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Temporal and spatial response of bacterioplankton lineages to annual convective overturn at the Bermuda Atlantic Time-series Study site

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Abstract

We used terminal restriction fragment length polymorphism (T-RFLP), clone library, phylogenetic, and bulk nucleic acid hybridization analyses to identify and characterize spatial and temporal patterns in marine bacterioplankton communities at the Bermuda Atlantic Time-series Study (BATS) site. Nonmetric multidimensional scaling of monthly surface and 200-m bacterial 16S rDNA T-RFLP fragments from 1992 to 2002 revealed temporal trends in bacterial community structure in different depth horizons. A 200-m 16S rRNA gene clone library was used to identify fragments increasing in relative abundance following mixing events and to link observed terminal restriction fragments with those predicted from sequence data. T-RFLP fragments matching those of cloned OCS116, SAR11, and marine *Actinobacteria* rRNA genes exhibited the strongest increases at 200 m following convective overturn, and fragments attributable to SAR11, SAR86, and SAR116 rRNA genes exhibited the strongest increases at the ocean surface during summer time periods. Variability in the distribution and relative abundance of fragments assigned to different SAR11 and SAR86 subclusters was also evident. Quantitative hybridization of extracted 16S rRNA with radiolabeled, taxon-specific oligonucleotide probes provided additional data supporting spatial and temporal patterns of lineage distributions and abundances suggested by ordination. Overall increases in the relative abundance of T-RFLP fragments attributable to the OCS116, SAR11, and marine *Actinobacteria* clusters following convective overturn suggest that members of these groups may play important roles in dissolved organic carbon dynamics at BATS.

Bacterioplankton are major biogeochemical agents responsible for mediating the flux of dissolved organic matter (DOM) (Ducklow 1993; Azam 1998) and subsequent cycling of nutrients. At the Bermuda Atlantic time-series study (BATS) site, regular annual patterns of nutrient distributions, DOM, and biological production are influenced by convective overturn (Hansell and Carlson 2001; Steinberg et al. 2001). Prokaryotic cell concentrations are greatest in the photic zone, reaching maximal concentrations of 5–10 $\times$ 10^8 cells L^{-1} in the summer period between 40 and 60 m. In the upper mesopelagic zone (140–250 m), cell abundances decrease significantly to 0.6–4 $\times$ 10^8 cells L^{-1} (Carlson et al. 1996). Despite the reduction in biomass at depth, a regular annual pattern of prokaryote cell dynamics occurs between 140 and 250 m at BATS, with cell concentrations increasing 20–260% during or shortly following convective overturn and persisting at elevated concentrations for several weeks (Carlson et al. 1996; Steinberg et al. 2001). Results from a simulated mixing study showed that mesopelagic bacterioplankton exhibited higher rates of surface-water dissolved organic carbon (DOC) degradation than did surface bacterioplankton. The resulting transformation of bacterial com-

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munity structure with subsequent DOM removal and enhanced bacterioplankton production suggest that mesopelagic community structure is a factor in mesopelagic bacterial and DOC dynamics after convective mixing at BATS (Carlson et al. 2004). However, few field data describing the spatial and temporal patterns of bacterial community structure of the upper mesopelagic in oceanic waters exist.

Ribosomal RNA gene cloning and abundance estimates indicate that bacterioplankton populations are stratified and that diverse lineages with mostly unknown phenotypes dominate marine microbial communities. The SAR11 clade, *Archaea* group I, and the SAR86 and *Chloroflexi*-related SAR202 clusters were recovered in early cloning studies from the Atlantic and Pacific Oceans (Giovannoni et al. 1990; DeLong 1992; Fuhrman et al. 1992) and are among the more predominant gene lineages in seawater (Giovannoni and Rappé 2000). Fluorescence in situ hybridization (FISH) cell counts have generally agreed with gene abundance estimates and have indicated that SAR11 cells can account for 35% of surface microbial communities in the Atlantic Ocean (Morris et al. 2002), and *Archaea* group I cells can account for 39% of mesopelagic and bathypelagic microbial communities in the Pacific Ocean (Karner et al. 2001). Cell counts for the SAR86 group reached 10% of surface counts in the North Sea (Eilers et al. 2000), and members of the SAR202 group accounted for 10% of all 4′-6-Diamidino-2-phenylindole (DAPI) staining cells in the Atlantic and Pacific Oceans between 500 and 4,000 m (Morris et al. 2004). Far less is known about the distribution and abundance of other bacterioplankton gene lineages that are also consistently recovered in clone library studies (Giovannoni and Rappé 2000).

Nonmetric multidimensional scaling (NMS) extracts predominant trends in community composition from a set of samples and indicates species contributions to predominant trends (Kruskal 1964; Mather 1976). When combined with community fingerprinting methods such as denaturing gradient gel electrophoresis (Muyzer et al. 1993), length heterogeneity polymerase chain reaction (PCR) (Suzuki et al. 1998), and terminal restriction fragment length polymorphism (T-RFLP) analyses (Liu et al. 1997), NMS can identify relative 16S rRNA gene variation among both major and minor lineages occurring in a set of samples. While less specific than direct cell counts or quantitative PCR estimates, T-RFLP analyses have been used to identify differences between prokaryotic communities in response to various environmental factors. For example, Moeseneder et al. (2001) used T-RFLP to compare attached and free-living bacterial communities of the eastern Mediterranean Sea, while González and coworkers (2000) used this method to fingerprint bacterioplankton communities associated with an eddy-induced oceanic algal bloom in the North Atlantic Ocean. Here we use NMS, T-RFLP, rDNA cloning, and phylogenetic analyses to identify and characterize bacterioplankton community shifts from monthly surface and 200-m samples collected at the BATS site between 1992 and 2002.

