RESEARCH ARTICLE

Temporal patterns of microbial community structure in the Mid-Atlantic Bight

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Abstract

Although open ocean time-series sites have been areas of microbial research for years, relatively little is known about the population dynamics of bacterioplankton communities in the coastal ocean on kilometer spatial and seasonal temporal scales. To gain a better understanding of microbial community variability, monthly samples of bacterial biomass were collected in 1995–1996 along a 34-km transect near the Long-Term Ecosystem Observatory (LEO-15) off the New Jersey coast. Surface and bottom sampling was performed at seven stations along a transect line with depths ranging from 1 to 35 m ($n = 178$). Microbial populations were fingerprinted using ribosomal 16S rRNA genes and terminal restriction fragment length polymorphism analysis. Results from cluster analysis revealed distinct temporal patterns among the bacterioplankton communities in the Mid-Atlantic Bight rather than grouping by sample location or depth. Principal components analysis models supported the temporal patterns. In addition, partial least squares regression modeling could not discern a significant correlation from traditional oceanographic physical and phytoplankton nutrient parameters on overall bacterial community variability patterns at LEO-15. These results suggest factors not traditionally measured during oceanographic studies are structuring coastal microbial communities.

Introduction

Microbial diversity using nucleic acid-based analyses has been extensively reported for oceanic samples (Mullins *et al.*, 1995; Rappe *et al.*, 1997, 2000; Murray *et al.*, 1998; Crump *et al.*, 1999; Moeseneder *et al.*, 1999, 2001; Bano & Hollibaugh, 2002). However, the degree of bacterial community variability on different temporal and spatial scales in the coastal ocean has not been well documented. Early investigations by Murray *et al.* (1998) on temporal/spatial variability, focusing on Archaea, utilized weekly samples at two depths near Anvers Island in Antarctica ($n = 36$) to demonstrate a decrease in Archaeal RNA hybridization signal from winter to summer and changes with season and depth using denatured gradient gel electrophoresis (DGGE) analysis. Later work by the same group (Murray *et al.*, 1999), with increased sampling intensity in the Santa Barbara Channel at two mooring sites [three to five depths (0–300 m); biweekly samples ($n = 207$)] found a depth correlation with domain-specific (Archaeal) and group-specific (Crenarchaeal and Euryarchaeal) probes. However, there was no significant correlation observed with season and limited resolution of various members of the community using this broad specificity, probing approach. For bacterial populations, Moeseneder *et al.* (1999) utilized a terminal restriction fragment length polymorphism (TRFLP) method with greater resolution and demonstrated spatial separation for two sites in the Adriatic and two sites in the Aegean seas using spring and summer surface samples ($n = 20$). In contrast, Bano & Hollibaugh (2002) (investigated Arctic coastal systems using DGGE) reported on primarily single-depth samples during spring/fall (March–May or August–October; $n = 11$) and described seasonal variability in various bacterial subdivisions e.g. the *Gammaproteobacteria*.

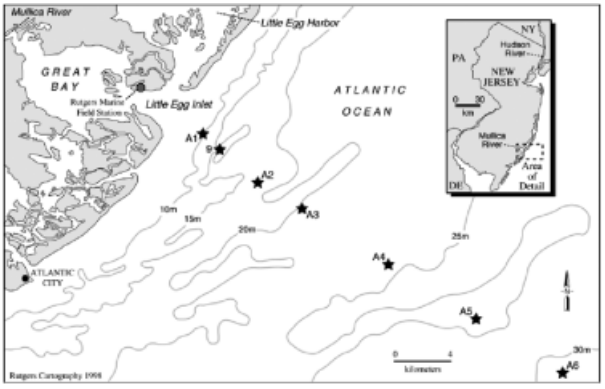

Fig. 1. Bathymetry map of the 34-km transect line from the study site off the Southern Coast of New Jersey.

Likewise, Crump & Hobbie (2005) and Kan *et al.* (2006) used DGGE to investigate estuarine samples from two Massachusetts rivers ($n = 20$) or the Chesapeake Bay ($n = 36$) and found temporal differences in microbial community structure were greater than the spatial differences. Finally, (Fuhrman *et al.*, 2006) collected an extensive series of monthly samples at a single site and depth in the San Pedro Channel at the San Pedro Ocean Time Series (SPOTS) site ($n = 54$). These researchers utilized Automated rRNA (gene) Intergenic Spacer Analysis (ARISA) and discriminant function analysis to find robust relationships between abiotic, biotic, nutrient, and ecosystem function for many of the OTUs discovered in their time series. Although each of these reports describe samples across temporal and spatial gradients in their study areas, many of these investigations have incorporated a relatively small number of environmental samples to reach their conclusions ($n < 30$) with only a few studies (Hollibaugh *et al.*, 2002; Fuhrman *et al.*, 2006) sampling the coastal ocean bacteria at higher densities ($n < 60$).

