*Hydrobiologia* **401:** 139–148, 1999.
*J.P. Zehr & M.A. Voytek (eds), Molecular Ecology of Aquatic Communities.*



# Variability in bacterial community structure during upwelling in the coastal ocean

L. J. Kerkhof[1], M. A. Voytek[2], R. M. Sherrell[1], D. Millie[3,*] & O. Schofield[1]
[1]*Institute of Marine and Coastal Sciences, 71 Dudley Rd. Cook College, Rutgers University, New Brunswick, NJ 08901-8521, U.S.A. E-mail: kerkhof@ahab.rutgers.edu*
[2]*United States Geological Survey, MS430, 12201 Sunrise Valley Drive, Reston, VA 20192, U.S.A.*
[3]*Southern Regional Research Center, Agricultural Research Service, New Orleans, LA 70179, U.S.A.*

*Key words:* marine bacteria, phytoplankton, upwelling, rRNA clonal library, specific co-occurrence

**Abstract**

Over the last 30 years, investigations at the community level of marine bacteria and phytoplankton populations suggest they are tightly coupled. However, traditional oceanographic approaches cannot assess whether associations between specific bacteria and phytoplankton exist. Recently, molecular based approaches have been implemented to characterize specific members of different marine bacterial communities. Yet, few molecular-based studies have examined coastal upwelling situations. This is important since upwelling systems provide a unique opportunity for analyzing the association between specific bacteria and specific phytoplankton in the ocean. It is widely believed that upwelling can lead to changes in phytoplankton populations (blooms). Thus, if specific associations exist, we would expect to observe changes in the bacterial population triggered by the bloom. In this paper, we present preliminary data from coastal waters off New Jersey that confirm a shift in bacterial communities during a 1995 upwelling event recorded at a long-term earth observatory (LEO-15) in the Mid-Atlantic Bight. Using PCR amplification and cloning, specific bacterial 16S ribosomal RNA sequences were found which were present in upwelling samples during a phytoplankton bloom, but were not detected in non-bloom samples (surface seawater, offshore sites or sediment samples) collected at the same time or in the same area. These findings are consistent with the notion of specific associations between bacteria and phytoplankton in the ocean. However, further examination of episodic events, such as coastal upwelling, are needed to confirm the existence of specific associations. Additionally, experiments need to be performed to elucidate the mechanisms leading to the specific linkages between a group of bacteria and a group of phytoplankton.

## Introduction

Bacteria and phytoplankton have a profound impact on the marine ecosystem since their combined activity accounts for nearly all of the biogeochemical cycling that occurs in the ocean (Goldhaber & Kaplan, 1974; Van Es & Meyer-Reil, 1982; Carpenter & Capone, 1983). For example, the importance of bacteria as consumers of organic carbon in marine food webs has long been recognized (Pomeroy, 1974; Fuhrman & Azam, 1980; Cho & Azam; 1988). Additionally, coupling between total bacteria and phytoplankton communities has been hypothesized and most often related to the extracellular release of photosynthetically produced dissolved organic carbon (for reviews see, Cole et al., 1988; Baines & Pace, 1991). These studies have shown that 30–50% of the bacterial carbon demand can be met from the direct excretion of photosynthetically-fixed carbon *in situ* in many oceanographic situations. The coupling between bacterial activity and phytoplankton is particularly important in the waters of the Middle Atlantic Bight (MAB). Only 5–10% of the particulate matter produced on the continental shelf is exported off the shelf, and

* Author for correspondence.

the remainder is remineralized and/or consumed by heterotrophic processes *in situ* (Kemp et al., 1994; Falkowski et al., 1994; Biscaye et al., 1994).

Although many of these traditional studies suggest a coupling between bacterial and phytoplankton populations overall, the whole-population methodology can not address whether specific associations exist between these two groups. If specific associations between bacteria and phytoplankton exist, changes in both these populations should result from physical forcing, such as upwelling events, on short time scales. For example, upwelling can very often lead to mono-specific phytoplankton blooms in the ocean. In a bloom, a diverse community of phytoplankton shifts to a community dominated by a few members of the phytoplankton community, i.e. phytoplankton diversity plummets. It is anticipated that bacterial diversity will likewise plummet during phytoplankton blooms if specific associations exist, since a few members of the bacterial community will also come to dominate during the bloom. Furthermore, these specific bacterial groups should be readily detectable in waters experiencing the bloom and otherwise absent or present at reduced numbers comparable to the 'host' phytoplankton species.

The MAB provides a unique opportunity to apply new methodologies and approaches to determine whether specific bacteria are associated with specific phytoplankton. Specifically, the region experiences episodic upwelling of offshore bottom waters into surface layers (Hicks & Miller, 1980). This upwelling is initiated by strong alongshore winds and can last for days or weeks (Glenn et al., 1996), leading to phytoplankton blooms and accumulation of organic matter (Warsh, 1987). An extensive array of oceanographic sensors have recently been put in place to monitor these upwelling events in real time (von Alt et al., 1997).

In this paper, we describe changes in a coastal planktonic community during an upwelling event in 1995 using 16S rRNA analysis (for review, Amann et al., 1995; Torsvik et al., 1996; Head et al., 1998; and Hugenholtz et al., 1998). On July 15, an upwelling began and lasted approximately 3 weeks. Initially, a small band of cold water was detectable near shore on the sea surface by satellite. By July 27, the upwelling feature was fully developed with colder surface water extending 80 km up and down the coast and 20 km offshore from the Rutgers Marine Field Station. Within a few days (August 4), the winds began to shift and a relaxation of upwelling became detectable. The entire upwelling feature was destroyed when Hurricane Felix began impacting the New Jersey coast on August 15,1995.

