

## Molecular analysis of picocyanobacterial community structure along an Arabian Sea transect reveals distinct spatial separation of lineages

Nicholas J. Fuller

Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, United Kingdom

Glen A. Tarran

Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth, PL1 3DH, United Kingdom

Marian Yallop

School of Biological Sciences, University of Bristol, Bristol, BS8 1UG, United Kingdom

Karen M. Orcutt<sup>1</sup> and David J. Scanlan<sup>2</sup>

Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, United Kingdom

### Abstract

We investigated the community structure of *Synechococcus* and *Prochlorococcus* along a transect in the Arabian Sea during September 2001. The transect spanned contrasting oceanic conditions, allowing investigation of the effects of both horizontal and vertical environmental gradients over relatively large spatial scales on picocyanobacterial population structure. We applied previously developed oligonucleotide probes specific for different *Prochlorococcus* ecotypes and *Synechococcus* clades by hybridization to 'oxygenic phototroph' 16S ribosomal DNA polymerase chain reaction amplicons. Flow cytometry data showed that, in general, the picocyanobacterial community was dominated by *Prochlorococcus* in the southern oligotrophic waters and by *Synechococcus* in the northern mesotrophic waters. Molecular analysis of these picophytoplankton communities, however, revealed more specific spatial separation of lineages along the transect, with *Prochlorococcus* in southern surface waters being dominated by the high light-adapted ecotype, while low light-adapted (LL) ecotypes were confined to deeper waters below the surface-mixed layer. Interestingly, between Sta. 2 to 4, the LL genotype MIT9303 appeared to be partitioned at the very base of the euphotic zone, beneath other LL genotypes. Most of the central and northern parts of the transect were dominated by *Synechococcus* genotypes of the clade II lineage. A significant exception was in the mesotrophic upwelling region, where genotypes representative of clades V/VII/VII dominated. Members of the recently discovered clades IX and X were found in subsurface samples in warm, coastal waters. We propose that discrete differences, both horizontally and vertically, in a suite of environmental parameters along the transect provide optimal growth conditions for specific genotypes in a particular patch of water, giving rise to distinct spatial compartmentalization of picocyanobacterial lineages.

Picocyanobacteria of the genera *Prochlorococcus* and *Synechococcus* dominate the prokaryotic component of the picophytoplankton of open-ocean environments, contributing over 50% of primary production in some areas (e.g., see Li 1994; Veldhuis et al. 1997; Brown et al. 1999). Work using both cultured isolates and natural populations has shown the two genera to be genetically (Toledo et al. 1999; Rocap et al. 2002; Fuller et al. 2003) and physiologically (see Moore et al. 1995; Palenik 2001; Scanlan 2003) diverse,

comprising a number of closely related genotypes or ecotypes. Indeed, it appears that this genetic diversity underlies their ecological success. Thus, for *Prochlorococcus*, phylogenetically distinct high light (HL)- and low light (LL)-adapted ecotypes exist, these being defined by specific photosynthetic pigment characteristics, photophysiology, and nutrient acquisition profiles (Moore and Chisholm 1999; Moore et al. 2002; Bailey et al. 2005). These HL and LL ecotypes exhibit specific depth-dependent distributions (West and Scanlan 1999; West et al. 2001; Steglich et al. 2003) with the idea that at least vertically down a stratified water column, partitioning of genotypes allows for competitive growth over a broader range of conditions than could be achieved by a genetically homogeneous population. Even for marine *Synechococcus*, for which the relationship between diversity and physiology is not yet well defined, but in which at least 10 genetic lineages have been defined (Fuller et al. 2003), there is evidence for the specific partitioning of strains, as indicated by the presence of a serotype found preferentially in surface waters (Toledo and Palenik 2003). Evidence thus far indicates, then, that the environmental gradients of light, nutrients, and

<sup>1</sup> Present address: Darling Marine Center, University of Maine, Walpole, Maine 04573.

<sup>2</sup> Corresponding author (D.J.Scanlan@warwick.ac.uk).

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temperature play a key role in determining picocyanobacterial community structure at least vertically down a water column. However, little is known about how the community structure of these organisms changes over large spatial scales; indeed, little is known about the effects of horizontal gradients of nutrients, as dictated by changes in physical conditions (e.g., upwelling or frontal regions), on this structure. This is important, since the global mapping of picocyanobacterial ecotype/genotype distributions provides a framework upon which we can begin to explain how the microdiversity within these two groups provides the diversity in function required to occupy such vast habitats. Certainly, as a result of the recent surfeit of genomic data (Dufresne et al. 2003; Palenik et al. 2003; Rocap et al. 2003), this ecological distribution information will enable researchers to more accurately interpret genomic, and indeed physiological (see Partensky et al. 1999b; Scanlan 2003), data from cultured isolates.