Here, we present analysis of temporal and spatial patterns of bacterioplankton communities ($n = 178$) achieved through approximately monthly sampling off the coast of New Jersey in 1995 and 1996 along a 34-km transect at the Long-Term Ecosystem Observatory (LEO-15) (von Alt & Grassle, 1992). The bacterial communities were analyzed in conjunction with traditional oceanographic measurements (salinity, temperature, N, P, etc). Our results demonstrate that temporal signals are the primary forcing factor controlling the variability of coastal bacterial communities in the Mid-Atlantic Bight (MAB). These results suggest that while the physical/chemical regime in the MAB can be heterogeneous, the bacterial community is much more homogeneous. Furthermore, phytoplankton cycles and water column responses, as indicated using traditional hydrographic measurements, do not appear to be directly structuring microbial communities. Rather, additional environmental factors, perhaps operating on discordant time scales, must be influencing the composition of microbial populations in this region of the coastal ocean.

Materials and methods

Sample collection

Water column samples were obtained along a transect line off the New Jersey Coast (Fig. 1) at roughly monthly intervals in 1995 (8 of 12 months) and 1996 (9 of 12 months), as weather conditions allowed. Physical properties were measured using a Falmouth Scientific FSI conductivity –temperature-depth (CTD) package equipped with a platinum resistance thermometer, a conductivity sensor, and a titanium pressure sensor at each station. Water samples were collected with Niskin bottles *c.* 1 m below the surface and 1 m above the bottom. At the offshore stations (A5 and A6) an additional mid-water depth sample was collected and a few surface samples were collected inshore of station A1 which were designated S1-3. From each Niskin bottle, oxygen samples were collected in prewashed 300-mL BOD bottles, fixed with $MnCl_2$ and alkaline-NaI solutions, and measured using Winkler titration. The oxygen samples were stored in the dark and analyzed on the day following sample collection. Nutrient samples were pumped directly out of the Niskin bottle through 0.45-mm Supor Gelman Sciences filters and collected in either glass (phosphate) or polystyrene (ammonia and nitrate+nitrite) tubes for analysis on a LaChat AE automated analyzer. Nutrient samples were kept refrigerated until analysis the day following collection. Carbon dioxide samples were analyzed on a single-operator multiparameter metabolic analyzer (SOMMA) that was calibrated with known volumes of high purity CO_2 gas. Alkalinity samples were also taken and analyzed using Gran titration. The pH was calculated from total CO_2 and alkalinity. The complete details of all chemical analyses and calculations have been published previously (Boehme *et al.*, 1998).

DNA-based analyses

Bacterial biomass was captured on 0.2-μm Supor Gelman filters on board from 500 to 1000 mL of seawater originating from the same Niskin bottles as oxygen and nutrient samples, frozen in the field and stored at $-80\,^{\circ}C$ until extraction. Because of funding and time constraints, only half the number of water column bacterial samples were processed and analyzed from the 1995 sampling ($n = 61$) compared with the 1996 sampling ($n = 116$). For each 0.2-μm filter, total genomic DNA was purified using a previously described phenol/chloroform extraction with minor modifications (Scala & Kerkhof, 2000; Nelson, 2002). Extracted DNA was further purified in cesium chloride density

gradients ($1\,g\,mL^{-1}$) centrifuged for 16 h at 225 000 ***g*** in a Beckman XL-100 ultracentrifuge using a TLA120 rotor. DNA bands were collected by pipetting directly from the gradient and cesium chloride and ethidium bromide were removed by drop dialysis using 0.025-μm filters from Millipore Corp. (Bedford, MA) for 1 h against 10 mM Tris (pH 8.2). Purified DNA was visualized on a 1% agarose gel and quantified using 250 ng of λ HinDIII DNA mass standard and a Fotodyne image analyzer (Fotodyne Inc., Hartland, WI) as described previously (Kerkhof, 1997).

PCR

16S rRNA target genes were amplified using standard eubacterial primers 27F labeled with (6)-carboxy-fluorescein (FAM) and 1525R (Lane, 1991). Each 50 μL PCR reaction contained 0.2 pM (each) primer, dNTPs, 5 μL of 10 × Red Taq reaction buffer (Sigma-Genosys, Woodlands, TX), 1 U of Red-Taq polymerase (Sigma-Genosys), and between 5 and 10 ng genomic DNA. The amplification conditions were as follows: an initial melting step at 94 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 90 s, and a final extension step at 72 °C for 10 min in a Perkin-Elmer Gene Amp PCR system 2400 or 9700 thermal cycler (Perkin-Elmer, Foster City, CA). Amplified DNA was visualized, sized, and quantified as described above.

Restriction digests and TRFLP analysis

Fluorescently labeled PCR products (25 ng) were digested with 1 U of Mnl1 at 37 °C for 6 h for TRFLP analysis (Avaniss-Aghajani *et al.*, 1994). DNA was precipitated with 2 vol. of 95% ethanol followed by centrifugation at 16 000 ***g*** at 4 °C using an Eppendorf microcentrifuge for 15 min. Pelleted DNA was rinsed twice with 250 μL of 70% ethanol and dried in a vacuum centrifuge for 5 min. Precipitated restriction digests were resuspended in a solution containing 14.7 μL deionized formamide and 0.3 μL of TAMRA 500-bp size standard for 15 min and denatured at 94 °C for 3 min. TRFLP profiles were generated using an ABI Prism 310 autoanalyzer (Perkin-Elmer Applied Biosystems) run in Genescan mode with a peak detection of 50 arbitrary fluorescent units.