NMS ordination and bulk nucleic acid hybridization analyses identified similar spatial and temporal patterns in relative rDNA and rRNA abundances, respectively. Increases in the abundance of T-RFLP fragments matching cloned OCS116, SAR11, and marine *Actinobacteria* rRNA genes coincided with temporal increases in total microbial cellular abundance following annual patterns of deep convective mixing at BATS (February–May). Additional patterns in community structure indicated summer (June–September) ocean surface increases in fragments matching cloned SAR11, SAR86, and SAR116 rRNA genes. Monthly 16S rRNA abundance estimates supported previously reported patterns for SAR11, SAR202, and SAR324 distributions in seawater (Giovannoni et al. 1996; Field et al. 1997; Wright et al. 1997), lending additional support to the ordination results reported here. The temporal increase in SAR11, marine *Actinobacteria*, and OCS116 clusters documented here suggests that representatives of these groups may play important roles in organic and inorganic nutrient cycling associated with deep convective mixing at BATS.

Materials and methods

Sample collection and nucleic acid extraction—Monthly surface and 200-m North Atlantic Ocean seawater samples were collected at the BATS site (32°N, 64°W) from August 1992 to February 1994, and September 1997 to December 2002. High molecular weight (HMW) RNA and DNA from samples collected prior to 1997 were obtained as described previously (Giovannoni et al. 1996). All other seawater samples were filtered through 0.2-μm polysulfone filters (Supor-200; Pall) housed in an in situ water transfer system (McLane). HMW RNA and DNA was extracted and processed as described previously (Giovannoni et al. 1996).

PCR, restriction digest, and T-RFLP analysis of 16S rDNA—Ribosomal RNA genes from mixed communities were amplified as previously described (Morris et al. 2004), with the following modifications: 16S rRNA genes were amplified by PCR with *Taq* polymerase (Fermentas) and variations of commonly used bacterial primers, 8F-FAM (Morris et al. 2004) and 519R (5′-GWATTACCGCGGCKGCTG-3′). The 8F-FAM primer was 5′ end labeled with the phosphoramidite fluorochrome 5-carboxy-fluorescein (6-FAM). Amplifications were performed in a PTC-0200 thermocycler (MJ Research) using the following conditions: 28 cycles, annealing at 55°C for 1 min, elongation at 72°C for 2 min, and denaturation at 94°C for 30 s.

PCR products were restricted for 6 h at 37°C in digests containing the appropriate buffer, 10 mmol L^{-1} $MgCl_2$, and 10 units *Bsu*R1 (*Hae*III) restriction enzyme (Fermentas). PCR and restriction digest cleanup were performed by transferring restricted amplicons to a MultiScreen 96-well filtration plate (Millipore) containing Amersham Sephadex G-50 superfine (Amersham). MultiScreen 96-well plates were prepared as described by the manufacturer (Millipore). Terminal restriction fragments were resolved on an ABI 3100 genetic analyzer (Applied Biosystems), and ABI Genotyper software was used to size fragments based on an internal lane standard with 23 discrete fragments ranging from 50 to 1,000 base pairs (bp) in length (Bioventures MapMarker 1000).

Table 1. Cloned 16S rDNA terminal restriction fragment lengths in nucleotides (A) observed on an ABI 3100 fragment analyzer and (B) predicted from gene sequences, and corresponding Pearson and Kendall correlations with ordination axes (Fig. 2) The number of clones exhibiting similar fragment lengths is listed for the first sequenced representative under total. No data (ND) indicates gene clones with observed fragment lengths smaller than the minimum standard used for T-RFLP analyses (50 bp) and those not present in final ordination matrices. T-RFLP designations are based on similarities with cloned 16S rDNA sequences identified by T-RFLP, sequencing, and phylogenetic analyses. Accession numbers for previously published sequences are in bold.

Clone ID	Accession numbers	T-RFLP fragment designation	Fragment length		Total	Complete axis 2	Surface axis 1	200 m axis 1
			A	B				
D92_09	AY923000	SAR11	ND	39	7	ND	ND	ND
D92_20	AY923001	Uncultured *gamma*	ND	39	7	ND	ND	ND
D92_33	AY923002	*Alteromonas*	ND	39	7	ND	ND	ND
D92_11	AY923003	SAR11 subgroup Ia	113	117	7	0.66	0.61	−0.73
D92_72	AY923004	SAR86	187	188	1	0.41	0.52	ND
D92_37	AY923005	SAR86	188	189	2	0.73	0.46	−0.64
D92_82	AY923006	SAR116	192	193	2	0.16	0.44	0.30
D92_17	AY923007	Uncultured *gamma*	193	193	2	−0.03	0.12	−0.62
D92_84	AY923008	*Roseobacter*	194	195	1	0.14	0.12	0.03
D92_26	AY923009	SAR316	202	203	1	−0.09	ND	ND
D92_04	AY923010	SAR11 subgroup Ib	227	228	10	0.48	0.77	−0.26
D92_46	AY923011	SAR11 subgroup Ib	228	230	1	−0.13	−0.78	−0.26
D92_22	**AY534099**	SAR202	257	258	1	0.04	−0.31	−0.45
D92_36	**AY534100**	SAR202	260	263	1	−0.06	ND	−0.19
D92_87	AY923012	SAR202	155	155	1	−0.53	−0.04	−0.05
D92_53	AY923013	*Cytophaga*	286	287	2	ND	ND	ND
D92_01	AY923014	SAR11 subgroup II	291	293	11	0.44	−0.49	−0.35
D92_34	AY923015	SAR11 subgroup II	291	293	11	0.44	−0.49	−0.35
D92_31	AY923016	SAR11 subgroup Ib	292	294	5	−0.59	0.54	0.19
D92_03	AY923017	OCS11	302	303	5	0.14	−0.70	−0.83
D92_63	AY923018	OCS11	302	303	5	0.14	−0.70	−0.83
D92_65	AY923019	OCS11	302	303	5	0.14	−0.70	−0.83
D92_92	AY923020	OCS11	302	303	5	0.14	−0.70	−0.83
D92_25	AY923021	*Prasinophyte* chloroplast	303	304	1	0.05	−0.38	−0.35
D92_02	AY923022	*Alcanivorax*	311	311	1	0.62	0.61	−0.47
D92_74	AY923023	Uncultured *gamma*	325	326	1	−0.05	−0.42	−0.06
D92_32	AY923024	Marine *Actinobacteria* cluster	328	330	2	0.08	−0.64	−0.65
D92_10	AY923025	*Prasinophyte* chloroplast	388	390	1	0.15	−0.59	−0.54
D92_78	AY923026	SAR324	405	405	2	−0.72	ND	0.39

