock and syringe fittings prior to heating, and detergent lysis occured entirely within the filter capsule. The STE and SDS containing bacterial DNA were then pushed out of the capsule filters with positive air pressure into microcentrifuge tubes. Bacterial cells were heat-lysed in a boiling water bath for 5 min, then centrifuged for 2 min at 3,000 × g to remove excess cell and filter debris. The supernatant was then transferred into a polycarbonate ultracentrifuge tube and precipiated overnight in 70% EtOH and 750 mmol L⁻¹ CH$_3$COONH$_4$. After precipitation, DNA was centrifuged at 15,000 × g for 1 h, dried, and resuspended in 200 μL deionized H$_2$O. The resuspension was then extracted sequentially with 200 μL phenol, phenol : chloroform (1 : 1) and finally chloroform : isoamyl alcohol (24 : 1), each time centrifuging the samples at 15,000 × g for 2 min and removing the solvent phase. After phenol:chloroform extraction, samples were reprecipitated overnight in 70% EtOH and 950 mmol L⁻¹ NH$_4$Oac. Reprecipitated samples were centrifuged at 15,000 × g, dried, then resuspended in 150 μL TE buffer (10 mmol L⁻¹ Tris, 1 mmol L⁻¹ EDTA). After extraction, DNA was quantified by Pico Green fluorescence (Molecular Probes Inc) on a Stratagene MX-3000 Quantitative PCR/Fluorometer and diluted to 2.5 ng μL⁻¹.

Automated rRNA intergenic spacer analysis—ARISA was conducted on 10 ng extracted DNA (Fisher and

Triplett 1999). The ITS region (plus about 285 bases of 16S and 23S rRNA) of DNA extracts was amplified using the polymerase chain reaction (PCR). PCR was carried out in 50 μL reactions containing 1× PCR buffer, 2.5 mmol L^{-1} MgCl$_2$, 1.25 mmol L^{-1} deoxynucleotide triphosphates (dNTPs) (Promega PCR Nucleotide Mix), 0.8 μmol L^{-1} each of universal primer 1392 F (5'-G(C/T)ACA-CACCGCCCGT-3') and bacterial-specific 23S-115R (5'-GGGTT(C/G/T)CCCCATTC(A/G)G-3'), labeled with a 5'-phosphoramidite (TET) tag (Fisher and Triplett 1999), bovine serum albumin (BSA) (40 ng μL^{-1} final concentration; Sigma #7030), and 2.5 U *Taq* DNA polymerase (Promega). Thermocycling in a Perkin Elmer 9600 comprised an initial heating step at 95°C for 3 min, followed by 30 cycles of denature at 95°C for 30 s, anneal at 55°C for 30 s, and extend at 72°C for 45 s, and a final extension step of 7 min at 72°C. PCR products were purified using Clean & Concentrator-5 Kits (Zymo Research) to remove primers and salts. Amplicons were then quantified again by Pico Green Fluorescence and diluted to 5 ng μL^{-1}. Products (1.5 μL) were then electrophoresed for 4.5 h at 100 V on an ABI 377XL automated sequencer (Avaniss-Aghajani et al. 1994) with ROX-labelled ABI 1,720-bp standards or Bioventures 1,500-bp standards. Sequencer gels were analyzed by ABI Genescan software, and analysis outputs transferred to Microsoft Excel for subsequent processing.

Statistical analysis of microbial assemblage fingerprints—Exported Genescan outputs of fragment length and fluorescence for each fingerprint were aligned against a list of all possible fragment lengths (400 to 1,200 bp in this analysis) using an Excel Macro (AAArray Data; Kitts pers. comm.). Fragments less than 5 times baseline fluorescence in height were eliminated because they could not be distinguished from instrument "noise". With these criteria, a fingerprint peak, or operational taxonomic unit (OTU) exceeded the baseline when it contributed > 0.09% of total amplified DNA fluorescence (Hewson and Fuhrman 2004). The area under each peak was then expressed as a percentage of the total integrated area under the electropherogram.