Data analysis

Terminal restriction fragment (TRF) data was rounded to the nearest whole base pair and aligned manually into an excel spreadsheet for analysis. The presence/absence of TRFLP peaks was recorded on all peaks comprising 90% of the total TRFLP peak area for any particular profile, Sorensen's similarity indices were calculated, and the data was clustered using an unweighted-pair-group mean-average (UPGMA) with the COMPAH 96 software (Berardesco *et al.*, 1998). UNSCRAMBLER software v 7.8 (http://www.camo.com) was used to create principal components and partial least squares regression models. For principal components analysis (PCA), the presence/absence of the 60 largest TRF peaks by area was used for input *X*-variables. Nearly all TRFLP profiles from the dataset were utilized to create PCA models ($n = 157$). For partial least squares regression analysis, chemical, and physical parameters [pH, temperature (°C), $[CO_2]$, $[O_2]$, $[PO_4]$, $[NO_3+NO_2]$, and $[NH_4^+]$] were included as independent (*X*) variables, and the presence/absence of TRF peaks as response (*Y*) variables, or dependent variables. Full cross-validation was used for all samples, and all samples were standardized for one SD. This process was carried out iteratively for each individual variable (Clement *et al.*, 1998; McGarigal *et al.*, 2000; Braker *et al.*, 2001).

Results

Water column properties

Temperature at the study site off the New Jersey Coast ranged from 2.5 to 24 °C, while salinity ranged from 28.5 to 33.4 ppt. However, plots of temperature vs. salinity indicate that surface and bottom waters represent separate water masses only during spring and summer (Fig. 2). In contrast, the nutrient fields demonstrate distinct offshore gradients in ammonium, nitrate, and phosphate during winter months and in oxygen during the summer (Fig. 3). Gradients with respect to depth were also generally apparent in the measured nutrients and oxygen (except NO_3/NO_2) throughout

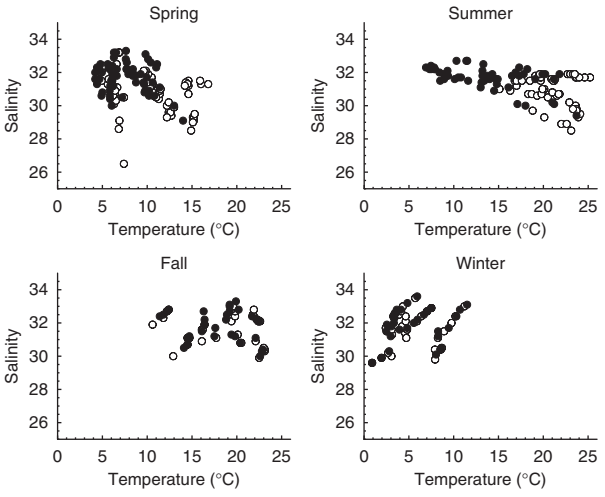

Fig. 2. Temperature vs. salinity plots of different seasons from a 2-year data set in the Mid-Atlantic Bight (1995–1996). Surface samples are presented (○), bottom samples (●).