Cloning—A bacterial 16S rRNA gene clone library was constructed from bacterioplankton collected from 200 m at the BATS site in February 1992 as described previously (Giovannoni et. al 1996; Morris et al. 2004). In short, ribosomal RNA genes were amplified from environmental DNA for cloning by PCR with *Taq* polymerase (Fermentas) and variations of commonly used bacterial primers, 8F and 1492R (Morris et al. 2004). Amplifications were performed in a PTC-0200 thermocycler (MJ Research) using the following conditions: 35 cycles, annealing at 55°C for 1 min, elongation at 72°C for 2 min, and denaturation at 94°C for 30 s. A single band of the predicted length was observed by agarose gel electrophoresis. A clone library was constructed using the resulting mixed-template amplicon and the pGEM-T-Easy (Promega) vector following the manufacturer's instructions. The clones were assigned the prefix D92 and numbered discontinuously from 1 to 96. All clones were stored at −20°C in Luria Bertani broth containing 10% glycerol (w/v). The clone library was screened using a streamlined protocol (Vergin et al. 2001) modified for T-RFLP analysis. Cloned 16S rDNA fragments were amplified, restricted, cleaned, and resolved as described above for environmental samples. In short, plasmid DNA was extracted and 16S rDNA inserts were amplified using the 8F-FAM labeled and 519R primers used for environmental T-RFLP analyses. Clones exhibiting similar terminal restriction fragments were grouped together, and group representatives were selected for sequencing (Table 1).

Sequencing and phylogenetic analysis—Cloned 16S rRNA genes were grouped according to terminal restriction fragment length, and representatives were sequenced and used for phylogenetic analysis. Only clones that contained full-length inserts (~1,500 bp), and for which there was a single or dominant observed terminal restriction fragment, were selected for sequencing (Table 1). 16S rRNA gene sequences were compared with sequences available in the GenBank database by Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1997) and aligned with the ARB software package and database (Ludwig et al. 2004). Only unambiguously aligned nucleotide positions were used for phylogenetic analyses. Three hundred sixty-four nucleotide positions (*E. coli* numbering, 83–503) were used for SAR11 phylogenetic analyses with PAUP* 4.0 beta 10 (Swofford

2002). Phylogenetic trees were inferred by neighbor-joining analyses (Saitou and Nei 1987) using Juke–Cantor evolutionary distance correction. The tree topology was also compared with the phylogenetic trees inferred by both maximum parsimony with a heuristic search and maximum likelihood with a heuristic search, a transition/transversion ratio of 1.7326, and nucleotide frequencies estimated from the data. The neighbor-joining and parsimony trees were evaluated by bootstrap analyses based on 1,000 and 100 resamplings, respectively. Phylogenetic trees other than the SAR11 tree were inferred with the ARB software package, using Juke–Cantor evolutionary distance correction and neighbor-joining analyses (Saitou and Nei 1987). The resulting neighbor-joining trees were evaluated by bootstrap analyses based on 1,000 resamplings with PAUP* 4.0 beta 10 (Swofford 2002). Short sequences (less than 800 bp) were inserted into phylogenetic trees using the ARB database parsimony insertion tool.

Statistical analysis of community structure—Nonmetric multidimensional scaling searches for the best positions of *n* entities (samples) on *k* dimensions (axes) that minimize stress of the final configuration. Calculations are based on a distance matrix generated from the species matrix, and stress is measured as the departure from monotonicity between distance in the original space and distance in ordination space (McCune and Grace 2002). Sorensen distance and autopilot slow and thorough options were selected for NMS analyses of T-RFLP data using a random starting configuration and the software package PC-ORD (McCune and Grace 2002). Analysis of an initial data matrix (data not shown) indicated that the predominant trends in bacterioplankton community structure at BATS had spatial and temporal components. Sample D92 (February 1992, 200 m) showed the strongest 200-m increase in fragments contributing to the temporal trend following convective overturn and was chosen for subsequent 16S rRNA gene cloning and sequencing. A total of 95 surface samples and 94 samples from 200 m were screened by T-RFLP. 16S rDNA restriction fragments were identified in 167 of 189 sample amplification and *Hae*III restriction digest reactions. Normal analyses were performed on complete (164 by 213), surface (81 by 114), and 200-m (83 by 157) data matrices. The following data adjustments were performed in the order listed: (1) fragments occurring in fewer than 10 samples were excluded; (2) fragments were transformed into relative units by dividing integrated fragment areas by the total for their corresponding sample; (3) samples with an average distance lying more than three standard deviations from the mean distance among sample units were excluded. Dimensionality was determined by assigning a priori categorical variables to surface and 200-m samples from postmixing (February–May), summer (June–September), and winter (October–January) time periods. Categories were based on suspected patterns associated with deep convective mixing and known patterns in community structure. The final stress obtained for complete, surface, and 200-m ordinations was 16.0, 13.1, and 15.9, respectively. A stable two-dimensional solution was identified for all ordinations, with instability near zero. The probability of a similar stress obtained by chance (0.0196) was determined for 40 real and 50 randomized Monte Carlo runs. Axes were rotated to maximize orthogonality (>99%).