Bacterioplankton assemblage fingerprints were compared by taking into account the presence or absence of different OTUs in fingerprints (i.e., the Sorensen Index), as well as the relative proportion of DNA associated with each OTU (the Whittaker Index). Sorensen's Index was calculated manually according to the following equation:

$$\text{Sorensen} = 2W/(a_1 + a_2)$$

where W is the number of shared OTUs between populations 1 and 2, and a$_1$ and a$_2$ are the total numbers of different OTUs in populations 1 and 2, respectively. Whole fingerprints (i.e., all OTUs each comprising >0.09% of total amplified DNA) were analyzed by calculating Whittaker's index of association (S$_w$) using the following equation:

$$S_w = 1 - \sum_{i=1,2}^{n} |b_{i1} - b_{i2}| \quad \text{(Whittaker 1952)}$$

where b_1 and b_2 are the percentage contributions to amplified DNA of the *i*th OTU in samples 1 and 2, respectively. S_w scales from 0 (completely different) to 1 (identical). The Shannon–Wiener diversity index (H') was calculated for each assemblage fingerprint (Table 1) using the following equation:

$$H' = \sum_{i=1}^{n} p_i \log_e (p_i)$$

where p_i is the percentage contribution to total amplified DNA of the *i*th OTU within each fingerprint.

To account for uncertainty in fragment size, ARISA fingerprints were binned ± 1 bp from 400 to 700 bp, ± 2 bp from 700 to 1,000 bp, and ± 5 bp > 1,000 bp. Because binning starting at a single frame may cause OTUs only 1 bp from each other to appear different in our analyses, we binned 10 times, each time beginning the bin frame at +1 bp (Hewson and Fuhrman 2006). The Sorensen and Whittaker Indices were calculated for all pairwise comparisons in all 10 bins, and maximum pairwise similarity used in subsequent analyses. Monte Carlo simulated assemblages with randomly sized OTUs (between 400 and 1,200 bp) and between 10 and 100 OTUs per assemblage were constructed using the random function in Microsoft Excel. With our binning protocols, such unrelated assemblages randomly shared on average a Sorensen Index of 0.22 ± 0.12 (mean ± SE), with 95% of pairwise comparisons sharing a Sorensen Index >0.46, whereas fingerprints shared a Whittaker Index of 0.09 by chance alone (Hewson and Fuhrman 2006). Cluster analysis was conducted using the XLStat (AddinSoft SARL) program using the Sorensen or Whittaker Index and clustering via unweighted pair-group mean average method. The pairwise distance between sampling locations based upon latitude and longitude was determined using the Le Progiciel R statistical analysis program (University of Montreal).

Results

Physicochemical characteristics of water masses—There was substantial variability in temperature/salinity characteristics between different locations and sampling times at 500 m; however, there was little variability deeper (800–3,000 m) within the same ocean gyre (Fig. 2). The 500-m samples from the SPOTS station grouped together on temperature/salinity plots; however, they were on three dates less similar than the other 24 months. North Atlantic Ocean samples had the most variable temperature/ salinity characteristics at 500 m, possibly because of mixing or sinking blooms from within the North Brazil Current, a fast and northwards-moving (75 cm s^{-1}) current that advects water from the offshore Amazon River Plume. In the North Pacific, temperature/salinity characteristics were more variable than at the SPOTS station, and grouped primarily by sampling time (i.e., June 2002, July 2003, and September/October 2002 clustered differently). At 1,000 m, most North Pacific samples had relatively invariant temperature/salinity characteristics, whereas those in the
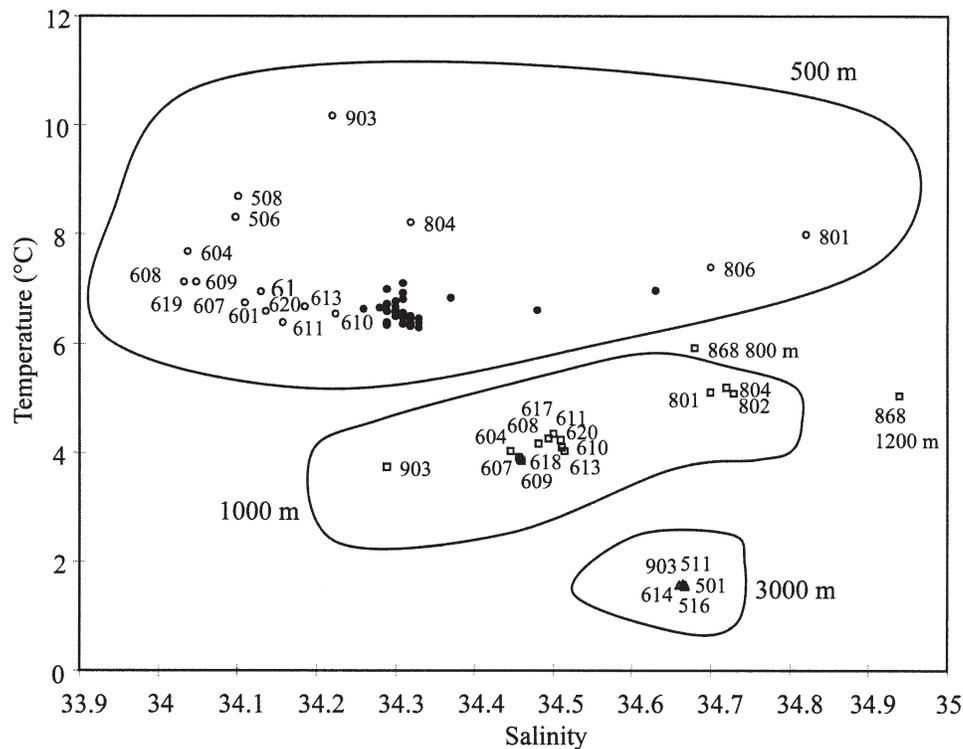
Fig. 2. Temperature/salinity characteristics of meso- and bathypelagic water samples collected in the North Pacific Ocean (stations 506–508 in June 2002, stations 601–620 in September/October 2002, station 903 in July 2003), and in the North Atlantic Ocean (stations 801–868 in April/May 2003). The same depth intervals are gated. Data from the San Pedro Ocean Time Series station (solid circles) is not labeled for clarity. Note that stations 868 had samples at 800 m and 1,200 m, which lie outside of gated areas.