On July 17 and August 9, 1995, we fortuitously collected bacteria from upwelling and non-upwelling sites during a routine sampling cruise along a 34 km transect line. Differences in microbial diversity, as well as changes in individual bacterial species composition were observed during this upwelling event. Specifically, microbial diversity was found to decrease during the onset of upwelling. However, ten 16S rRNA genes were found in two upwelling/phytoplankton bloom gene libraries (9 m depth) that were not observed in six different gene libraries from samples where the phytoplankton concentrations were low (surface seawater, surface and bottom at an offshore site, and 2 sediment samples). This study suggests that distinct populations of bacteria are present during coastal upwelling and implies that physical events can drive phytoplankton community composition and bacterial diversity in the coastal ocean. Furthermore, our data provide important molecular markers for use in the future to ascertain whether the associations observed between specific bacteria and phytoplankton in this study are robust or circumstantial.

## Methods

### *Field samples*

Our routine, monthly sampling in 1995 covered a 34 km transect (10 stations) off the coast of New Jersey. Water was collected using Niskin bottles at two depths (1 m below the surface and 2 m above the bottom; see Figure 1). The depth of the water column at our study area is between 5 and 35 m. Seawater samples (500–1000 ml) were filtered onto 0.2 $\mu$m polysulfone filters to obtain bacterial and phytoplankton biomass. All filtered samples were placed at $<0$ °C at sea and stored at $-80$ °C in the laboratory until processing.

For the upwelling study described in this paper, biomass samples were characterized at two stations collected 4 and 19 km offshore (Station 9 and A4; see Figure 1). The samples were collected early (July 17) and late (August 9) during upwelling, and the stations were selected to represent samples within and outside the upwelling feature. Physical and chemical data for this area are described in Glenn et al. (1996) and Boehme et al. (1998).

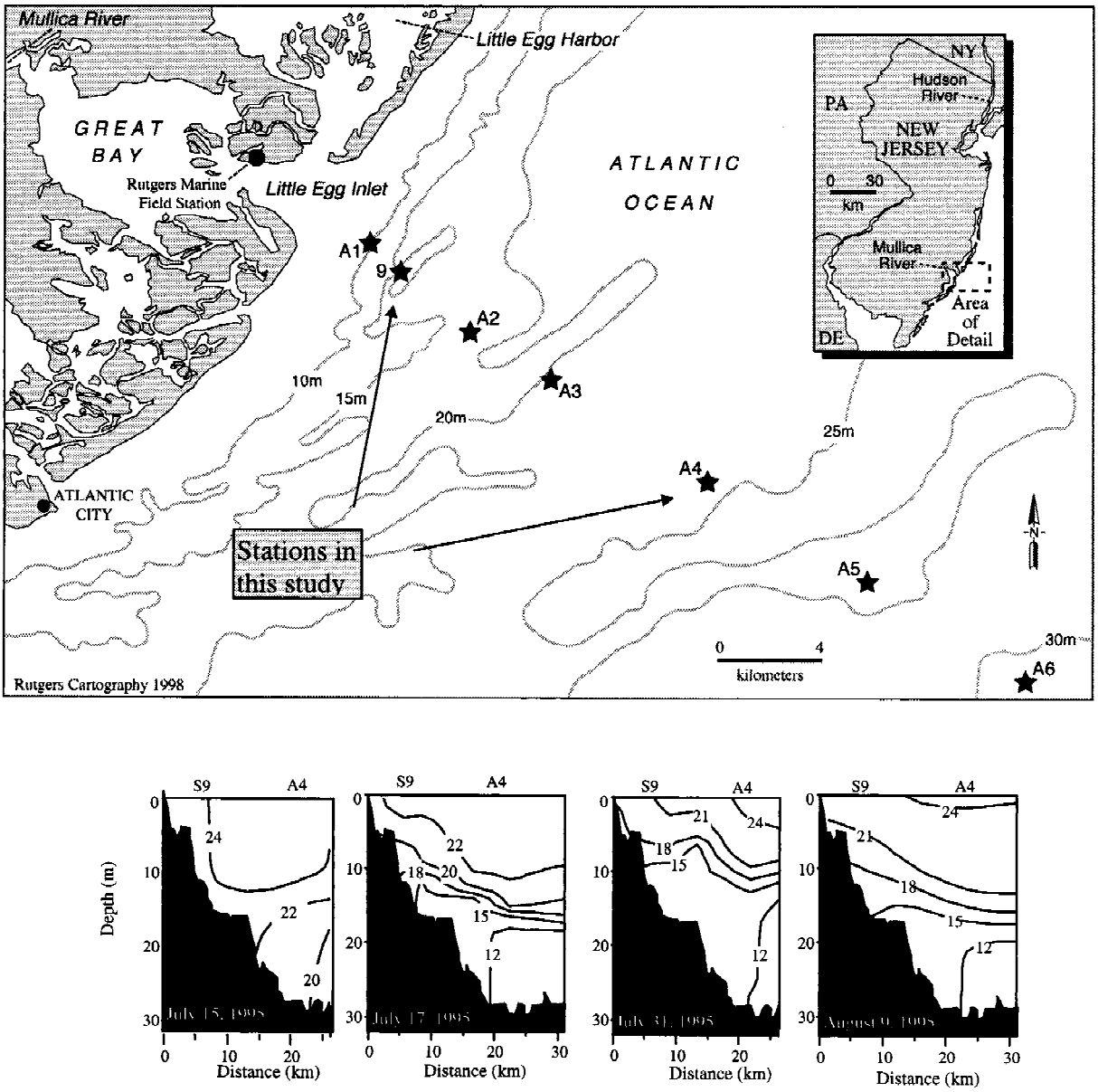


*Figure 1.* Map of study area off the New Jersey Coast indicating stations along transect line. Stations 9 and A4 are indicated. Below are CTD profiles obtained during the upwelling event. Samples were collected on July 17 and August 9, 1995.

*DNA extractions and PCR amplification of 16S rRNA genes*

Bacterial biomass samples were subjected to a modified phenol/chloroform extraction as previously described (Kerkhof & Ward, 1993). Amplification of 16S rDNA was done using standard eubacterial primers [27 Forward (5′ cua cua cua cua AGA GTT TGA TCC TGG CTC AG 3′) and 1525 Reverse (5′ cau cau cau cau AAG GAG GTG WTC CAR CC 3′)] (Lane et al., 1991) which have a uracil-rich cloning site. The following amplification parameters were used: initial denaturation at 95 °C for 5 min, then 94 °C for 0.5 min, 55 °C for 0.5 min and 72 °C for 1.5 min for 22 cycles, and a final extension step at 72 °C for 10

min. The amplifications were performed in a Perkin-Elmer Gene Amp PCR system 2400 thermal cycler (Perkin-Elmer, Foster City, CA).