With this in mind we describe here an analysis of picocyanobacterial community structure along a transect in the Arabian Sea using previously developed oligonucleotide probes (West and Scanlan 1999; Fuller et al. 2003, 2005) specific for different *Prochlorococcus* ecotypes and *Synechococcus* clades. The Arabian Sea is a complex oceanic basin containing a diversity of biogeochemical provinces as a result of marked seasonal changes due to monsoon activity (e.g., see Burkill et al. 1993b). During September 2001, the transect spanned oligotrophic, stratified southern waters through mesotrophic, downwelling central waters and crossed a region of monsoonal upwelling off the northeast coast of Oman. Hence, the cruise allowed analysis of waters of differing nutrient regimes and physical structures. These contrasting hydrographic regions represented an ideal situation for evaluating picophytoplankton community structure as a function of markedly differing environmental regimes encompassing both vertical and horizontal gradients.

## Materials and methods

**Sampling**—Seawater for DNA extraction was collected aboard the RRS *Charles Darwin* (cruise 132) along a transect in the Arabian Sea during September 2001, as previously described (Fuller et al. 2006).

**Measurement of inorganic nutrients**—Nitrate, soluble reactive phosphate, and ammonium were measured along the transect, as previously described (Fuller et al. 2006).

**Flow cytometric analysis**—*Prochlorococcus* and *Synechococcus* were enumerated from 2.2-mL seawater samples using a FACSort™ flow cytometer (Becton Dickinson). This instrument counted the cells in the samples and also measured chlorophyll fluorescence (>650 nm), phycoerythrin fluorescence (585 ± 21 nm), and side scatter (light scattered at 90° to the plane of the vertically polarized argon ion laser exciting at 488 nm). Data acquisition was triggered on chlorophyll fluorescence, using laboratory cultures to set rejection gates for background noise. Samples were analyzed for 3 min at a flow rate of 95 ±

2.6 μL min<sup>-1</sup>, and data were stored in 'listmode' format. The 'listmode' data were read using WinMDI (version 2.8) flow cytometry analysis software (Joseph Trotter) to produce scatter-plots of side scatter versus chlorophyll or phycoerythrin fluorescence, from which cell counts were made.

**Polymerase chain reaction (PCR) amplification**—The oxygenic phototroph-specific primers OXY107F and OXY1313R (West et al. 2001) were used for PCR amplification from environmental DNA and from control strains for dot blot hybridization, following the PCR protocol described previously (Fuller et al. 2003).

**Dot blot hybridization**—16S ribosomal DNA (rDNA) amplicons from Arabian Sea environmental DNA and from control strains were purified, blotted onto nylon membranes, and hybridized to oligonucleotide probes specific for the different *Prochlorococcus* ecotypes and *Synechococcus* clades, following the method of Fuller et al. (2003, 2005). Oligonucleotide probes used in this study were labeled as previously described (Fuller et al. 2003). Probe sequences are shown in Table 1. Hybridization was quantified by using a Fujifilm FLA-5000 phosphorimager and Total Lab software (Phoretix). Relative hybridization values (%) were calculated as previously described (Fuller et al. 2003).

**Calculation of relative abundance**—Relative abundance values (cells mL<sup>-1</sup>) were calculated as follows:

$$C_{RA} = (C_{RH} / \Sigma C_{RHn}) \times N$$

where  $C_{RA}$  is the relative abundance value from a specific cyanobacterial probe,  $C_{RH}$  is the relative hybridization signal (%) from that same probe,  $\Sigma C_{RHn}$  is the sum of the relative hybridization signals (%) from all the individual probes representing that genus (*Synechococcus* or *Prochlorococcus*), and  $N$  is the cell count (cells mL<sup>-1</sup>), by flow cytometry, of that same genus (*Synechococcus* or *Prochlorococcus*). For *Prochlorococcus*,  $\Sigma C_{RHn}$  is derived from the sum of the relative hybridization values from all *Prochlorococcus* probes, with the exclusion of the MIT9303-specific probe (MIT1023R), as this strain is detected by the LL probe (DPRO634R). For *Synechococcus*,  $\Sigma C_{RHn}$  is derived from the sum of the relative hybridization values from the probes specific for clades I–X.