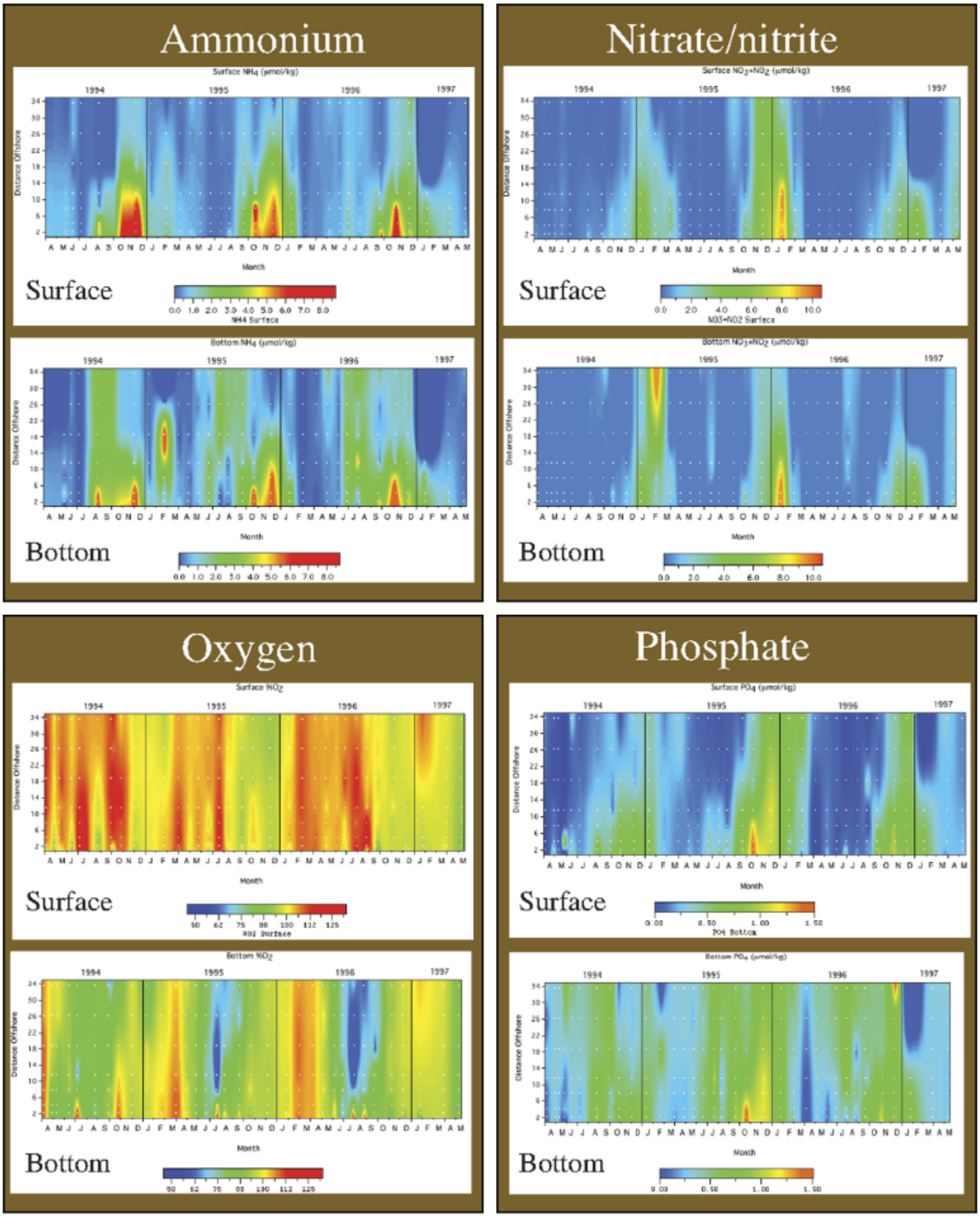

Fig. 3. Nutrient fields plotted against distance offshore in the Mid-Atlantic Bight study area over a 3-year time frame. Surface and bottom samples are indicated.

much of the year. The nutrient fields suggest that surface/bottom and onshore/offshore represent separate water masses with different physical/chemical properties.

Cluster analysis of TRFLP data

A total of 62 TRFLP profiles were obtained from 1995 and 116 profiles were generated from 1996. These fingerprints contained 121 operational taxonomic units (OTU) (average 29 ± 9 OTU per sample) in 1995 and 157 OTUs (average 28 ± 10 OTU per sample) in 1996. To ascertain whether microbial communities are more similar in time or in space during the sample period, a cluster dendrogram using Sorensen's similarity index was constructed using the COMPAH96 program (Berardesco *et al.*, 1998) and the TRFLP fingerprints (Figs 4 and 5). Samples with 3-month intervals have been color coded for ease in discerning temporal patterns. The time periods are Winter (blue: December, January, February), Spring (green: March, April, May), Summer (red: June, July, August), and Fall (grey: September, October, November). Several major temporal clusters were observed in the 1995 dendrogram in the winter, summer, and fall (Fig. 4). Each seasonal cluster formed between the 57% and the 69% similarity level. Surprisingly, the level of spatial or depth partitioning in the 1995 sample set was very limited. Specifically, a subgroup of offshore stations (A5, A6) was observed in the bottom half of the winter 1/95, 12/95 offshore cluster with similarities ranging from 0.69 to 0.87. However, other offshore samples appear to partition with inshore sites in this winter cluster. In addition, there were a few 7/95 offshore samples (station A5, A6 bottom) that partitioned into a subgroup coinciding with a hypoxic event that occurred during the summer of 1995 at the LEO-15 site (Fig. 3). For most of the cluster analysis, onshore/offshore and surface/bottom samples were co-mingled. These results indicate that spatial/depth grouping is very weak and that major temporal clusters are more well defined.