*Cloning, screening and sequencing*

The cloning of 16S rDNA was done using the Cloneamp System (Gibco BRL, Gaithersberg, MD) as per the manufacturer's instruction using 22 cycle amplifications. Colonies were screened by restriction enzyme analysis (Hae III, Boehringer-Mannheim (BMB), Indianapolis IN) on extracted plasmids or by PCR amplification of insert 16S rDNA and digestion with Rsa I and Msp I (BMB). The restriction digests were analyzed using Methaphor agarose (FMC Corp., Rockland, ME). Estimates of microbial diversity in these samples were made using rarefaction curves by tallying the number of unique 16S rRNA restriction patterns vs. the total number of clones screened within a 16S rRNA clonal library (Sakano & Kerkhof, 1998). The shapes of these curves reflect the underlying diversity in the bacterial population.

Plasmids bearing a unique restriction banding pattern were re-purified using a Flexiprep kit (Pharmacia, Piscataway, NJ). DNA sequence was determined by automated techniques (Perkin Elmer-ABI, Foster City, CA) with the following primers: 27 Forward (5′ AGA GTT TGA TCC TGG CTC AG3′), and 519 Reverse (5′ GWA TTA CCG CGG CKG CTG 3′), (Lane, 1991). Determinination of the phylogenetic affiliation of all RFLP unique clones in each library was performed by BLAST (Altschul et al., 1990) search and phylogenetic tree re-construction sequences and alignments in the Ribosomal Database Project (Maidak et al., 1994).

Initial screening of all unique RFLP clones from the six small ribosomal subunit libraries was done by phylogenetic analysis of unambiguously aligned sequence (321–375 bp) from a total of 470 bp sequence. The initial phylogenetic trees were re-constructed by the Neighbor joining method to identify all 16S rRNA clones common to both upwelling and non-upwelling samples and those clones bearing >99% identity to other clones in the library. This initial screening made it possible to identify the 16S rRNA genes unique to the bloom conditions. Final phylogenetic tree re-construction on the bloom-specific 16S rRNA genes was based on 294 bp of aligned sequence using fastDNAml (Olsen et al., 1994) and 100 bootstrap replications. The sequences have been submitted to Genbank and have the accession numbers: AF111859–AF111867.

*Phytoplankton Pigment Analysis.*

Pigments were extracted in acetone and concentrated before HPLC analysis. Chlorophylls and carotenoids were separated on a Vydac reverse-phase, C18 column using a binary gradient system (after Van Heukelem et al., 1992) optimizing for separation of the carotenoids, zeaxanthin and lutein. Pigment peaks were identified by comparing retention times and absorption spectra (380–670 nm) with those of authentic standards and of known pigments from purified algal cultures maintained at the Southern Regional Research Center (SRRC, Millie et al., 1993).

## Results

Sea surface temperature from AVHRR images and CTD data indicated a coastal upwelling event occurred off New Jersey between July and August, 1995. A large difference in surface temperature was observed with the coldest surface water (between 18.7 and 25.5 °C) detectable along the coast. Station 9 is centered within this upwelling feature and station 4 is along the offshore edge during the greatest extent of upwelling (data not shown). CTD profiles (Figure 1) confirmed a shoaling of isotherms towards the surface during our sampling periods. Chemical analysis on July 17 detected minimal differences between nutrients at Station 9 for surface and bottom samples (0.11–0.13 $\mu$mol/kg nitrate + nitrite, () 0.12–0.13 $\mu$mol/kg ammonium, and 0.39–0.53 $\mu$mol/kg phosphate). However, Station A4 values during this same period had higher nutrients in the bottom waters (0.12–1.64 $\mu$mol/kg nitrate + nitrite), 0.14–2.78 $\mu$mol/kg ammonium,; and 0.12–0.85 $\mu$mol/kg phosphate. In August, Station 9 had higher values of some nutrients in bottom samples (0.11–0.13 $\mu$mol/kg nitrate + nitrite, 0.23–0.72 $\mu$mol/kg ammonium, and 0.46–0.56 $\mu$mol/kg phosphate). Station A4 values during this same period were 0.03–0.53 $\mu$mol/kg nitrate + nitrite, 0.15–2.07 $\mu$mol/kg ammonium, and 0.12–0.45 $\mu$mol/kg phosphate. These data suggest that the sources of upwelled waters in our study area are from offshore bottom sites, such as Station A4, and that nutrient were brought to the bottom waters at Station 9. A more detailed report of nutrient dynamics for a 2-year time series in this area is presented in Boehme et al. (1998).