**Construction of contour plots**—Arabian Sea data of physical, chemical, and biotic parameters were plotted as contour plots using Sigma Plot (version 8.0) software. Data were interpolated using the running average method with 0.01 sampling proportion and "nearest neighbors" as the bandwidth method.

**Statistical analyses**—Multivariate statistical analysis of the physical, chemical, and biotic data from the transect was performed with the *Synechococcus* and *Prochlorococcus* relative abundance data set using redundancy analysis (RDA; Ter Braak 1994). Environmental (explanatory)

Table 1. 16S rDNA oligonucleotide probes used in this study.

Probe	Sequence (5'–3')	Target organism(s)	Reference
SYN1006	CTC TCA AGT TTC CAA GAG	<i>Synechococcus</i> clade I	Fuller et al. 2003
SYN1006RS	CTC TCC TGT TTC CAA GAG	<i>Synechococcus</i> clade II	Fuller et al. 2003
SYN262	GAT GCC TTG GTA GGC CTT	<i>Synechococcus</i> clade III	Fuller et al. 2003
SYN635	AAG CCC CTC AGT TTC CAC	<i>Synechococcus</i> clade IV	Fuller et al. 2003
SYN1280	GAG CCA CGG TTT ATG AGA	<i>Synechococcus</i> clades V, VI, & VII	Fuller et al. 2003
SYN620	CAC TGC CAC GAT GGA GTT	<i>Synechococcus</i> clade VIII	Fuller et al. 2003
SYN1000	GGT TTC CCA GAA ATT CGC	<i>Synechococcus</i> clade IX	Fuller et al. 2003
SYN1007	ACC CTC CGG TTT CCC AGA	<i>Synechococcus</i> clade X	Fuller et al. 2003
SYN1258	TTG TCC TCG CGA ACT TGC	<i>Synechococcus</i> clades I–VII & X, <i>Prochlorococcus</i> sp. MIT9303	Fuller et al. 2003
SIPRO634R	GCC GAT CAG TTT CCA CTG	HLI <i>Prochlorococcus</i>	West and Scanlan 1999
S2PRO640R	ATA CTC AAG CCT TTC AGT TT	HLII <i>Prochlorococcus</i>	Fuller et al. 2005
DPRO634R	GCC AAT CAG TTT CCA CTG	LL <i>Prochlorococcus</i> (except SS120)	West and Scanlan 1999
SARG634R	GCC CTT CAG TTT CCA CTG	<i>Prochlorococcus</i> sp. SS120	West and Scanlan 1999
MIT1023R	TGC GTT CCC AAA GGC ACT	<i>Prochlorococcus</i> sp. MIT9303	West and Scanlan 1999
EUB338	GCT GCC TCC CGT AGG AGT	Eubacteria	Amann et al. 1995

variables were log (n + 1) transformed prior to analysis as these variables were measured on different scales. Response variables (abundance data) were square-root transformed prior to analysis to reduce the variance. Environmental variables were selected by a Forward Selection option with Monte Carlo restrictions. Explanatory variables with a *p* value of <0.05 were considered significant.

## Results

**Picocyanobacterial abundance**—Flow cytometric enumeration of picocyanobacteria along the transect revealed a general spatial separation of dominant genus, with *Prochlorococcus* dominating in the oligotrophic southern four stations and *Synechococcus* dominating in the mesotrophic northern seven stations (Fig. 1A,B). However, *Prochlorococcus* were also found alongside *Synechococcus* in the warm coastal waters in the north. Picocyanobacterial counts generally increased toward the deep chlorophyll maximum (DCM). *Prochlorococcus* counts were around  $10^5$  cells mL<sup>-1</sup> in surface waters of Sta. 1 to 4, with a maximum observed at 50 m at Sta. 4 ( $3.7 \times 10^5$  cells mL<sup>-1</sup>). *Synechococcus* counts were generally around  $10^4$  cells mL<sup>-1</sup> at Sta. 1 to 4 and  $10^5$  cells mL<sup>-1</sup> further north, reaching a maximum at the surface (1 m) of upwelled waters of Sta. 9 ( $6.7 \times 10^5$  cells mL<sup>-1</sup>).