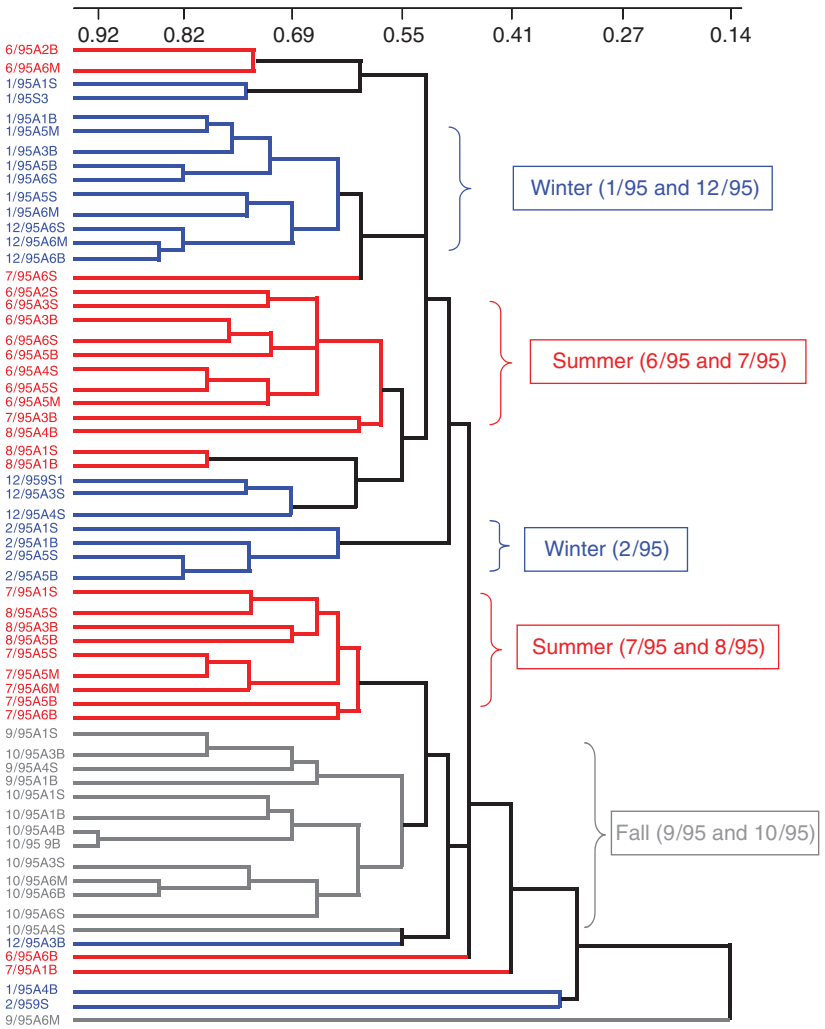

Fig. 4. COMPAH analysis of bacterial TRFLP fingerprints from 1995 ($n = 61$). Samples are color coded in 3-month increments. The percent similarity is indicated by the scale bar at the top.

The outliers were those samples for which seasonal and spatial grouping was not readily discernible. One bacterial community sample (7/95 A1B) corresponded with a phytoplankton bloom and high fluorometry measurements that occurred during the collection of the time series with distinct microbial populations, as reported previously (Kerkhof *et al.*, 1999). COMPAH analysis of this TRFLP profile revealed that it contained 21 OTUs and was 43% similar to other TRFLP profiles in 1995. Several unique TRFs (83, 103, 120, 123, and 172 bp) with frequencies $< 4\%$ were present at this station during the phytoplankton bloom. Another sample (9/95 A6 M) grouped at the 0.2 similarity level to all other samples in the dendrogram. This community sample contained the lowest number of OTUs in the sample set ($n = 10$) and harbored low-frequency TRFs, (50 and 367 bp), occurring rarely in 6.5% and 0.5% of all TRFLP profiles, which can account for the low similarity with respect to the full data set.

As in the 1995 dendrogram, the 1996 dendrogram was divided into major seasonal clades (Fig. 5 and Table 1 for figure labels) with similarities from 56% to 77%. The major winter clade for 2/96 formed at the 0.71 level of similarity while the 12/96 samples clustered at the 0.56 level. The fall clade formed at the 0.58 similarity level and was almost entirely composed of 10/96 samples. There were two branches of this cluster consisting of near shore and offshore samples (Table 1). The 10/96 nearshore and offshore samples separated at the 0.76 and 0.84 similarity level, forming