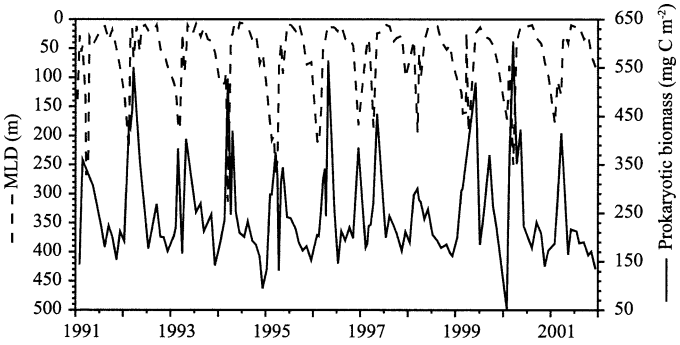

Fig. 1. Seasonality of the mixed layer depth (MLD) and integrated prokaryote biomass between 140 and 250 m at BATS. Prokaryote biomass was estimated from cell abundance using a carbon conversion factor of 1×10^{-14} g C cell^{-1}. MLD was determined as the depth where sigma-*t* is equal to sea surface sigma-*t* plus an increment in sigma-*t* equivalent to a 0.2°C temperature decrease (Sprintall and Tomczak 1992).

RNA profiles—RNA profiles expressed as the ratio of specific probe hybridization to bacterial 338R probe hybridization of blotted RNA proportions (100, 50, 20, and 10 ng) cross linked to Zetaprobe (Bio-Rad) nylon membranes were performed as previously described (Giovannoni et al. 1996). Two oligonucleotide probes targeted the SAR11 clade, SAR11–441R and SAR11–542R (Morris et al. 2002); one probe targeted the SAR202 cluster, SAR202–312R (Morris et al. 2004); and one probe targeted the marine *Actinobacteria* cluster, OM1–195R (5′-ATCTTAGTTCGGCTGACCTT-3′). Stringency conditions used for this study were empirically determined. Hybridization wash temperatures were 42°C and 45°C in 2.0 × saline-sodium phosphate-EDTA buffer (SSPE)/0.1% sodium dodecyl sulfate (SDS), and 37°C in 0.2 × SSPE/0.1% SDS for SAR11, SAR202, and marine *Actinobacteria* probes, respectively. The specific hybridization value for SAR11 was determined by comparison with the SAR11 positive control strains, HTCC1040 and HTCC1062 (Rappé et al. 2002).

Nucleotide sequence accession numbers—Gene sequences were deposited in Genbank and given accession numbers AY923000 through AY923026

Results

Prokaryote dynamics in the upper mesopelagic—Throughout the BATS record, integrated stocks of the upper mesopelagic prokaryote biomass (140–250 m) increased 60–260% during or shortly following convective overturn and persisted at elevated concentrations for several weeks (Fig. 1). From 1992 to 2001 (excluding 1994 and 1998) there was a temporal lag between annual convective overturn and an increase in prokaryote stocks in the upper mesopelagic zone (Fig. 1). Ordination of the complete T-RFLP data set indicated variability in relative fragment abundances among samples with depth, and during or following deep convective