North Atlantic Ocean were remarkably variable within the depth interval from 800 to 1,200 m. Station 868 was investigated primarily because of unusual and vertically heterogeneous bacterial production measured at that station during an earlier sampling, which was originally ascribed to the presence of aggregates in a sinking bloom that originated from the Amazon River plume. At 3,000 m, all samples fell within a very narrow temperature/salinity window, indicating that the same water mass was sampled on all three cruises in the North Pacific Ocean.

Bacterioplankton abundance and production—Bacterioplankton production was greatest at 500 m (11.5–14.7×10^3 cells mL^{-1} d^{-1}), and least in 3,000 m (0.3×10^3 cells mL^{-1} d^{-1}; Table 2), indicating turnover times from 4.2 d to 3 yr. Between the North Pacific and Atlantic Ocean basins, production was statistically indistinguishable within different depth intervals; however, production was significantly lower at the SPOTS station at 500 m than elsewhere. Bacterial abundance at 500 m was nearly fourfold higher, but more variable at $2.72 \pm 1.35 \times 10^5$ cells mL^{-1}, than at depth ($1,000$ m $= 6.87 \pm 1.36 \times 10^4$ cells mL^{-1}).

ARISA fingerprint characteristics—Fingerprints each contained 15–117 OTUs and had Shannon–Wiener indices of 0.22–1.66; however, they were heterogeneous with depth (Table 1). The greatest variability in fingerprint richness and diversity was found in the 500-m samples, and surprisingly, fingerprints from 1,000 m were both less rich and diverse on average than were either the 500-m or the 3,000-m samples, the latter of which had the highest Shannon–Wiener indices of all depths sampled.

Spatial and temporal similarity between ARISA fingerprints—Assemblage fingerprints from the SPOTS station were variable from month to month, with each fingerprint sharing a Sorensen Index of 0.60–0.91 with previous months (Fig. 3). The mean similarity for all months was a Sorensen Index of 0.68 ± 0.01 and a Whittaker Index of 0.68 ± 0.01 (Table 2). In contrast, open-ocean samples shared on average a Sorensen Index of 0.48 ± 0.01 and a Whittaker Index of 0.38 ± 0.01 at 500 m, a Sorensen Index of 0.42 ± 0.02 and a Whittaker Index of 0.50 ± 0.02

Table 1. ARISA fingerprint characteristics for meso- to bathypelagic seawater samples. S_{ave} = mean total fingerprint richness (i.e., no. of OTUs), H_{ave} = mean Shannon–Wiener Index. Data reflect fingerprints from all ocean basins and dates (mean $\pm$ SE).