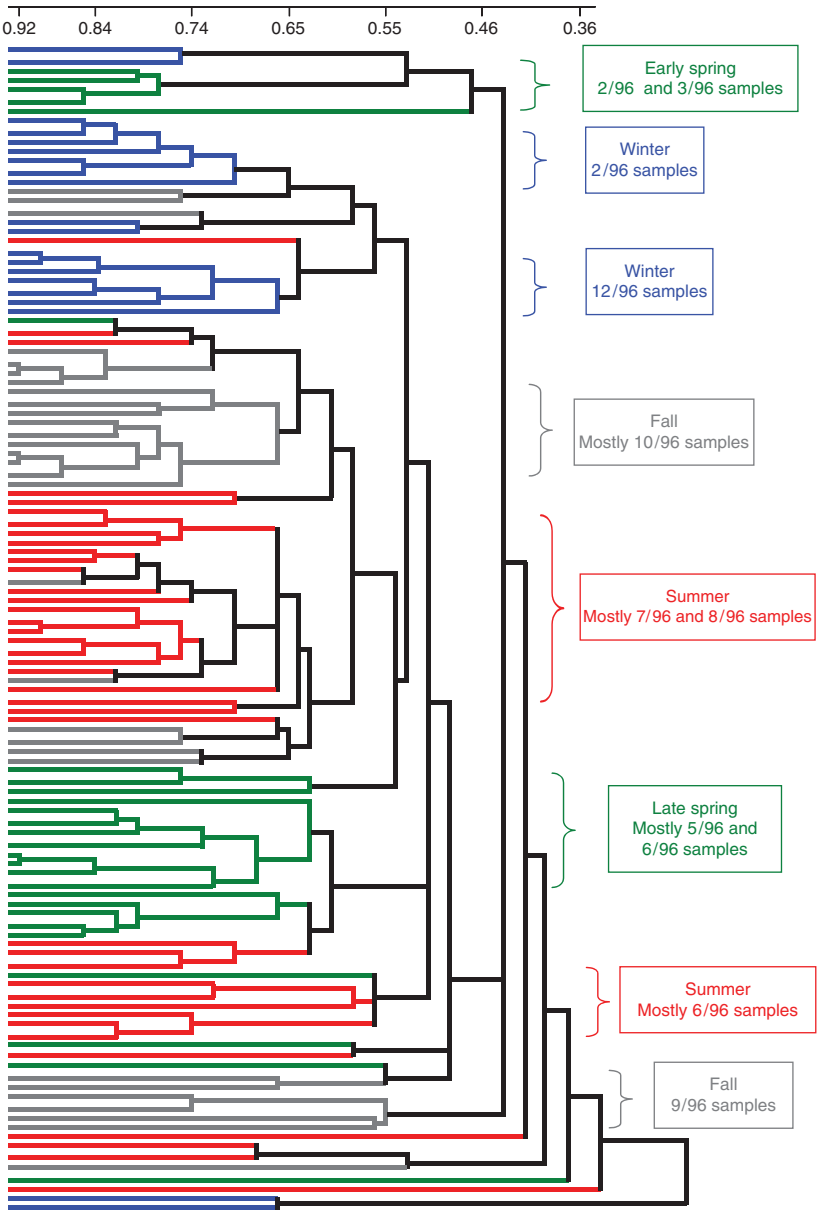

Fig. 5. COMPAH analysis of bacterial TRFLP fingerprints from 1996 ($n = 116$). Samples are color coded in 3-month increments and the sample labels are provided in Table 1.

Table 1. Profile labels for the clusters in Fig. 5

Early spring (g)	Winter (b)	Fall (gr)	Summer (r)	Spring (g)	Summer/fall (r)/(gr)
2/96A1S (b)	2/96A3S (b)	6/96A3S (r)	7/96A4B (r)	5/96A3S (g)	5/96A2B (g)
2/96A1B (b)	2/96A6S (b)	6/96A1S (r)	7/96A5B (r)	5/96A3B (g)	6/96A2S (r)
3/96A5S (g)	2/96A6M (b)	10/96A2S (gr)	6/96A1B (r)	5/96A4SD (g)	6/969SD (r)
3/96A6B (g)	2/96A4B (b)	10/969S1 (gr)	6/969S (r)	5/96A2S (g)	6/96A6M (r)
3/96A5M (g)	2/96A3B (b)	10/969B1 (gr)	7/96A2B (r)	5/96A4B (g)	6/96A5M (r)
3/96A5B(g)	2/96A4S (b)	10/969B2 (gr)	7/96A4S (r)	5/96A6B (g)	6/96A5B (r)
3/969S (g)	2/96A6B (b)	9/96A6B (gr)	7/96A1S (r)	5/96A5B (g)	6/96A6B (r)
	10/96A1B (gr)	10/96A3S (gr)	8/96A6B (r)	5/96A4BD (g)	3/96A4B (g)
	10/96A2B (gr)	10/96A3B (gr)	8/96A4B (r)	5/969S (g)	7/96A2S (r)
	10/96A1S (gr)	10/96A4S (gr)	9/96A4B (gr)	5/969B (g)	3/969B (g)
	12/969S2 (b)	10/96A6S (gr)	7/96A1B (r)	5/969SD (g)	9/96A1S (gr)
	12/969B2 (b)	10/96A5S (gr)	8/96A6S (r)	5/969BD (g)	9/96A3B (gr)
	6/96A3B (r)	10/96A5M (gr)	8/96A1S (r)	5/96A4S (g)	9/96A5S (gr)
	12/96A2S (b)	10/96A6M (gr)	8/969S (r)	5/96A5S (g)	9/96A5M (gr)
	12/96A2B (b)	10/96A5B (gr)	8/969B (r)	5/96A5M (g)	9/96A6S (gr)
	12/96A3S (b)	10/96A6B (gr)	8/96A1B (r)	5/96A6S (g)	9/96A6M (gr)
	12/96A5S (b)		8/96A2S (r)	5/96A6M (g)	
	12/96A6B (b)		8/96A2B (r)	6/96A4S (r)	
	12/96A6S (b)		8/96A3B (r)	6/96A5S (r)	
	12/96A3B (b)		9/96A2B (gr)	6/96A6S (r)	
			7/96A6S (r)		
			8/96A4S (r)		
			8/96C2S (r)		
			7/96A6B (r)		
			9/96A1B (gr)		
			9/96A3S (gr)		
			9/969B2 (gr)		
			9/96A2S (gr)		

Color coding for individual profiles is indicated by: (b), blue; (g), green; (r), red; (gr), grey.

two spatial clusters. The summer clade was a large and complex group consisting of 6/96, 7/96, 8/96, and a few 9/96 samples. Individual clusters split at the 0.69–0.73 similarity level within the larger grouping. A late-spring clade, composed of almost entirely of 5/96 and a few 6/96 offshore, surface samples formed at the 0.62 level, with intracluster similarities ranging from 0.62 to 0.92. This late spring cluster contained duplicate microbial biomass samples collected at stations 9 surface, 9 bottom, and A4 bottom (Fig. 5). Each of these duplicates clustered within the late spring grouping near the original sample with similarities ranging from 0.74 to 0.85. (This lower than expected similarity in the duplicates may reflect significant settling of particle-associated bacteria within the Niskin bottle during sampling processing on board ship because duplicates were collected after all microbial biomass/chemistry samples.)