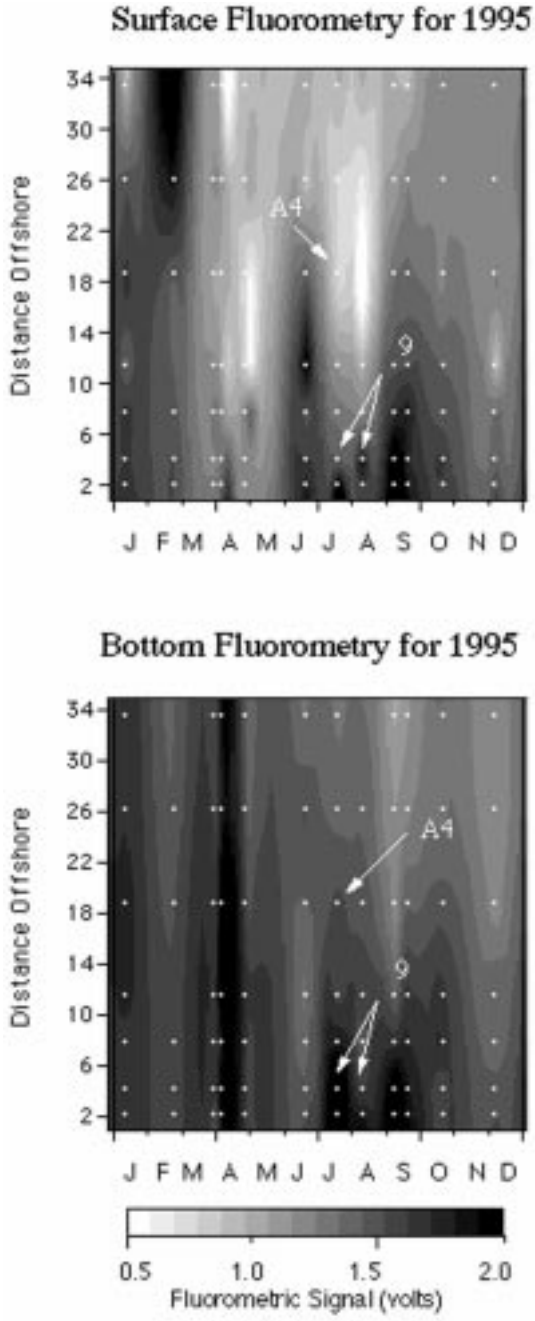


*Figure 2.* Annual survey of fluorescence along a 40 km transect offshore from New Jersey during 1995. Stations 9 and A4 sampled for this study are indicated.

Fluorometry data collected during 1995 indicated high fluorescence in bottom waters during the upwelling event (Figure 2). Surface water exhibited a reduced fluorescence throughout much of the year and during our sample period (Figure 2).

The greatest concentrations of chlorophyll *a* and other algal accessory pigments were observed in the summer (July 1995 and August 1995) in the bottom samples at our study site (Table 1). During the initial stages of upwelling, the chlorophyll *a* concentrations were 7-fold higher in bottom waters than in surface waters. The surface samples at these times contained extremely low concentrations of all algal pigments. By the end of the upwelling event, bottom waters had 100 times higher chlorophyll *a* concentrations than surface samples. The significant peridinin concentration in the bottom waters during July and August of 1995 indicated dinoflagellates were a major component of the phytoplankton population. The other major marker pigment present during 1995 was fucoxanthin, suggesting that diatoms were a dominant member of the phytoplankton community. Surface samples taken simultaneously, or offshore samples, had much lower pigment signals indicating lower concentrations of all phytoplankton in these waters during this time.

To determine the impact of the July–August 1995 upwelling event on microbial diversity, six clonal libraries of 16S rRNA genes were created for Station 9 and Station A4. Four libraries were created at Station 9 (surface and bottom in July 1995 and August 1995) and 2 libraries at Station A4 (surface and bottom in July 1995). Rarefaction curves were constructed from all libraries to estimate overall microbial diversity. A large drop in diversity is apparent in the Station 9 bottom July 1995 sample when compared with all other samples (Figure 3) concurrent with the initial stages of upwelling at our study site. However, an analysis of the phylogenetic affiliation of all clones in each library showed only minor changes in the relative percentages of the contributing members of the microbial community during upwelling suggesting minimal bias in the clonal libraries (Figure 4). At all sites/times, the small subunit (SSU) clones were comprised mainly of Proteobacteria sequences (80–90%). Representatives of the $\alpha$ and $\gamma$ subdivisions accounted for the bulk of the clones in each library. A small number of clones from the $\beta$ subdivision were also present in most of the marine libraries. Interestingly, a significant fraction of the SSU clones in the Station 9 bottom samples contained sequences from the $\epsilon$-subdivision Proteobacteria. SSU clones from this subdivision were not observed in the Station 9 surface or Station A4 samples (surface and bottom) containing lower algal pigment concentrations. Clones from the Cytophaga phyla were present in all libraries at lower abundance ($<$20%).

*Table 1.* Concentrations of pigments from station 9 time series samples Units in $\mu$g/l

| Date | Depth | Chl *a* | Chl *b* | Chl *c*1–*c*2 | Peridinin | Fuco | Diadino | Anth | Allo |
|---|---|---|---|---|---|---|---|---|---|
| Jan-95 | surface | - | - | - | - | 0.116 | - | - | - |
| | bottom | - | - | - | - | 0.190 | - | - | - |
| Feb-95 | surface | - | - | - | - | 0.049 | - | - | - |
| | bottom | - | - | - | - | 0.050 | - | - | - |
| Mar-95 | surface | 0.116 | - | - | - | 0.119 | - | - | - |
| | bottom | - | - | - | - | 0.073 | - | - | - |
| Apr-95 | surface | 0.088 | - | - | - | 0.073 | - | - | - |
| | bottom | - | - | - | - | - | - | - | - |
| May-95 | surface | 0.178 | - | 0.173 | - | 0.351 | 0.033 | - | 0.039 |
| | bottom | 0.279 | - | 0.203 | - | 0.294 | - | 0.048 | - |
| Jun-95 | surface | 0.119 | - | 0.094 | - | 0.112 | - | - | 0.099 |
| | bottom | - | - | - | - | 0.036 | - | - | |
| Jul-95 | surface | 0.049 | - | - | - | 0.133 | - | - | 0.036 |
| | bottom | 0.339 | - | 2.628 | 2.269 | 0.501 | 0.179 | 0.389 | - |
| Aug-95 | surface | - | - | 0.114 | 0.044 | 0.326 | - | - | - |
| | bottom | 4.853 | 0.294 | 6.42 | 8.151 | 1.338 | 0.437 | - | - |

Surface=1 m depth, bottom =15 m depth; Chl *a*=Chlorophyll *a*, Chl *b*=Chlorophyll *b*, Chl *c*1-*c*2=Chlorophyll *c* 1 and 2, fuco=fucoxanthin, anth=antherxanthin, allo=alloxanthin. - = below detection limit.