Depth interval	S_{ave}	Range	H_{ave}	Range
500 m	56 ± 4	15–117	1.35 ± 0.04	0.22–1.66
800–1,200 m	41 ± 5	16–84	1.19 ± 0.06	0.80–1.57
3,000 m	58 ± 7	26–78	1.47 ± 0.05	1.27–1.60

Table 2. Comparison of assemblages in North Pacific and Atlantic and San Pedro Basin. Within-basin mean similarity was calculated for all pairwise Sorensen's indices between assemblages from the same geographic region. Between-basin mean similarity was calculated from all pairwise comparisons between that basin and all other basins. Mean bacterial production was estimated by ^{3}H-leucine incorporation (Kirchman et al. 1985; Simon and Azam 1989) (mean $\pm$ SE). The similarity between two computer-generated random assemblages was on average a Sorensen Index of approximately 0.22 ± 0.12 (Hewson and Fuhrman 2006).

Ocean basin	Depth (m)	n	Within-basin mean similarity (Sorensen Index)	Between-basin mean similarity (Sorensen Index)	Mean bacterial production ($\times 10^3$ cells mL^{-1} d^{-1})
San Pedro Basin	500	24	0.68 ± 0.01	0.39 ± 0.01	3.3 ± 0.7
North Pacific	500	17	0.48 ± 0.01	0.37 ± 0.01	11.5 ± 4.6
	1,000	11	0.48 ± 0.02	0.31 ± 0.01	5.8 ± 1.8
	3,000	6	0.51 ± 0.02	0.49 ± 0.03	0.3 ± 0.1
North Atlantic	500	4	0.44 ± 0.03	0.44 ± 0.01	14.7 ± 7.4
	1,000	4	0.33 ± 0.03	0.31 ± 0.01	3.5 ± 1.4

at 800–1,200 m, and a Sorensen Index of 0.33 ± 0.01 and a Whittaker Index of 0.34 ± 0.03 at 3,000 m (Fig. 4). There were differences in fingerprint similarity between stations in each basin; however, the similarity between basins within each depth interval was greater than within-basin variability (Table 2). Within the North Pacific Ocean, variability in fingerprint similarity was not distinguishable between depth intervals, but in the North Atlantic Ocean, fingerprints at 1,000 m were significantly (Student's t-test, $p < 0.05$, $n = 4$) more variable than at 500 m. Fingerprints from open-ocean spatial locations shared fewer OTUs with each other than they did at the SPOTS station over 4 yr of sampling.

No significant linear regressions between separation distance and pairwise similarity were observed in the oceanic 500-m (North Pacific + North Atlantic Oceans) fingerprints, nor at 1,000 m in the North Pacific Ocean; however, significant and negative correlations were observed in the North Atlantic Ocean at 1,000 m and in the North Pacific Ocean at 3,000 m (Fig. 5).

Discussion

Fingerprints from the SPOTS station demonstrated remarkable similarity over 4 yr (Table 1); however, they shared even higher similarity seasonally during August 2000–July 2003 and September 2003–June 2004. Spatially, among 12 stations sampled on a cruise around Hawaii over 20 days, bacterioplankton assemblage composition also varied dramatically, typically sharing only about half of OTUs between stations at 500 m and less at 1,000 m (Fig. 4). Note that this temporal and spatial variability in assemblage fingerprints may be attributable to spatially heterogeneous water masses in the open ocean. Temporal changes at the SPOTS station are unlikely to have resulted from patchy water masses moving past the station because,
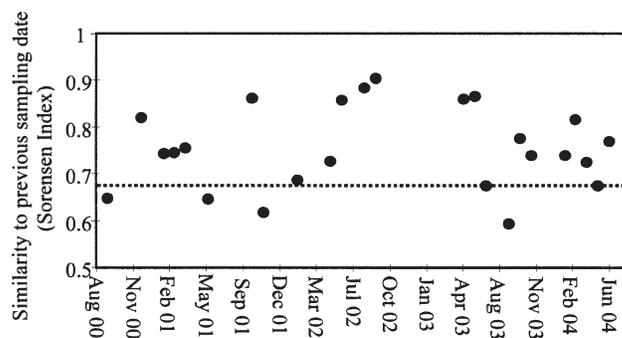