Efforts to combine the dataset to delineate distinct seasonal clusters over the entire 2-year period were not as successful as using the individual years, 1995 and 1996. An in-depth look at specific OTUs indicated that many TRFs were restricted to specific seasons in specific years (Fig. 6). For example, TRF 54 was detectable in the early Spring and Summer of 1995 while it was hardly detected in 1996. Conversely, TRFs 216 and 223 were more abundant in 1996 rather than 1995. Finally, the 204 bp peak was mainly observed in summer of both years, but was also detected in Fall of 1996. Overall, roughly 15% of the TRFLP peaks demonstrated a season pattern over the entire transect while 10% of the peaks were found in virtually every sample. The remainder of the peaks were largely restricted to a single season, but were not found over the entire transect.

Multivariate statistical analysis

PCA was used to assess patterns present across all variables (e.g. the presence/absence of TRFLP peak) and to account for variability in the total data set (i.e. all community profiles). Principle components 1 and 2 could only account for 14% and 8% of the total variability in TRF presence/absence (data not shown). However, distinct seasonal groups could be discerned in the PCA model, with a separation between summer and winter samples being most apparent (Fig. 7). Partial least squares (PLS) regression analysis was also used to determine if the variances in

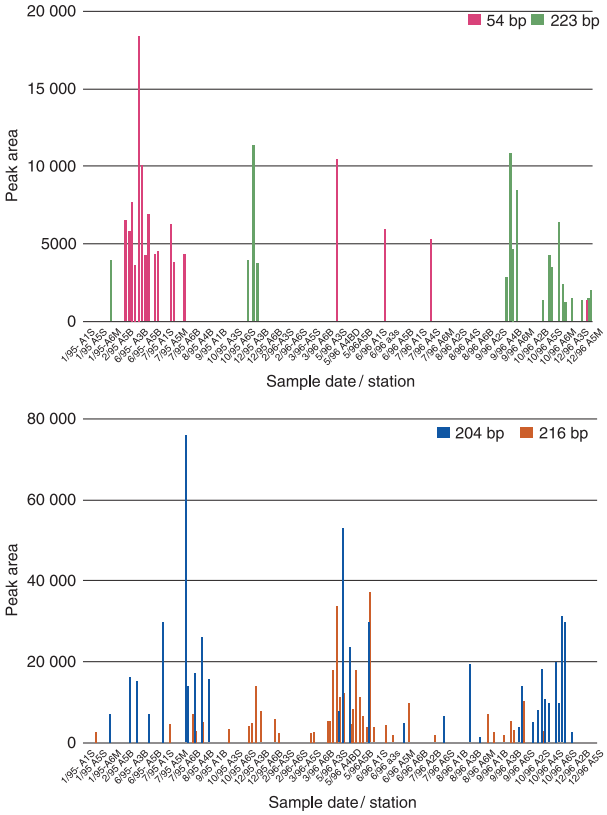

Fig. 6. Peak area vs. time of sampling for select TRFs. A 2-year sampling period is presented.

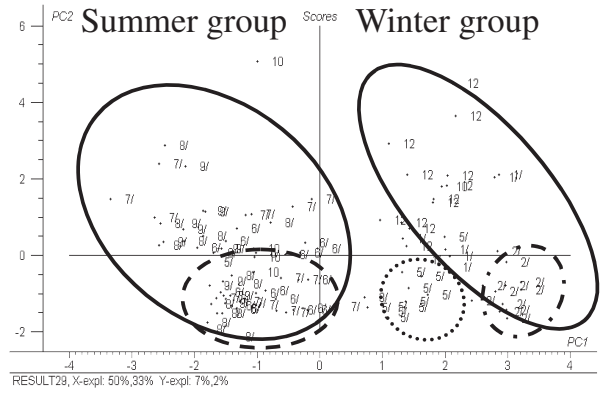

Fig. 7. Partial least squares regression scores plot of the major TRFs in the dataset ($n = 60$). The months of the various TRFLP profiles are shown and the seasonal clustering is indicated by dashed and closed circles.

the presence or absence of TRFs could be explained by the measured or derived chemical and physical parameters including pH, temperature, $[CO_2]$, $[O_2]$, $[PO_4]$, $[NO_3+NO_2]$, and $[NH_4^+]$. Salinity and depth were not included in the PLS analysis given the small range in data (5 ppt and 35 m). Figure 8 shows the results of PLS regression analysis of 157 TRF samples with respect to the

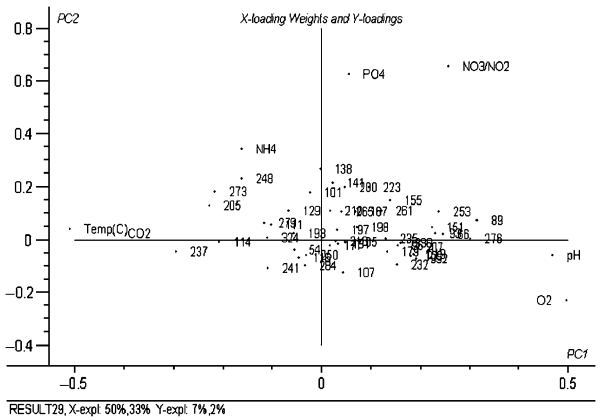

Fig. 8. *X*- and *Y*-loadings weight plot using the physical/chemical data and the major TRFs from the profiles in the dataset ($n = 157$). The individual TRFs are indicated.

chemical parameters in the dataset. The physical/chemical parameters aligned with principal component 1 (PC1) (i.e. CO_2, O_2, pH, and temperature) and with component 2 (PC2) (i.e NO_3+NO_2, NH_4^+, and PO_4) were associated with 50% and 33% of the variability in *X* variables, respectively. PC1 and PC2 explained 7% and 2% of the variability in the *Y* variables (the presence/absence of the TRFs), respectively. Two predictors (O_2 concentration and pH – a surrogate for microbial activity) exerted the largest influence on PC1 in the positive direction, while temperature ($^\circ$C – a surrogate for season) had the largest influence in the negative direction of PC1. PC2 demonstrated a meager influence on the microbial community (positively) by $[NO_3+NO_2]$ and $[PO_4]$ during the sampling period.