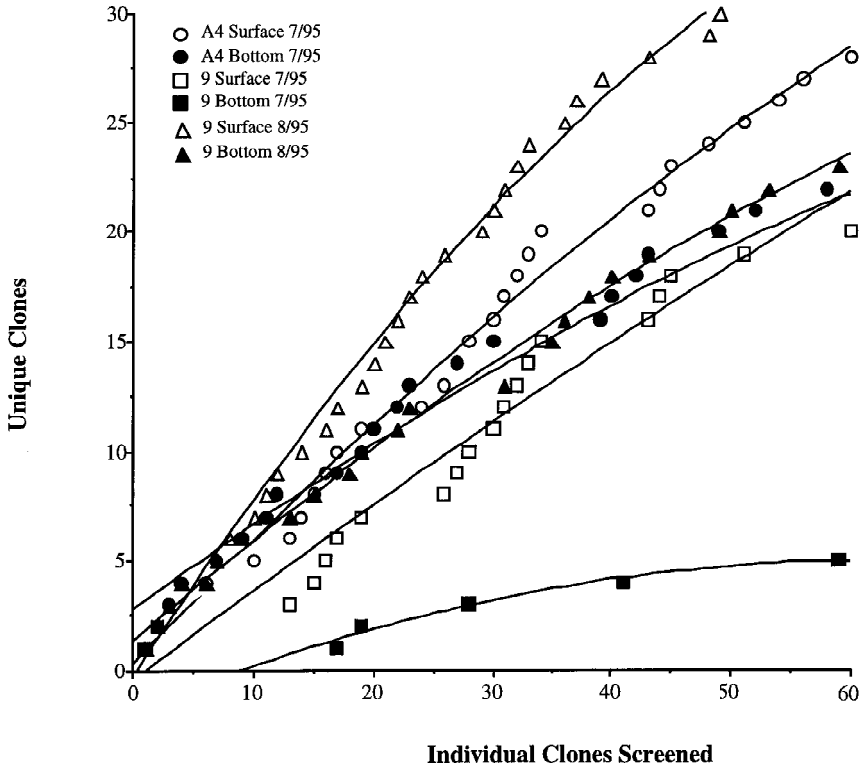


*Figure 3.* Rarefaction curve of surface and bottom samples during summer 1995. The samples are from: Station A4 Surface July 1995 (○), Station A4 Bottom July 1995 (●), Station 9 Surface July 1995 (□), Station 9 Bottom July 1995 (■), Station 9 Surface August 1995 (▲), and Station 9 Bottom August 1995.

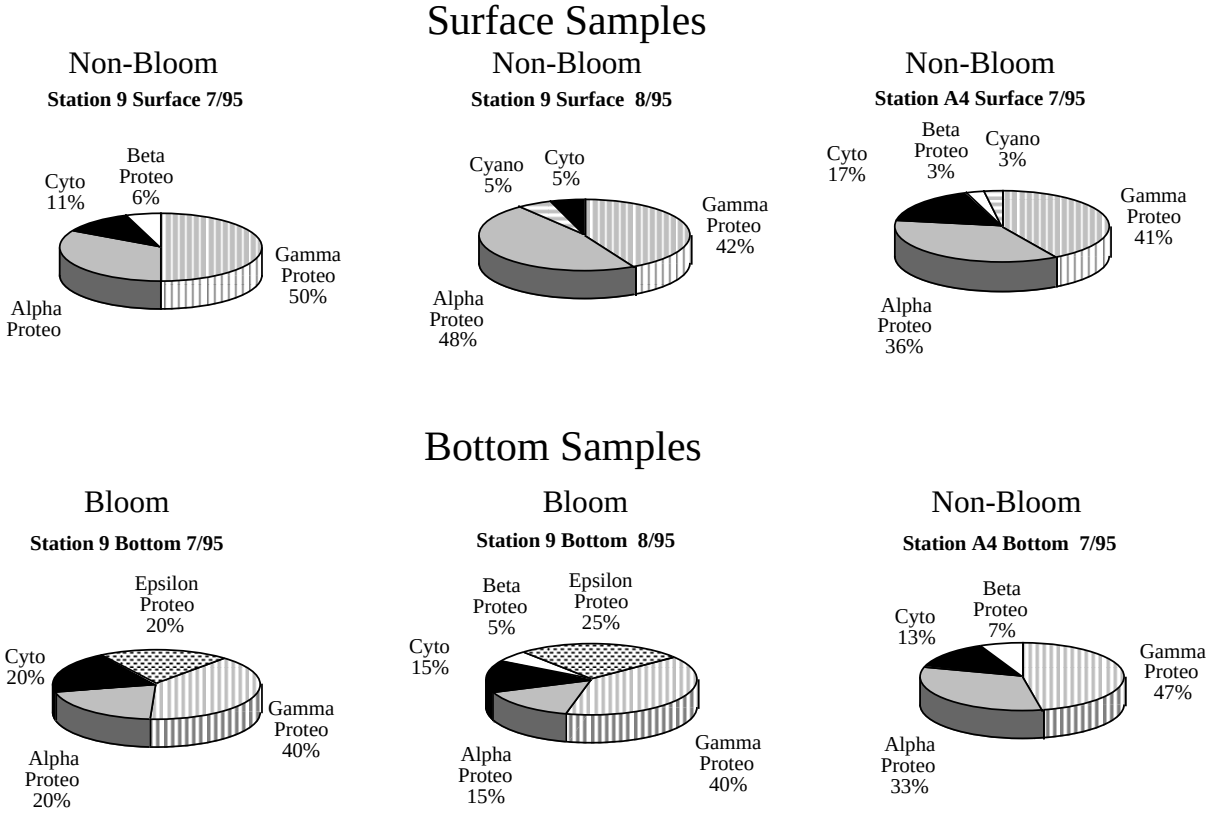


*Figure 4.* Proportion of phylogentic affiliation of the various clones observed in all 16S rRNA gene libraries collected in the study. Sites, times, and depths are indicated. Legend: Cyto= *Cytophaga*, Proteo=*Proteobacteria*, Cyano=*Cyanobacteria*.