Fig. 3. Similarity of bacterioplankton assemblages pairwise between subsequent sampling times at the San Pedro Ocean Time Series Station at 500 m. Comparison is based upon the Sorensen Index of assemblage similarity, which is the ratio of the number of shared phylotypes to the number of total phylotypes between two samples. Clustering was performed by Unweighted Pair-Group-Mean Average (UPGMA). The dotted line indicates average monthly similarity between assemblages at 500 m.

with the exception of 3 dates, temperature/salinity characteristics were relatively constant. In contrast, open-ocean stations at 500 m were extremely variable with respect to temperature/salinity characteristics. However, even stations with relatively similar temperature/salinity characteristics shared few OTUs or proportions of total amplified DNA fluorescence (e.g., stations 619, 608, and 607, which had almost identical T/S values).

Assemblages collected only short distances from other stations (or at the same station) and separated by only a few days were surprisingly dissimilar (e.g., 607 and 608 at 1,000 m), yet other stations located large distances away were remarkably similar (e.g., 608 and 610; Fig. 4). These results are congruent with pairwise similarity–distance plots (Fig. 5) that demonstrate heterogeneity in assemblages at 500 and 1,000 m in the North Pacific Ocean. In the North Atlantic, stations located beneath the low-salinity Amazon River plume were very dissimilar, sharing fewer than half of all OTUs between stations at 500 and 1,000 m, in the similarity range of unrelated communities (Hewson and Fuhrman 2006). Separation distance/time and pairwise similarity comparisons indicated that 500-m and 1,000-m bacterioplankton assemblage fingerprints were not shaped by regional-scale factors (Fig. 5), but rather may have been shaped by factors acting at spatial scales less than the distance between adjacent sampling stations (Hewson et al. in press). At 1,000 m in the North Atlantic Ocean and at 3,000 m in the North Pacific, however, significant and negative correlations between pairwise similarity and separation distance suggest that regional-scale (i.e., basin-scale) factors may also shape bacterioplankton assemblages.

The high average similarity in assemblages at 500 m in the San Pedro Basin possibly resulted from the laterally enclosed nature of the basin at that depth restricting motion. This is encouraging and suggests the interpretation that heterogeneity in assemblage composition within the North Pacific gyre is not simply an artifact of inherent variability of the fingerprinting method. Mesopelagic assemblages were less similar between ocean basins than
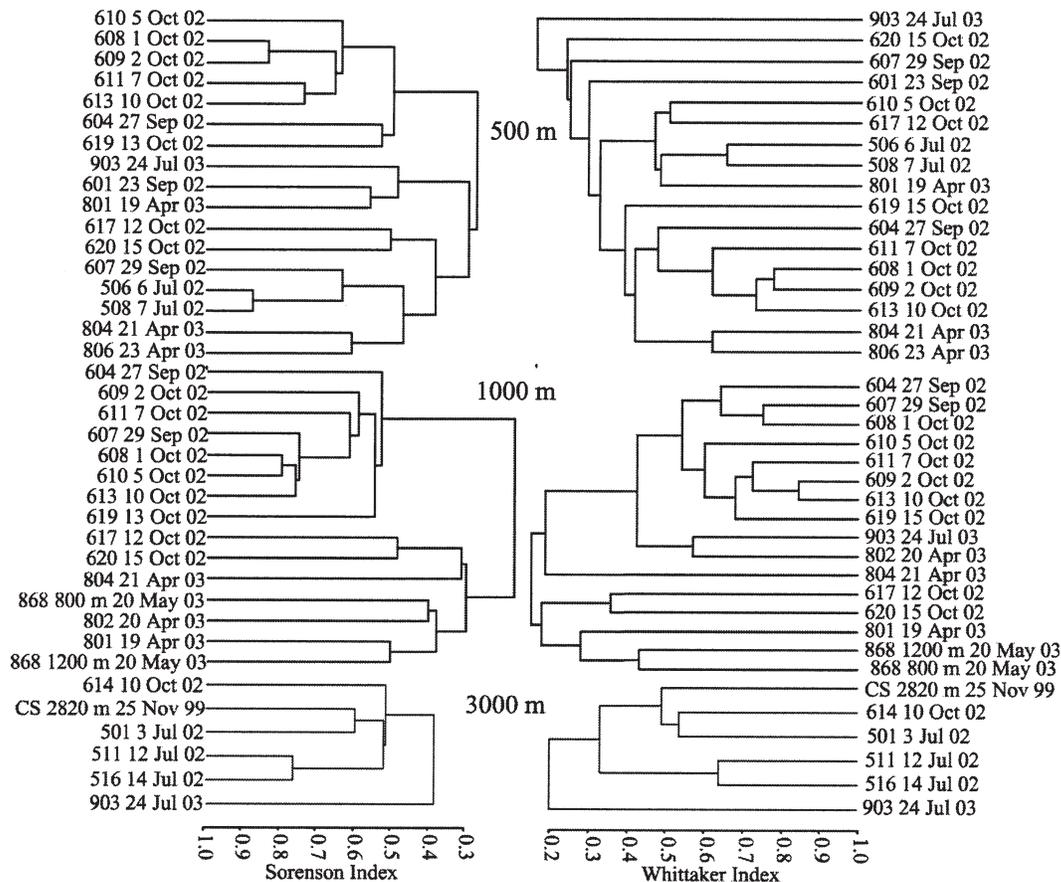
Fig. 4. Dendrogram showing relatedness of 500 m, 1,000 m and 3,000 m bacterial assemblages from the North Pacific, North Atlantic, and Coral Sea. Clustering was performed using the Sorensen Index and Whittaker Index by Unweighted Pair-Group-Mean Average (UPGMA).