**Prochlorococcus population structure**—Dot blot hybridization of oligonucleotide probes to PCR amplicons from total oxygenic phototrophs (cyanobacteria and plastids) was used to assess the structure of the *Prochlorococcus* community along the Arabian Sea transect (Fig. 2). HL ecotypes of type HLI and the LL genotype SS120 were not detected at all. Most of the *Prochlorococcus* hybridization signal was confined to the first four stations, a result that is similar to that exhibited by the flow cytometry data of *Prochlorococcus* cell abundance (Fig. 1A). The surface waters of these stations were dominated by the HLII ecotype, while waters below the surface-mixed layer (SML)

were dominated by LL ecotypes. HLII ecotypes were generally confined to surface waters, while LL ecotypes were generally confined to deeper waters below 50 m. HLII ecotypes were also detected at the surface of Sta. 10 and at Sta. 11, where they extended to the base of the euphotic zone, outnumbering LL ecotypes. LL ecotypes were observed at Sta. 8 and 10 just below the DCMs. The LL genotype MIT9303 was identified as being a contributor to the general LL signal and appeared to dominate the entire LL signal from Sta. 7 to 11. However, at Sta. 2, 3, and 4, MIT9303 only dominated the LL signal at the very base of the euphotic zone.

The dot blot data were used to calculate relative abundance values for the different *Prochlorococcus* ecotypes (Fig. 3) to express values as cells mL<sup>-1</sup>. The contour plots show similar patterns to those from relative hybridization data, and the HLII plot is very similar to the flow cytometry plot (Fig. 1A). However, it is clear that the peak of *Prochlorococcus* cells at Sta. 8, 50 m, solely comprised LL ecotypes, more specifically MIT9303 types. While relative hybridization values showed high signals of MIT9303 genotypes at 150 m from Sta. 1 to 3, relative abundance data revealed that the actual number of cells at this depth was much lower.

**Synechococcus population structure**—Dot blot hybridization of oligonucleotide probes to PCR amplicons from total oxygenic phototrophs (cyanobacteria and plastids) was used to assess the structure of the *Synechococcus* population along the Arabian Sea transect (Fig. 4). Clades IV and VIII were not detected at all. Clade II genotypes gave the greatest overall relative hybridization signal, and this was the dominant *Synechococcus* clade for most of the transect, reaching around 75% of the total oxygenic phototroph signal in surface waters of Sta. 5 and 11. Clade III genotypes generally had a similar distribution to clade II, but with a considerably lower signal. However, they showed distinct peaks at Sta. 6, 44 m; Sta. 9, 9 m; and Sta. 10, 27 m. Genotypes of clades V, VI, and VII were detected with low signal over much of the transect. However, there