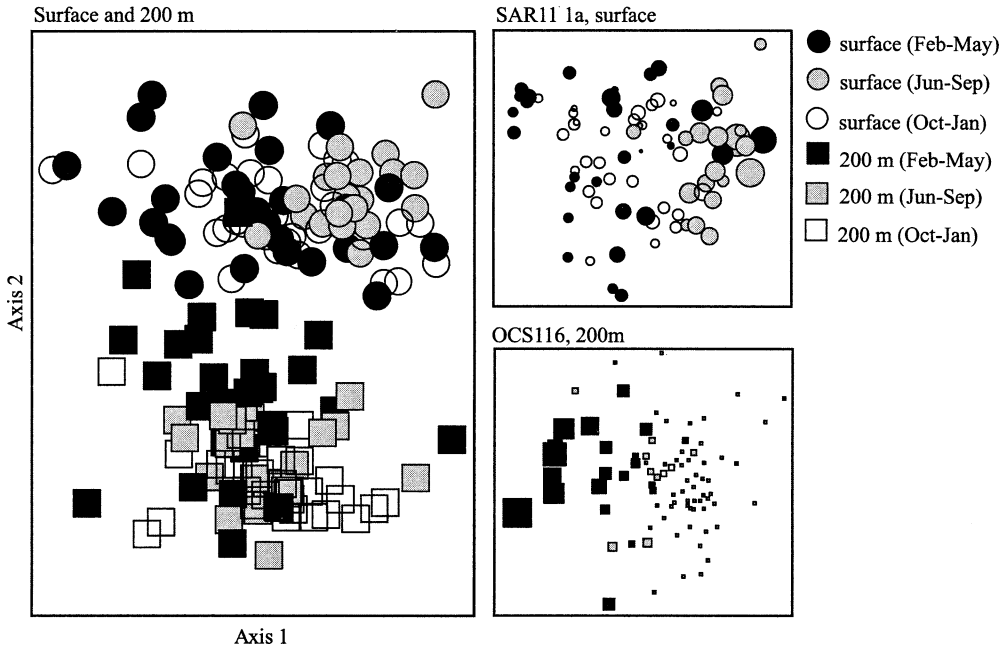

Fig. 2. Nonmetric multidimensional scaling (NMS) of bacterial 16S rDNA terminal restriction fragments collected at the BATS site between 1992 and 2002. Sample groupings indicate bacterioplankton variability in postmixing (February to May), summer (June to September), and winter (October–January) time periods for complete, surface, and 200-m T-RFLP data sets. Integrated fragment areas were divided by the total for their corresponding sample to transform the data into relative units. Larger symbol sizes correspond to increases in the relative abundance of SAR11 subgroup Ia and OCS116 fragments. Correlation coefficients in Table 1 indicate the strength (magnitude) and directionality (sign) of a fragment's contribution to an ordination axis. Positive and negative signs indicate an increase or decrease along an axis, respectively. February 200 m (D92) exhibited strong increases in fragments contributing to postmixing groupings and was selected for clone library analysis.

overturn (Fig. 2). Of the 212 T-RFLP fragments of distinct length identified in this study, 11 were unique to samples collected from the surface and 33 were unique to the upper mesopelagic zone. Fragments assigned to different SAR11 subgroups accounted for three of the most abundant fragments recovered (113, 228, and 292 bp). Combined, these fragments averaged 20% of total integrated fragment areas in $n = 158$ (113), $n = 129$ (228), and $n = 129$ (292) samples. Correlation coefficients from the complete ordination suggested that fragments matching cloned SAR11 and SAR86 cluster fragments were more abundant in surface waters. Fragments matching other cloned SAR11 fragments, and SAR202 and SAR324 cloned fragments were more abundant in the upper mesopelagic zone (Table 1). Correlation coefficients in Table 1 indicate the strength (magnitude) and directionality (sign) of a fragment's contribution to an ordination axis. Positive and negative signs indicate an increase or decrease along an axis, respectively.

Surface and 200-m data sets were ordinated separately to test the robustness of seasonal trends in community structure independently of variation with depth (Fig. 2). Nonmetric multidimensional scaling of depth-specific data matrices supported postmixing prokaryotic blooms in the 200-m depth horizon and identified specific fragment increases following mixing (Fig. 2; Table 1). Correlation coefficients with axis one indicated that a fragment with a predicted length of 303 bp (OCS116) exhibited the strongest contribution to ordination of samples along axis one (Table 1). Symbol sizes in Fig. 2 indicate relative differences in 303-bp fragment contributions to 200-m samples. Larger symbols indicate an increase in the relative abundance of 303-bp fragments to samples collected between February and May (solid squares). Similar contributions are indicated by strong negative correlation coefficients for SAR11 and marine *Actinobacteria* fragments (Table 1). Postmixing groupings (February–May) were less robust for the surface ordination (Fig. 2). However, summer sample groupings (June–September) supported known increases in relative SAR11 rRNA abundances during these time periods (Morris et al. 2002). Symbol sizes in Fig. 2 indicate relative differences in the 113-bp fragment (SAR11 subgroup 1a) contributions to surface samples. Larger symbols indicate an increase in the relative abundance of 113-bp fragments to samples collected between June and September. Similar contributions are indicated by strong positive correlation coefficients for fragments assigned to SAR86 and SAR116 gene clusters (Table 1).

16S rRNA terminal restriction fragment identification and phylogeny—Phylogenetic affiliations were assigned to terminal restriction fragments by direct comparison of observed and predicted fragment lengths obtained from 16S rDNA