within basins and in the same range as random possibilities. Similarities of 3,000-m assemblages were surprisingly heterogeneous; they shared on average only half of all OTUs, but shared about the same number of OTUs between sites in the same basin as between the North Pacific and Coral Sea.

Because of concerns about whether similarity differences were being driven by minor OTUs that drift above and below the detection threshold of ARISA, we compared the similarity between fingerprints based upon OTUs comprising each >0.5% or >1.0% of total amplified DNA. We found that using only these major OTUs in our analysis decreased mean similarity between assemblage fingerprints (calculated for all OTUs >0.09%) by a Sorensen Index of 0.03–0.13. This indicates that the low-similarity clustering patterns in the North Atlantic and Pacific were not driven by variations in the presence of minor OTUs.

The California Borderland is mesotrophic and has seasonal variable surface water productivity, experiencing extensive and intense algal blooms including red tides in spring (April–May), when portions of upwelled water from North of the site move with the California Current and impinge upon the Borderland region. Despite these variations at the surface, the relative constancy of deep-water bacterial assemblages in the San Pedro Basin (the

first sample set we analyzed) was initially not a surprise, because of the expected constancy of the deep sea. However, by comparison, the high variability between meso- and bathypelagic assemblages within the North Pacific gyre was quite unexpected, because there is low surface-water productivity year-round and little seasonality. Consequently, there are only small and not highly variable spatial inputs of sinking organic matter into the ocean interior (Hansell and Ducklow 2003).