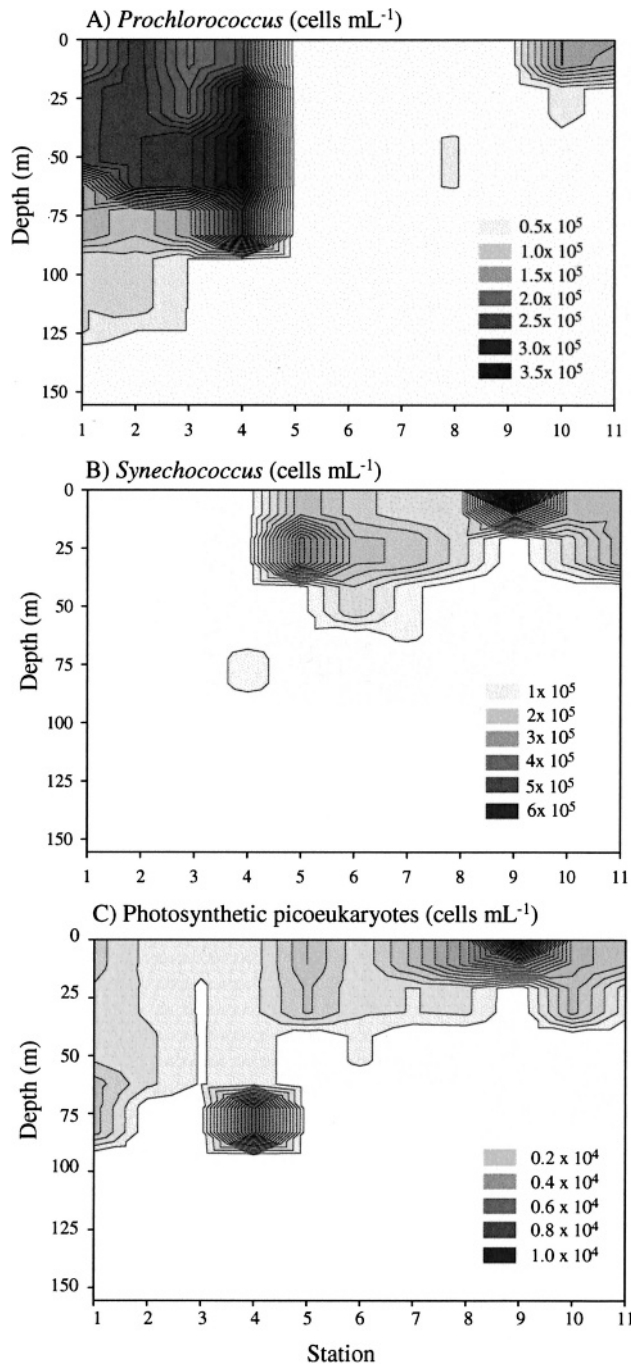


Fig. 1. Contour plots of the Arabian Sea transect, from Sta. 1 in the south to Sta. 11 in the north, of (A) *Prochlorococcus*, (B) *Synechococcus*, and (C) photosynthetic picoeukaryote abundance from flow cytometry analyses.

were two major peaks, at the surface of Sta. 9, where they dominated the *Synechococcus* population, reaching up to 53% of the total oxygenic phototrophic signal (10 times greater than the clade II signal), and at the DCM of Sta. 11 (27 m), where they reached 25%. Clade I genotypes were detected with low signal, particularly at the surface of Sta. 5 to 11. However, the greatest clade I relative hybridization value was below the SML at the upwelling station, Sta. 9.

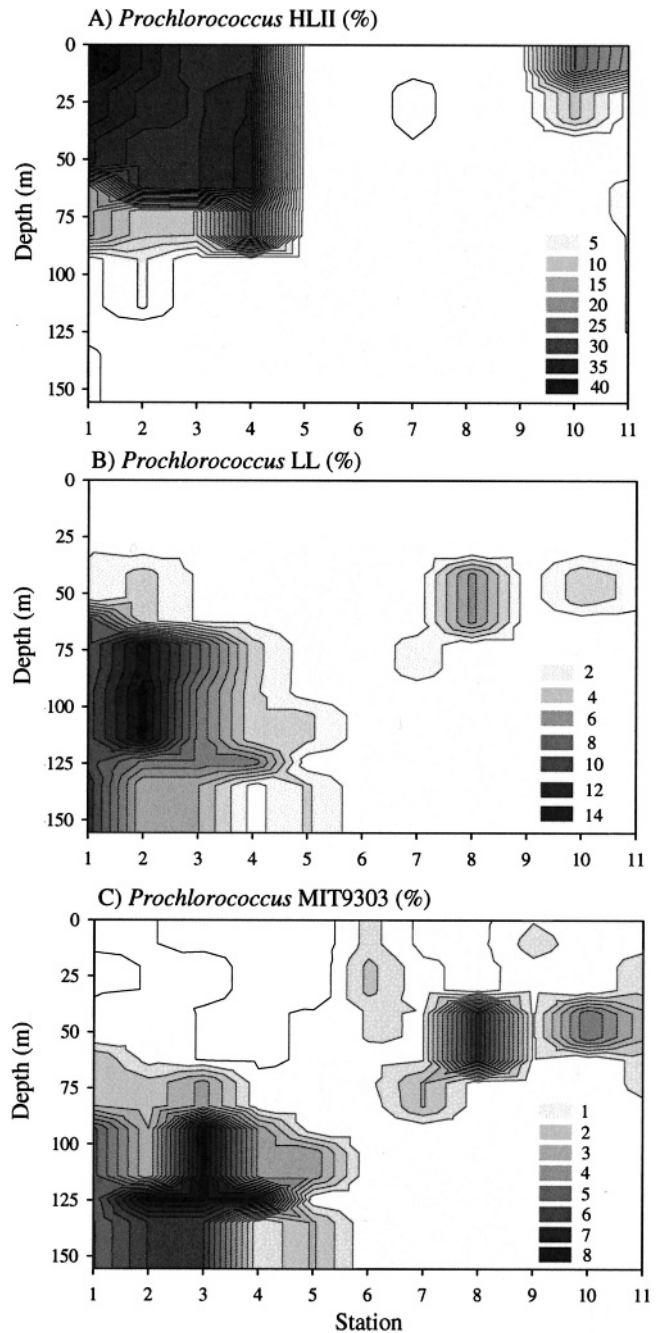


Fig. 2. Contour plots of the Arabian Sea transect, from Sta. 1 in the south to Sta. 11 in the north, of *Prochlorococcus* ecotypes, as % relative hybridization (as a proportion of all amplified by primers OXY107F and OXY1313R). (A) HLII, (B) LL, and (C) MIT9303.