To ascertain whether there were specific bacteria associated with the phytoplankton at Station 9 in July 1995 and August 1995, phylogenetic trees were reconstructed using sequences from the six 16S rRNA libraries. A total of 8 $\alpha$-subdivision, 12 $\gamma$-subdivision, and 6 $\epsilon$-subdivision SSU clones were observed in all six 16S rRNA libraries. Of the 26 Proteobacteria SSU clones, 9 were found to be unique to the Station 9 upwelling samples with 5 clones coming from the $\epsilon$-subdivision. None of these upwelling SSU clones have been observed in 16S rRNA genes libraries from sediments within a kilometer of Station 9 in November 1995 and May 1996 (Kerkhof, unpubl. data), suggesting that bottom re-suspension was not a significant source of the unique rDNA clones obtained from upwelling samples (data not shown).

The phylogenetic affiliations of the bloom-specific clones (highlighted in gray) are presented with most of their closest matches from Genbank in Figure 5. The designation of UPA or B refers to SSU clones present in July 1995 or August 1995, respectively. Many of the clones exhibited identity with rRNA genes obtained from the marine environment. For most UPA or UPB clones, the nearest matches in Genbank were <93% similar. The clones UPB A1 and UPA D3 were related to gene sequences from other marine 16S rRNA gene libraries (OM 27, <88% similar) or uncultured microorganisms (Lucinid endosymbionts, <90% similar). One 16S rRNA gene sequence, UPB C7, discovered in our study was related (<92% similar) to an algicidal bacterial strain capable of lysing *Gymnodinium* sp. (Yoshinaga et al., 1995). The clone UPB G7 was found to be between 96 and 98% similar to the SSU gene sequences OM 42, GAC 2, and BAL 28 from libraries generated from Atlantic and Pacific samples (Rappe et al., 1997; Gonzalez & Moran, 1997; and Pinhassi et al., 1997). Finally, 5 of the SSU clones from this study were related (<92% similarity) to the epsilon subdivision of the Proteobacteria. The closest isolates are indicated in Figure 5. Because this group of microorganisms have diverse metabolic capabilities, it is difficult to extrapolate the metabolic capabilities of the epsilon bacteria detected in this study.

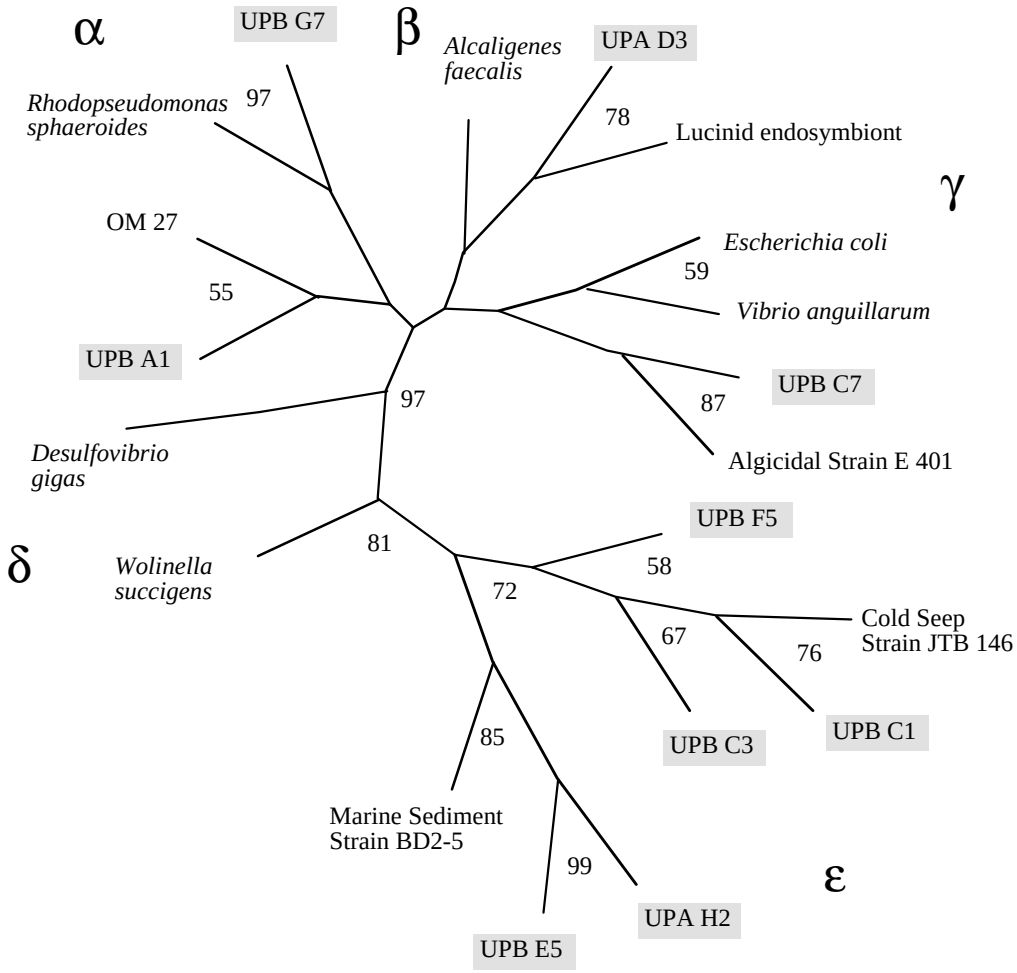


*Figure 5.* Maximum likelihood phylogenetic tree of Proteobacterial 16S rRNA clones re-constructed using 295 bp alignment. Clones unique to the upwelling condition are boxed in gray. The diatom (Diatom) and dinoflagellate (Dino) bloom associated clones are indicated.

**Discussion**

Other studies using a species approach to document the response of the microbial community to environmental changes have applied techniques including immunoassays, fatty acid analysis, or 5S rRNA analysis (Frostegard et al., 1993; Höfle & Brettar, 1995; Tuomi et al., 1997). Studies have also been performed to compare microbial communities from different aquatic habitats (Field et al., 1997; Rappe et al., 1997; Gonzalez & Moran, 1997; Pinhassi et al., 1997 and Methe' et al., 1998). The common thread in all of these reports is a switch from bulk measurements of bacteria to techniques that allow for detection of specific 'types' in response to environmental factors (e.g. depth, salinity, DOC, nutrients, heavy metals, virus abundance, protozoan grazing).