The deep ocean contains relatively few barriers to geographic dispersal, and therefore has been believed to contain homogeneous assemblages. We propose that, in contrast, physical barriers, such as enclosed basins bordered by sills, may cause the most homogeneous assemblages because of reduced microbial dispersal, irrespective of inputs of organic matter from overlying waters. There may also be an effect of low oxygen typical in such basins (18.5 ± 3.6 μmol L^{-1} 4 yr average at the SPOTS station 500 m; Michaels et al. unpubl. data available from the author). Sinking particulate organic matter may be hypersolubilized by native bacteria, and the rate of arrival to deep water is correlated with bacterial production and biomass (Cho and Azam 1988; Nagata et al. 2000; Hansell and Ducklow 2003). These observations fit with the conclusions that bacteria in this compartment primarily
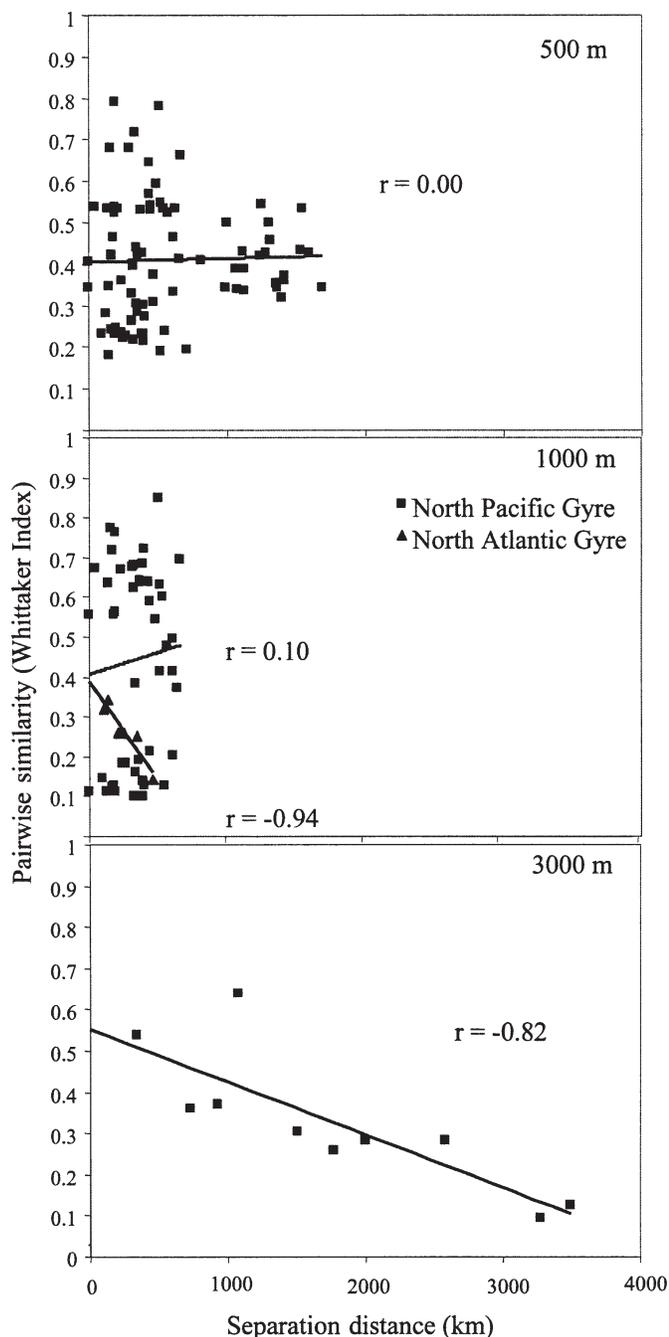
Fig. 5. Pairwise similarity (Whittaker Index) and separation distance compared for all samples at 500 m, 1,000 m, and 3,000 m. Correlation coefficients were not different from zero for the North Atlantic Ocean at 500 m when considered separately from the North Pacific Ocean samples. The correlation coefficients for 1,000 m in the North Atlantic Ocean and at 3,000 m for all locations were significant ($p < 0.05$).

consume carbon that sinks from surface waters (Cho and Azam 1988; Nagata et al. 2000), rather than that advected horizontally by lateral transport. Satellite imagery of primary production has demonstrated remarkable patchiness in mesoscale eddies over the surface of the oligotrophic oceans (Subramaniam et al. 1999), and Rossby waves have

been demonstrated to cause patchiness in surface water primary productivity in the North Pacific Ocean (Saka-moto et al. 2004). Consequently, variation in assemblage structure in the deep oligotrophic North Pacific and Atlantic may reflect both temporal and spatial variability in organic matter source, but also free dispersal of bacterial species. An alternative possibility is that there may be deep mesoscale eddies with stable microbial assemblages swirling past each other in complex patterns, like those seen at the surface.

We used a sensitive molecular fingerprinting technique, automated rRNA intergenic spacer analysis (ARISA; Fisher and Triplett 1999) to compare different deep-water bacterial communities. Such techniques endeavor to display the entire bacterial community in a single assay, separated into OTUs, with OTU resolution in this case near the species level (Fisher and Triplett 1999; Brown et al. 2005). PCR-based approaches to addressing microbial assemblage composition have been criticized because of unequal extraction of target cells (Polz et al. 1999), differences in amplification efficiency because of G + C content (Suzuki and Giovannoni 1996) and differences in *rrn* copy number per organism (Crosby and Criddle 2003). However, slow-growing bacterioplankton typically have compressed genomes containing one or two *rrn* copies (Klappenbach et al. 2000). Additionally, reports of proportional amplification in fingerprinting studies (Leuders and Friedrich 2003) and strong positive correlations between *Prochlorococcus* flow-cytometry counts and the relative proportion of amplified DNA associated with an ITS length similar to that in the *Prochlorococcus* genome indicates that ARISA is at least semiquantitative when used to analyze bacterioplankton communities (Brown et al. 2005).