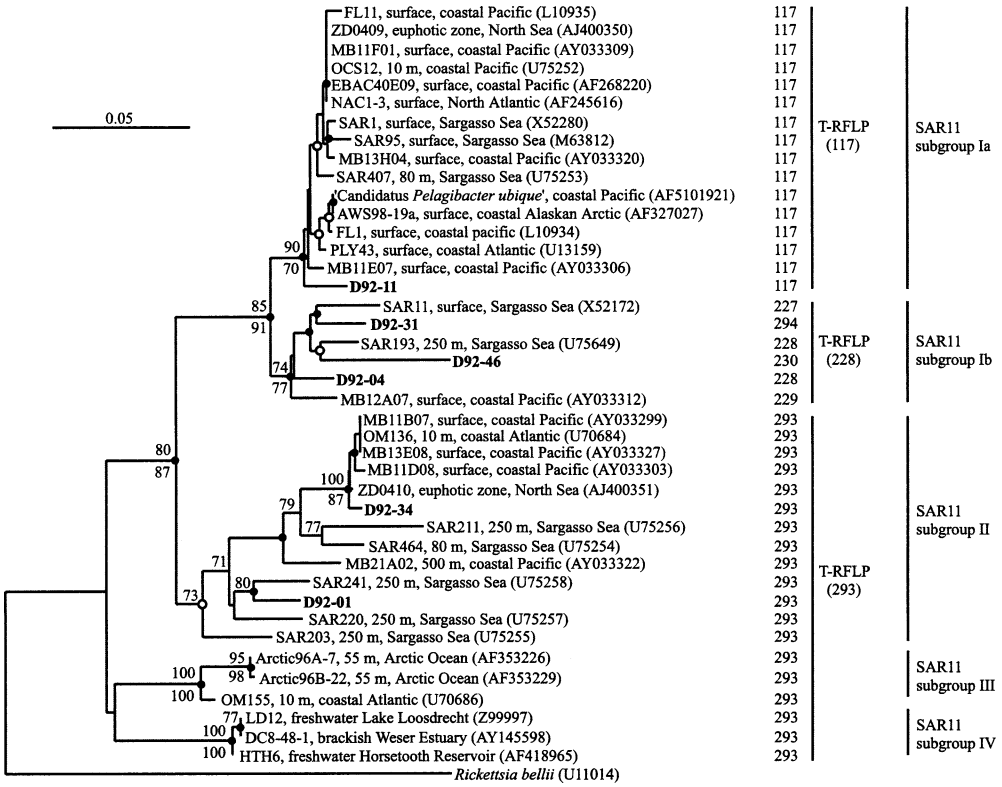

Fig. 3. Neighbor-joining 16S rRNA phylogenetic tree showing terminal restriction fragment sizes and relationships among representative members of the SAR11 clade. The scheme used for naming the SAR11 subgroups is an extension of nomenclature proposed by Suzuki et al (2001). Note that subgroups III and IV are found in brackish or fresh water, and therefore the 293-bp (predicted length) fragment is essentially monophyletic at BATS. Clones obtained in this study are shown in bold type. Representative sequences derived from public databases are labeled with clone names and accession numbers. Bootstrap proportions over 70% from both neighbor-joining (above nodes) and maximum parsimony (below nodes) are shown. The closed circles and open circles at each node indicate recovered nodes in three treeing methods and recovered nodes in two treeing methods, respectively. SAR11 subgroups Ia, Ib, II, III, and IV were designated by tree topology. *Rickettsia bellii* (U11014) was used as an outgroup. Terminal restriction fragment sizes expected from the 16S rRNA gene sequence data are shown to the right. Scale bar, 0.05 substitutions per nucleotide position.

clone library sequence data (Table 1). Sixty-eight clones in the D92 library were placed in 21 groups with unique fragment lengths, and representatives of each group were sequenced. Seven of the sequences, representing 35 clones, were placed in the SAR11 clade; four terminal restriction fragments (predicted lengths of 117, 228, 293 bp) accounted for most (28 of 35) of these clones (Table 1). The seven remaining SAR11 clones had predicted fragment lengths that were uninformative (<50 bp), 230, or 294 bp. Clones representing other well-characterized bacterioplankton lineages included members of the marine *Actinobacteria* cluster (Rappé et al. 1999), the *Chloroflexi*-related SAR202 cluster (Giovannoni et al. 1996), and the gamma Proteobacteria SAR86 cluster (Mullins et al. 1995). Representatives from other uncultured groups included five clones most closely related to the alpha Proteobacteria OCS116 (Rappé et al. 2000), uncultured gamma Proteobacteria affiliated with environmental clone ZD0417 (Zubkov et al. 2002), and a delta Proteobacteria (SAR324) (Wright et al. 1997). Clones closely related to groups well represented in culture collections included the genus *Alcanivorax,* the *Roseobacter* clade, the family *Flavobacteriaceae, Prasinophyte* chloroplasts, and the genus *Alteromonas* (Table 1).

Predicted terminal restriction fragment analyses of the SAR11, marine *Actinobacteria,* OCS116, SAR202, and SAR86 phylogenetic clusters using published and 16S rRNA genes sequenced in this study revealed unique, taxon-specific terminal restriction patterns using an isoschizomer of the *Hae*III restriction enzyme. Three distinct fragment types within the SAR11 clade were discriminated by 41 sequences and sequence-specific fragments (Fig. 3). The distribution of SAR11 T-RFLP fragments we report supports previous conclusions about depth-specific patterns of distribution among subgroups within the SAR11 clade (Field et al. 1997). However, there has been a substantial increase in the number of SAR11 sequences deposited in public databases since 1997, resulting in a more highly resolved SAR11 phylogeny (Garcia-Martinez and Rodriguez-Valeria 2000; Suzuki et al.