Indeed, at a depth of 50 m at Sta. 9, clade I genotypes dominated the *Synechococcus* signal. The recently discovered clade IX was virtually absent from the entire transect but had a clear peak at the DCM of Sta. 11, though it still only reached 3% of the total signal. Genotypes of the recently discovered clade X had a very similar distribution to clade IX, also peaking at the DCM of Sta. 11, but its measure was slightly more abundant, with a maximum

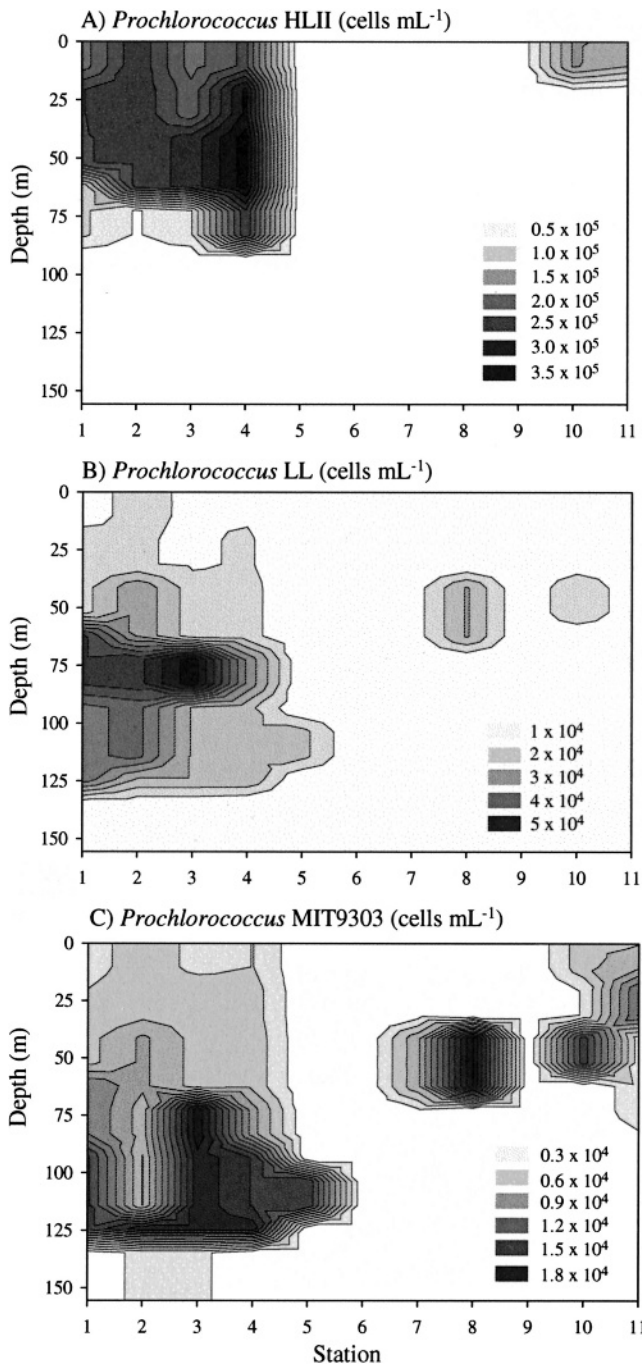


Fig. 3. Contour plots of the Arabian Sea transect, from Sta. 1 in the south to Sta. 11 in the north, of *Prochlorococcus* ecotypes, as relative abundance. (A) HLII, (B) LL, and (C) MIT9303.

signal of 8%. The plot of relative hybridization data for the “general MC-A *Synechococcus*” probe is similar to that of clade II, emphasizing that clade II was the dominant clade, but it also shows the peak of clades V, VI, and VII at the surface of Sta. 9.