In this study, we used a 16S rRNA-based approach to address the response of specific bacteria and phytoplankton to an episodic event, such as upwelling, and assess the effect of physical factors on microbial community structure. Although 16S rRNA gene characterization is now routine in many labs, potential biases in the clone and sequence approach still exist. Primarily, DNA extraction procedures can miss entire groups that are difficult to lyse such as Gram-positive bacteria. Additionally, large amounts of template DNA, high cycle numbers in the PCR amplification, and large amounts of transforming DNA are used to maximize the amount of colonies obtained during cloning. However, this strategy will confound any attempts at quantitative compositional analysis due to PCR (Alard et al., 1993; Suzuki & Giovannoni, 1996) or cloning biases resulting from

asymptotic transformation of *E. coli* (Hanahan, 1983; Dower et al. 1988). We have taken steps to minimize these biases inherent in the traditional approach. For example, our extraction procedure can successfully isolate Gram-positive 16S rRNA genes from marine sediments (Phelps et al., 1998). Furthermore, we used minimal template concentration (<10 ng of genomic DNA), low cycle numbers (20–25), and low transforming DNA concentrations(<6 ng) to create our clonal libraries for determining specific bacterial 16S rRNA genes associated with upwelling.

Previous research has demonstrated the bacterial community is linked to the phytoplankton community at a whole population level (Cole et al. 1988; Baines & Pace, 1991). Our data from an upwelling event off New Jersey is consistent with the concept of linkages between bacterial and phytoplankton assemblages. Primarily, shifts in bacterial populations were observed over the course of several weeks during upwelling in the MAB. Secondly, there appeared to be specific 16S rRNA phylotypes associated with phytoplankton bloom conditions that were not detected in samples where phytoplankton concentrations were low.

Although these differences in bacterial community assemblages co-occur with differences in algal pigment distributions, at present it is unclear what mechanism(s) control the bacterial and phytoplankton populations. Given the limitations of this study, we cannot yet determine how robust these associations are, or whether they represent physical structuring of microbial communities (advection) and/or biotic interactions between the bacteria and the phytoplankton. In order to firmly establish if specific bacterial groups are stimulated by various phytoplankton groups (e.g. diatoms, dinoflagellates), future studies during upwelling events in the MAB system should collect samples on ecologically relevant spatial and temporal scales and identify specific bacterial 16S genes associated with specific groups of phytoplankton. Furthermore, quantitative analysis on specific groups of bacteria and phytoplankton (abundance and growth rate analysis) are needed to verify any potential causal linkage. Ultimately, research such as described in this study will help to provide an understanding of the mechanisms connecting bacteria and phytoplankton in the ocean.

Given the specific linkages between bacterial and phytoplankton groups that were identified in this study, specific hypotheses can be tested in future studies: 1. Specific groups of bacteria and phytoplankton will be tightly coupled in time and space. That is, specific bacterial 16S rRNA genes will only become dominant during specific phytoplankton blooms and otherwise be minor constituents of the community. 2. 16S rRNA gene sequences identified within a specific phytoplankton bloom will undergo growth and senescence in phase with the bloom itself. 3. Specific associations are repeatable and can be observed in different bloom events either in the same year or from year to year. These hypotheses can be tested during episodic events (such as upwelling) which provide a physical/biological gradient over short spatial and temporal scales.

## Acknowledgments

This research was supported in part by funds from Rutgers University, (NOAA/NURP NA76RU0165 to L. K.) and ONR CoMOP Program (N0014-97-1-0797 to O.S.). The authors wish to especially thank Mike Crowley and Clare Reimers for supplying physical and chemical data and Gary Taghon for constructive comments on the manuscript. We are indebted to David Scala, Larry Biohem, Mark Moline, Joe Grymski, Sue Boehme, Robert DeKorsey and Rose Petrecca for help in sample collection or processing.

## References

Alard, P, O. Lantz, M. Sebagh, C. Clavo, D. Weill, G. Chavanel, A. Senik & B Charpentier, 1993. A versatile ELISA-PCR assay for mRNA quantitation from a few cells. Biotechniques 15: 730–737.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers & D. J. Lipman, 1990. Basic local alignment search tool. J. mol. Biol. 215: 403–410.

Amann, R. I., W. Ludwig, & K. H. Schleifer, 1995. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. Microbiol. Rev. 59: 143–169.

Baines, S. B. & M. L. Pace, 1991. The production of dissolved organic matter by phytoplankton and its importance to bacteria: Patterns across marine and freshwater systems. Limnol. Oceanogr. 36: 1078–1090.

Biscaye, P. E., C. N. Flagg & P. G. Falkowski, 1994. The shelf edge exchange processes experiment, SEEP-II: an introduction to hypotheses, results and conclusions. Deep Sea Res. Part II, 41: 231–255.

Boehme, S., C. Sabine & C. Reimers, 1998. $CO_2$ fluxes from a coastal transect: A time series approach. Mar. Chem. In Press.

Carpenter, E. J. & D. G. Capone, 1983. Nitrogen in the Marine Environment. Academic Press. London. 900 pp.

Cho, B. C. & F. Azam, 1988. Major role of bacteria in biochemical fluxes in the ocean's interior. Nature 332: 441–443.

Cole, J. J., S. Findlay & M. Pace, 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. Mar. Ecol. Prog. Ser. 43: 1–10.

Dower, W., J. Miller & C. Ragsdale, 1988. High efficiency transformation of *E. coli* by high voltage electroporation. Nucl. Acids. Res. 16: 6127–6144.

Falkowski, P. G., P. E. Biscaye & C. Sancetta, 1994. The lateral flux of biogenic particles from the eastern North American continental margin to the North Atlantic Ocean. Deep Sea Res. Part II, 41: 583–601.

Field, K. G., D. Gordon, T. Wright, M. Rappe, E. Urbach, K. Vergin & S. J. Giovanonni, 1997. Diversity and depth-specific distribution of SAR 11 cluster rRNA genes from marine planktonic bacteria. Appl. envir. Microbiol. 63: 63–70.