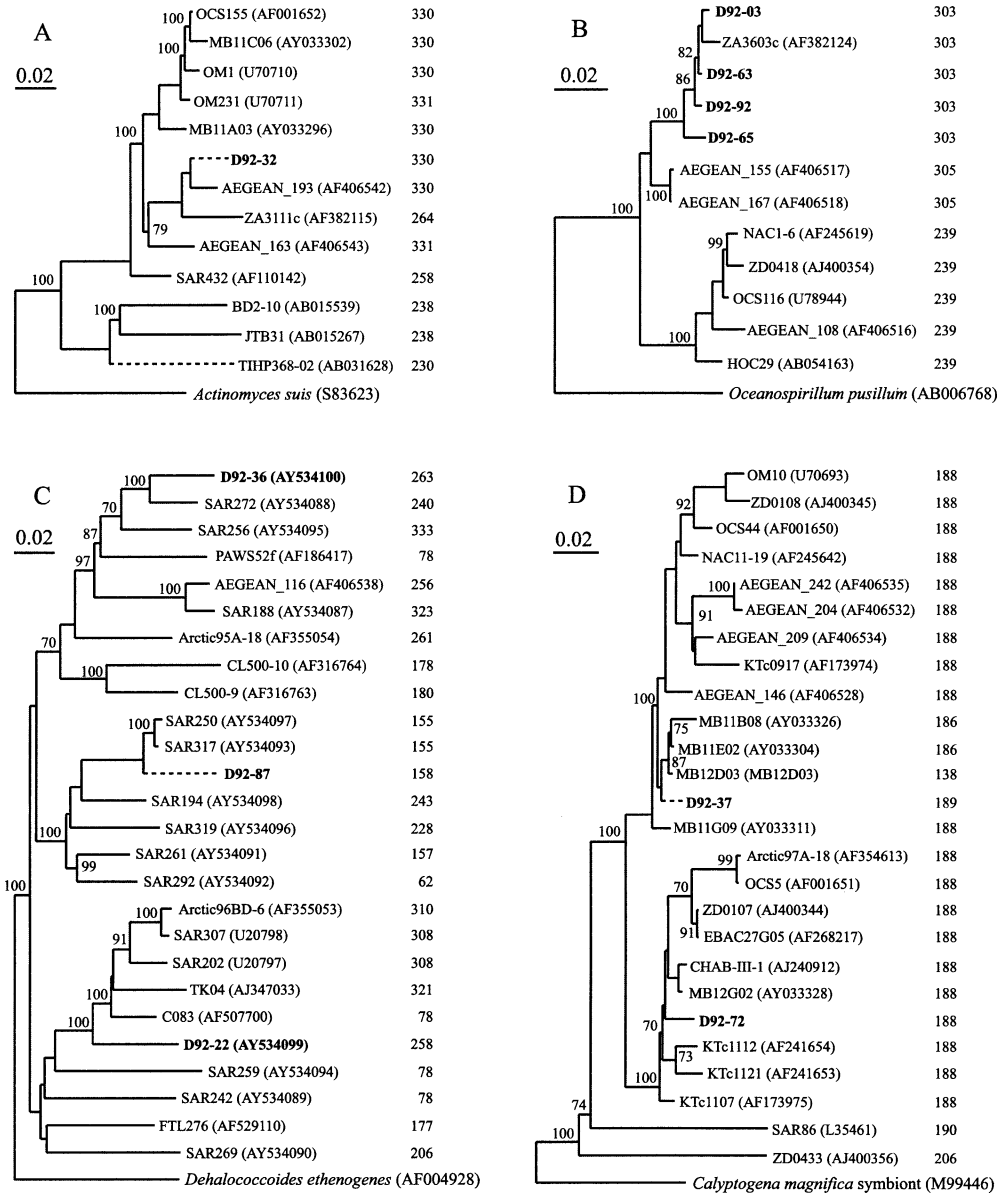

Fig. 4. Neighbor-joining 16S rRNA phylogenetic trees showing terminal restriction fragment sizes and relationships among representative members of the (A) marine *Actinobacteria,* (B) OCS116, (C) SAR202, and (D) SAR86 clusters. Clones obtained in this study are shown in bold type. Representative sequences derived from public databases are labeled with clone names and accession numbers. Sequences less than 800 bp were inserted in the tree using the ARB database parsimony insertion tool and are represented as dashed lines. Bootstrap proportions over 70% are shown. Sequences used as outgroups are italicized. Terminal restriction fragment sizes expected from the 16S rRNA gene sequence data are shown to the right of each tree. Scale bars, 0.02 substitutions per nucleotide position.

2001; Rappé and Giovannoni 2003). These analyses are consistent with our observations that there are three distinct terminal restriction fragment patterns (Ia, Ib, and II) correlated with predominant SAR11 subgroups at BATS. Of the SAR11 sequences analyzed, only two clones, D92-31 (294 bp) and D92-09 (39 bp), had predicted fragment lengths that were inconsistent with their phylogenetic position (Fig. 3). Marine *Actinobacteria,* OCS116, SAR202, and SAR86 terminal restriction fragments (Fig. 4A,B,C,D, respectively) exhibited differing degrees of uniformity within and between lineages. Ten of thirteen marine *Actinobacteria* cluster sequences analyzed were discriminated by 238-, 330-, or 331-bp terminal restriction fragments. Three unique sequences, ZA3111C (264 bp), SAR432 (258 bp), and TIHP368-02 (230 bp), had predicted fragment lengths uncharacteristic of closely related taxa. OCS116 cluster sequences had either 239-, 303-, or 305-bp predicted fragments (Fig. 4B). SAR202 sequences identified in this study were most closely

related to SAR202 subclusters one and three (Morris et al. 2004). Twenty-two unique terminal restriction fragments ranging from 62 to 333 bp in length were predicted among the diverse SAR202 sequences studied (Fig. 4C). Nearly all SAR86 cluster sequences (20 of 26) had predicted terminal restriction fragments of 188 bp (Fig. 4D). Two additional SAR86 sequences had predicted fragment lengths of 186 bp, and variants included 138-, 189-, 190-, and 206-bp representatives. Lineage designations assigned to terminal restriction fragments were unique among and between the phylogenetic lineages reported in this study. Closely related fragments identified within subclusters may have resulted from errors in peak calling, but single base differences in predicted fragment lengths obtained from cloned sequences and significant variability observed in contributions to ordination axes support fragment designations reported in this study.

Quantitative rRNA hybridizations—Integrated stocks of prokaryote biomass indicated increases in prokaryotic abundance in samples taken from 200 m following deep convective mixing at BATS (Fig. 5). Marine *Actinobacteria* exhibited increases in relative rRNA abundance at 200 m, which closely matched mixed layer depth (MLD) profiles (Fig. 5). A significant increase in marine *Actinobacteria* relative rRNA in surface waters was evident only in spring 2000, when the MLD was the greatest. An increase in the relative abundance of SAR11 rRNA in surface waters between March and September coincided with an increase in SAR11 T-RFLP terminal restriction fragment increases suggested by ordination (Fig. 2). Annual increase in the relative abundance of SAR11 rRNA at 200 m occurred slightly earlier in 1997 and closely matched MLD profiles (Fig. 5). Relative abundance estimates of SAR202 cluster rRNA agreed with ordination analyses as well, indicating greater abundance at 200 m, with no significant temporal increase observed in either depth horizon (Fig. 5).