Relative abundance data for the *Synechococcus* clades (Fig. 5) show very similar patterns to the relative hybridization data, except for clade I, where the proportional peak below the SML at Sta. 9 (Fig. 4A) is masked by a low

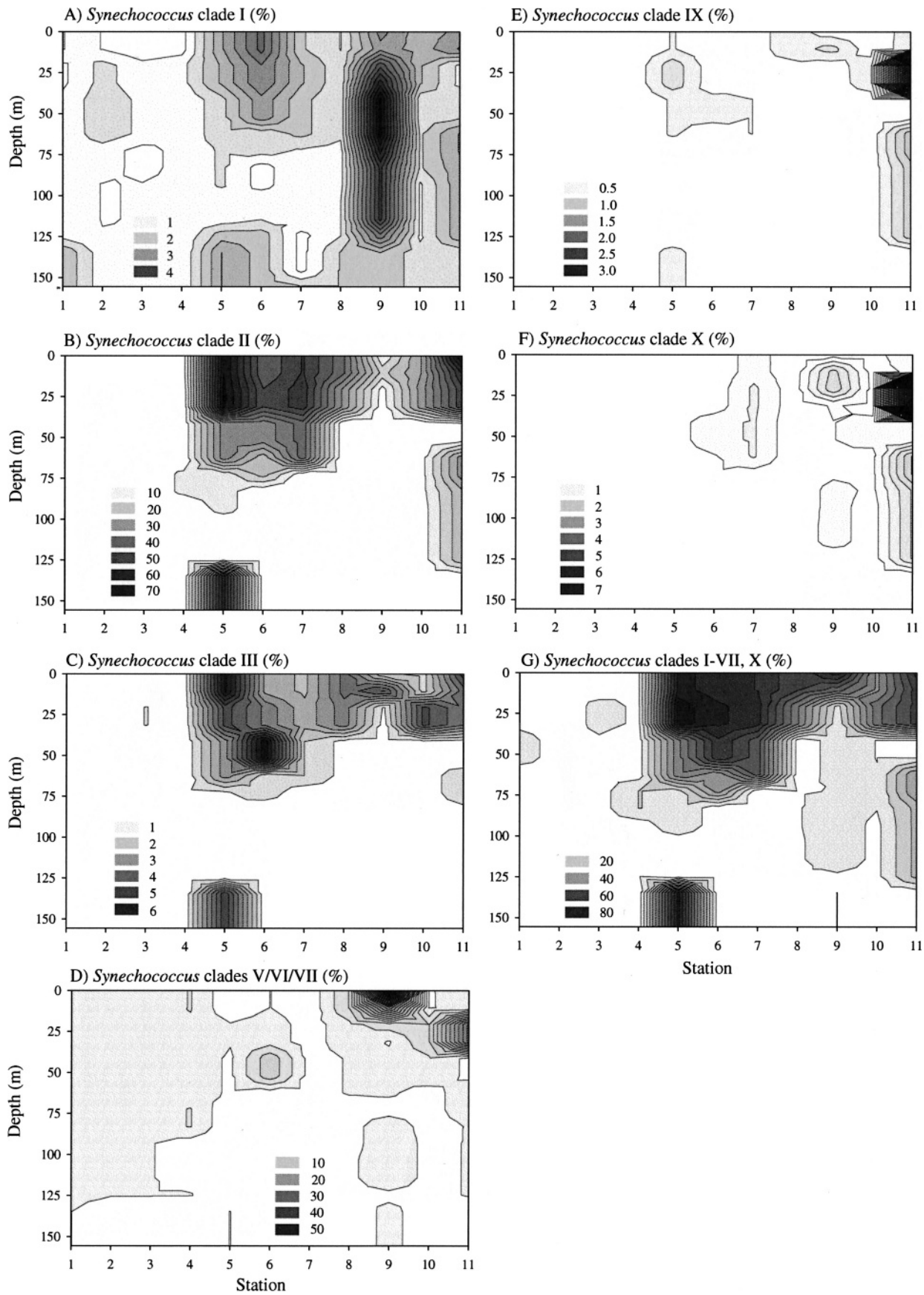
actual abundance, with a peak in actual numbers at the surface (1 m), coinciding with the peak of clades V, VI, and VII. Furthermore, clade III is now seen to be most abundant at this same station, but slightly deeper, at 9 m. The peaks in relative hybridization from several clades at Sta. 5, 150 m, are shown not to be peaks in actual cell numbers.