PCR used in this study focused upon bacteria domain, thought to make up 60% of prokaryotic abundance, if we can extrapolate from a few local studies (Fuhrman and Ouverney 1998) and a North Pacific long-term study (Karner et al. 2001). ARISA of the Archaea domain would probably miss several taxa because of the prevalence of unlinked 16S–23S genes in marine Crenarcheotes (Moreira et al. 2004). Similarly, this analysis may not include bacteria with unlinked *ssu* and *lsu* genes (e.g., Planctomy-cetes; Menke et al. 1991). Recent genomic study of a *Chloroflexi* relative, similar to the SAR-202 cluster that is common in mesopelagic waters (Morris et al. 2004), revealed unlinked 16S and 23S rRNA genes, suggesting this may be a common feature of *Chloroflexi* relatives (Seshadri et al. 2005). We are also aware of criticism that the primer set used in this study may not amplify all bacteria in terrestrial environments (Cardinale et al. 2004). Our results form part of a global diversity dataset generated in our laboratory, amplified using the Fisher and Triplett (1999) primers, which allows intercomparison between studies (Brown et al. 2005; Hewson and Fuhrman 2004). The amplification of 7 replicate surface seawater samples collected within ca. 1 km of each other, using our primers, is repeatable because fingerprints shared >89% of OTU and a Whittaker Index of >0.90 (Hewson et al. in press). Our conclusions do not rely on complete coverage of taxonomic diversity by the fingerprints (e.g., the absence of

Archaea or bacteria with unlinked *ssu* and *lsu* rRNA genes), but rather upon reasonably good coverage and consistency to permit comparisons, because spatial variability in these components is independent of spatial variability in bacteria amplified by ARISA.

Furthermore, our results apply to free-living bacteria <ca. 1.2 μm, because samples for fingerprinting analyses were prefiltered to remove larger particles. Although undoubtedly differences in the particle-associated assemblages (e.g., those associated with marine snow and particulate organic matter) may exist, analysis of their assemblages is made more difficult than analysis of the free-living assemblage because of the potential inclusion of eukaryotic plastids (in phytoplankton and potentially in the guts of zooplankton). Plastid DNA is readily amplified with the universal and bacterial primers utilized in this study, and consequently would appear to be a distinct OTU in fingerprint. Variability in fingerprints from station to station could occur in larger-size fractions because, with low abundance of micro- and mesozooplankton in meso- and bathypelagic waters, stochastic effects of random rare inclusions in filtered volumes (4.5 L–20 L) may not allow a consistent population to be sampled from location to location. In any case, our study focused on the free individual bacteria.

These results demonstrate that mesopelagic bacterial assemblages are stable over time at a single location under a relatively productive regime, yet remarkably variable spatially beneath less productive open-ocean conditions. Although some of this variability may be because of sampling of different water masses, even those assemblages within the same or similar water masses are dissimilar. Our results contrast with paradigms based on slow-growing microorganisms with doubling times of months or years (Jannasch et al. 1970; Cho and Azam 1988; Nagata et al. 2000) and therefore hypothetically stable communities in the deep ocean. This variability in open-ocean mesopelagic bacterioplankton assemblages may be a consequence of spatially heterogeneous resource inputs because of the "rain" of organic matter from overlying waters, compared to mesopelagic waters below more productive regimes where the supply of organic matter is more consistent over time. Finally, these results demonstrate that assemblages are probably not heavily influenced by older organic matter sources transported by global deep-water circulation patterns. Thus, the remarkable deep-sea diversity patterns we observed imply highly localized and patchy rather than basin-wide or regional control of deep-sea biogeochemical processes, and suggest that large-scale genomic sequencing efforts need to consider this high variability in deeper pelagic environments.

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