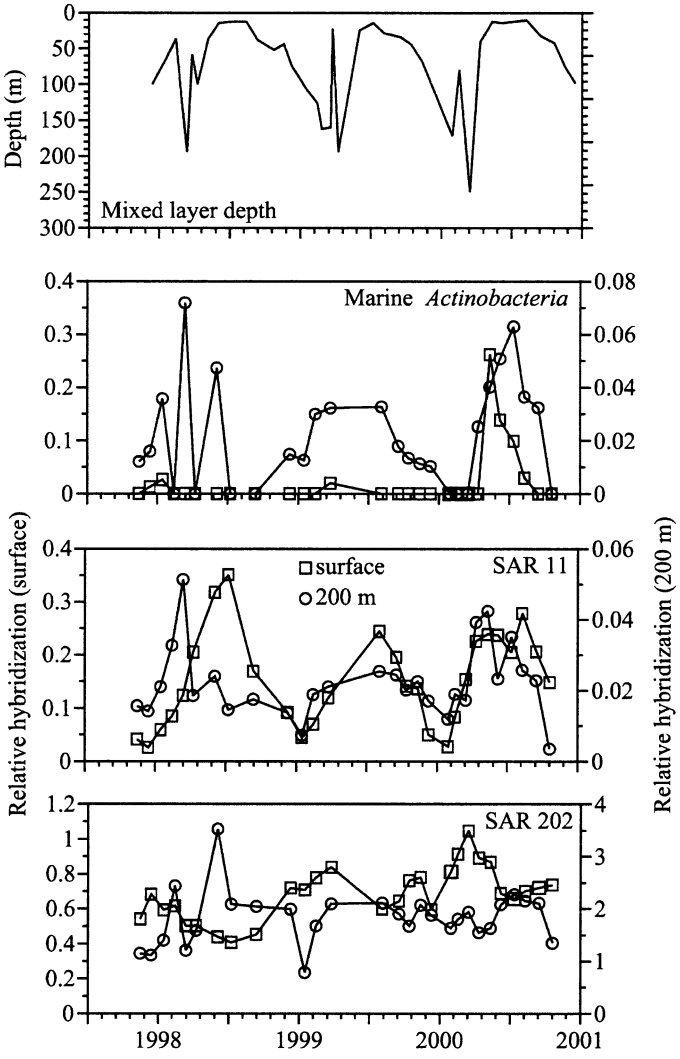

Fig. 5. Relative SAR11, SAR202, and marine *Actinobacteria* cluster 16S rRNA abundances. Monthly surface and 200-m seawater samples collected at the BATS site from November 1997 to October 2000 are shown. Mixed layer depth was determined as the depth where sigma-*t* is equal to sea surface sigma-*t* plus an increment in sigma-*t* equivalent to a 0.2°C temperature decrease (Sprintall and Tomczak 1992).

Discussion

Repeatable spatial and temporal patterns in T-RFLP fragments observed at BATS suggest that shifts in upper mesopelagic microbial communities are coupled to DOC and nutrient dynamics in the North Atlantic Ocean. Specific increases in fragments related to cloned OCS116, SAR11, and marine *Actinobacteria* genes in the mesopelagic zone supported increases in bacterioplankton biomass following convective mixing. While some of the observed increases may be due to the physical process of mixing, the observed dynamics in 16S rRNA abundance in the upper mesopelagic zone provides compelling evidence that suggests that patterns in community structure, as well as prokaryotic biomass, change in response to deep convective mixing; how these are related to environmental factors such as nutrient dynamics, light field, or organic matter cycling is yet to be determined.

We used taxon-specific relative abundance estimates obtained via T-RFLP to document patterns of bacterioplankton community variation. Terminal restriction fragments similar to SAR11 cloned fragments were identified in every sample and accounted for an average of 20% of total fragment areas. Terminal restriction fragments assigned to the marine *Actinobacteria* and OCS116 clusters were far less abundant, averaging 1.4% ($n = 120$) and 1.1% ($n = 93$) of total fragment areas, respectively. Identifying variability in both abundant (e.g., SAR11) and less abundant (e.g., marine *Actinobacteria* and OCS116) lineages was facilitated by a community-based approach. However, the primers and restriction enzyme used in this study do not target *Archaea* or differentiate all bacterioplankton lineages. Although some microbial lineages are missed using this approach, rRNA hybridization for *Archaea* and other predominant bacterioplankton lineages suggested that they did not contribute significantly to the variability in community structure reported here.

Both rRNA- and rDNA-based analyses provide evidence for a surface bacterioplankton community shift associated

with high light conditions during summer time periods. Environmental genomic studies have linked members of the SAR86 cluster with rhodopsin-like gene sequences (Beja et al. 2000). Additional studies have shown that diverse sequences related to rhodopsins are abundant in the oceans (de la Torre et al. 2003; Venter et al. 2004). This suggests that bacterioplankton lineages other than SAR86 may contain rhodopsin-based light-driven proton pumps. Community structural analysis of the surface rDNA T-RFLP data set supported summer increases in the relative abundance of fragments matching cloned SAR11, SAR86, and SAR116 genes (Table 1). Evidence of widely distributed proteorhodopsins in seawater and distribution and abundance estimates for the SAR86, SAR11, and SAR116 lineages suggests that representatives of these groups may contain adaptations similar to other phototrophic bacterioplankton.

While future quantitative distribution and abundance estimates are needed, community response data reported here are in agreement with postmixing prokaryotic blooms and experimental simulations (Carlson et al. 2004). These data identify lineage-specific increases in relative 16S rDNA abundances following deep convective mixing and correspond to patterns of DOC cycling (Hansell and Carlson 2001) in the upper mesopelagic zone at BATS. However, the effect of varying light and nutrient fields on lineage-specific variability requires further investigation.

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