Discussion

Currently, we know little about the physical and chemical conditions of various water masses that are the driving forces structuring microbial communities in the coastal ocean. It is widely assumed that the traditional physical and chemical gradients commonly measured in the ocean (e.g. temperature, salinity, phytoplankton nutrients, etc.) select for the various members of the bacterial community. However, which physical/chemical gradient is critical for structuring a particular microbial community is not well known. In our seasonal study, we initially attempted to determine whether time or space was the primary driver for organizing the microbial community in the Mid-Atlantic Bight. Although substantial gradients in physical and phytoplankton nutrient parameters were observed along our 34 km transect off coastal New Jersey (Fig. 3), the primary forcing factor for the coastal bacteria appeared to be temporal. The formation of major seasonal and minor spatial groups in this study was demonstrated by UPGMA analysis using

Sorensen's similarity indices based on the presence or absence of TRFs. Additionally, the results from multivariate statistical analyses agreed well with UPGMA data with PCA and PLS models confirming the seasonal effect on bacterioplankton communities based on TRFLP profiles. These results are consistent with the findings of temporal forcing in vibrioplankton off Plum Island, MA (Thompson *et al*., 2005) and in coastal communities of bacterioplankton off the California coast (Fuhrman *et al*., 2006).

Interestingly, the PCA analysis of routine oceanographic chemical measurements (e.g. ammonium, nitrate, phosphate) did not correlate well with the variability seen in the bacterial community for the MAB as has been observed in the California time series (Fuhrman *et al*., 2006). Perhaps this difference in findings results from our samples originating in a shallow coastal ocean system or from multiple points and depths along a transect line where changes in physical/chemical parameters could be observed within a relatively homogeneous bacterial population. Our results suggest that biological phenomena such as changes in quality/quantity of DOM, bacterial grazing, and competition among bacterial groups that are not traditionally measured could be responsible for the seasonal patterns seen in the MAB microbial community dataset. There are examples of different sources of DOM stimulating different members of the bacterial community (Wawrik *et al*., 2005; Judd *et al*., 2006) as can be seen during the standard microbiological practice of enrichment culture. Another instance of a biological phenomenon was observed at Station 1 during a short-lived, upwelling event and phytoplankton bloom in July, 1995 (Kerkhof *et al*., 1999). Analysis of the 7/95 Station A1 bottom TRFLP profile revealed the presence of several unique TRFs as well as a marked increase in the relative proportion of a 250-bp TRF, representing the *Roseobacter* lineage (Nelson, 2002). It is possible that the major phylotype(s) represented by TRFs in this study are responding to ephemeral biological events, such as phytoplankton blooms (Kerkhof *et al*., 1999; Gonzalez *et al*., 2000).

Another potential explanation that could account for some of the seasonal pattern that was observed was preferential amplification of 16S rRNA gene templates (Suzuki & Giovannoni, 1996). This possibility was addressed by analyzing the relative abundance of the dominant TRFs in our study. One would expect that if one TRF or a group of TRFs were preferentially amplified, that this would be observed in all 178 of our TRFLP profiles. However, we found several examples in which the relative proportion of the high-frequency TRFs was either zero or much lower than the average relative abundances for that particular peak (data not shown; Nelson, 2002), indicating that PCR bias played a minor role in the present study.

In conclusion, it appears that there are significant temporal forcing factors that structure microbial communities in the coastal ocean. In fact, nearly 50% of the microbial community can turn over within weeks between the different seasons in the Mid-Atlantic Bight. However, those regulators are not the traditional physical/chemical parameters typically measured during oceanographic investigations. The results of the current study agree with those of Scala and Kerkhof, which reported that it is likely that there is no one single regulator that is responsible for bacterial community dynamics in sediments (Scala & Kerkhof, 2000). Some of these seasonal populations drivers of bacteria at the LEO-15 site could include: changes in overall quality or quantity of organic matter, shifts in grazing pressure for distinct microbial populations, or advection of larger microbial biomes into the study site. At present, there is evidence of little to no overall change in DOC quantity or quality in the Mid-Atlantic Bight with season or over the depths encountered during this study (Aluwihare *et al*., 2002; Del Vecchio & Blough, 2004). However, none of these hypotheses has been directly tested as a driver for structuring microbial communities in the Mid-Atlantic Bight. Our results underscore the need for a wider array of chemical and physical data collection to understand bacterioplankton community dynamics and illustrate how time series analysis of larger spatial regions can lead to testable hypotheses to discern the mechanisms controlling bacterial community structure and function.

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