Frostegard, A., A. Tunlid & E. Baath, 1993. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Appl. envir. Microbiol. 59: 3605–3617.

Fuhrman, J. & F. Azam, 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. Appl. envir. Microbiol. 39: 1085–1095.

Goldhaber, M. & I. Kaplan, 1974. The Sulfur Cycle. In The Sea. Goldberg E., (ed.), John Wiley and Sons, New York: 569–655.

Glenn, S. M., M. F. Crowley, D. B. Haidvogel & Y. H. Song, 1996. Underwater observatory captures coastal upwelling events off New Jersey. EOS 77: 233–236.

Gonzalez, J. & M. A. Moran, 1997. Numerical dominance of a group of bacteria in the alpha-subclass of the class Proteobacteria in coastal seawater. Appl. envir. Microbiol. 63: 4237–4242.

Hanahan, D, 1983. Studies on transformation of *E. coli* with plasmids. J. mol. Biol. 166: 557–580.

Head, I. M., J. R. Saunders & R. W. Pickup, 1998. Microbial evolution, diversity, and ecology: A decade of ribosomal RNA analysis of uncultivated microorganisms. Micr. Ecol. 35: 1–21.

Hicks, D. C. & J. R. Miller, 1980. Meteorological forcing and bottom water movement off the northern New Jersey coast, Estuar. coast. Sci. II. 563–571.

Hofle, M. G. & I. Brettar, 1995. Taxonomic diversity and metabolic activity of microbial communities in the water column of the central Baltic Sea. Limnol. Oceanogr. 40: 865–874.

Hugenholtz, P., B. M. Goebel & N. R. Pace, 1998. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. J. Bact. 180: 4765–4774.

Kemp, P. F., P. G. Falkowski & C. D. Wirick, 1994. Modeling vertical oxygen and carbon flux during stratified spring and summer conditions on the continental shelf, Middle Atlantic Bight, eastern U.S.A. Deep Sea Res. Part II, 41: 629.

Kerkhof, L. & B. B Ward, 1993. Comparison of nucleic acid hybridization and fluorometry for measurement of the relationship between RNA/DNA ratio and growth rate in a marine bacterium. Appl. envir. Microbiol. 59: 1303–1309.

Lane, D. J., 1991. 16S/23S rRNA sequencing, In Stackebrandt E. & M. Goodfellow (eds), Nucleic Acid Techniques in Bacterial Systematics. John Wiley & Sons: 115–175.

Maidak B. L., G. J. Olsen, N. Larsen, R. Overbeek, M. J. McCaughey & C. R. Woese, 1997. The RDP (Ribosomal Database Project). Nucl. Acids Res. 25: 109–111.

Methe, B. A., W. D. Hiorns & J. P. Zehr, 1998. Contrasts between marine and freshwater bacterial community composition: Analyses of communities in Lake George and six other Adirondack lakes. Limnol. Oceanogr. 43: 368–374.

Millie, D., H. Paerl, J. Hurley & G. Kirkpatrick, 1993. Algal pigment determinations in aquatic ecosystems: Analytical evaluations, applications, and recommendations. Current Topic in Bot. Res. 1: 1–13.

Olsen, G. J., H. Matsuda, R. Hagstrom & R. Overbeek, 1994. FastDNAml – a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. Computer Applications in the Biosciences 10: 41–48.

Phelps, C., L. Kerkhof & L. Young, 1998. Molecular characterization of a sulfate-reducing consortium which mineralizes benzene. FEMS Micr. Ecol. 27: 269–279.

Pinhassi, J., U. Zweifel & A. Hagstrom, 1997. Dominant marine bacterioplankton species found among colony-forming bacteria. Appl. envir. Microbiol. 63: 3359–3366.

Pomeroy, L. R., 1974. The ocean's food web, a changing paradigm. Bioscience 24: 499.

Rappe, M. S., P. F. Kemp, & S. J. Giovannoni, 1997. Phylogenetic diversity of marine coastal picoplankton 16S rRNA genes cloned from the continental shelf off Cape Hatteras, North Carolina. Limnol. Oceanogr. 42: 811–826.

Reynolds, C. S., 1980. Phytoplankton assemblages and their periodicity in stratifying lake systems. Holarctic Ecol. 3: 141–159.

Sakano, Y. & L. Kerkhof, 1998. Assessing changes in microbial community structure during operation of an ammonia biofilter using molecular tools. Appl. envir. Microbiol. 64: 4877–4882.

Suzuki, M. T. & S. J. Giovannoni, 1996. Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. Appl. envir. Microbiol. 62: 625–30.

Tuomi, P., T. Torsvik, M. Heldal & G. Bratbak, 1997. Bacterial population dynamics in a meromictic lake. Appl. envir. Microbiol. 63: 2181–2188.

Torsvik, V., R. Sorheim & J. Goksoyr, 1996. Total bacterial diversity in soil and sediment communities – A review. J. industrial Microbiol. 17: 170–178.

Van Es, F. & L. Meyer-Reil, 1982. Biomass and Metabolic Activity of Heterotrophic Marine Bacteria. In: K. Marshall, (eds), Advances in Microbial Ecology Plenum, New York: 111–169.

Van Heukelem, L., A. J. Lewitus, T. M. Kana & N. E. Craft, 1992. High-performance liquid chromatography of phytoplankton pigments using a polymeric reversed-phase $C^{18}$ column. J. Phycol. 28: 867–872.

Von Alt, C., M. DeLuca, S. Glenn, J. Grassle & D. Haidvogel, 1997. LEO-15: Monitoring and managing coastal resources. Sea Technology 38: 10–16.

Warsh, C., 1987. NOAA's Northeast monitoring program (NEMP): A report on progress of the first five years (1979–84) and a plan for the future. NOAA Technical Memorandum NMFS-F/NEC-44. Northeast Fisheries Center, Woods Hole.

Yoshinaga, I., T. Kawai & Y. Ishida, 1995. Lysis of *Gymnodinium nagasakiense* by marine bacteria. Proceedings of the International Conference On Harmful Algal Blooms: 687–692.