**Multivariate analysis**—RDA was performed on a suite of environmental variables and the *Prochlorococcus* ecotype/*Synechococcus* clade relative abundance data (Fig. 6) in order to define the environmental parameters that allowed proliferation of specific picocyanobacterial groups along the transect. The first two RDA axes explained 55% of the variance in the species data and 81.4% of the variance in the relative abundance data, with respect to the environmental variables. Key significant explanatory variables on Axis 1 were salinity, chlorophyll *a* (Chl *a*, as a surrogate measure of overall trophic status), and two pigments, alloxanthin and fucoxanthin. Temperature was a major significant variable on Axis 2. *Synechococcus* clades V–VII, IX, and X showed a very strong positive correlation with temperature, while there was a strong correlation of *Synechococcus* clades II and III with salinity. Conversely, both HLII and LL *Prochlorococcus* ecotypes correlated negatively with salinity. Additionally, *Synechococcus* clades generally showed a positive correlation with algal biomass (Chl *a*) and various other pigments, with clades I and III demonstrating a strong positive correlation with fucoxanthin, while clades V–VII, IX, and X showed a strong positive correlation with alloxanthin.

## Discussion

The distribution of organisms is a fundamental issue in ecology, with implications not only for issues of species diversity but also for those of ubiquitous versus endemic distribution and, hence, niche adaptation. Although there are numerous studies of picocyanobacterial population distribution over relatively large spatial scales using flow cytometric analysis (reviewed by Partensky et al. 1999a), we have a poor knowledge of the taxonomic composition of these populations. Describing community structure at the genetic level, and ultimately understanding why this structure occurs, is implicit to explaining why a particular organism is present in a particular place at a particular time. For marine *Synechococcus* and *Prochlorococcus* this is especially important, since we already know that there is considerable phylogenetic (see Scanlan and West 2002) and phenotypic (see Moore et al. 1998, 2005; Scanlan 2003) diversity among these genera.

The present study was part of an investigation to determine the composition of the total picophytoplankton population (i.e., including photosynthetic picoeukaryotes [PPEs], see the accompanying article, in this issue) along a transect in the Arabian Sea under different conditions of environmental forcing. The general patterns of *Prochlorococcus* and *Synechococcus* abundance, as determined by flow cytometry data, are comparable with those described in previous studies in this oceanic region (see Burkill et al.



1993a; Campbell et al. 1998; Tarran et al. 1999), namely a numerical dominance of *Prochlorococcus* in more oligotrophic stratified areas with surface nitrate concentrations below  $0.1 \mu\text{mol L}^{-1}$  and surface temperatures above  $27^\circ\text{C}$ . In contrast, *Synechococcus* cell abundance generally co-varied with PPE abundance (compare Fig. 1B and C), and numbers were highest in regions of upwelling or intense mixing.

*Prochlorococcus* populations in southern oligotrophic waters were dominated by the HLII ecotype (Figs. 2A, 3A). This ecotype was confined to surface waters in this region, a feature contrasting with that observed in the Gulf of Aqaba, Red Sea, where HLII genotypes dominated throughout a stratified water column (West et al. 2001; Fuller et al. 2005). Interestingly, Zinser et al. (2006) also found that the HLII ecotype ( $\equiv$ eMIT9312 in Zinser et al. [2006]) dominated the surface mixed layer at two stations in the North Atlantic Ocean, but at an eastern site, HLII ecotype abundance dropped off dramatically below the mixed layer (30 m), whereas at the western site it maintained high abundance down to 100 m. The specific environmental conditions that allow for these two differing population profiles for this ecotype are still unclear. However, the fact that HLII ecotypes can dominate at depth, and outnumber LL ecotypes at such a time, might indicate that factors such as selective grazing or viral lysis of LL ecotypes or local physical seeding mechanisms that preferentially bring HL ecotypes into a water column (factors that have been proposed previously; Fuller et al. 2005) are relevant on larger scales.

Certainly, the fact that HLII genotypes dominate this Arabian Sea region, while HLI genotypes are absent or at least undetectable, is consistent with what we have observed in the Gulf of Aqaba (West et al. 2001; Fuller et al. 2005), indicating that the HLII ecotype is the dominant HL population throughout this region. Conversely, HLI and HLII ecotypes show differential dominance in water columns in the North Atlantic (West and Scanlan 1999; Zinser et al. 2006). Upon analyzing all of this population data collectively from these differing oceanic regimes, it appears that water column temperature may play a key role in dictating which HL ecotype dominates, with the HLII ecotype generally occupying warmer waters, and coincident with regions of high stratification.

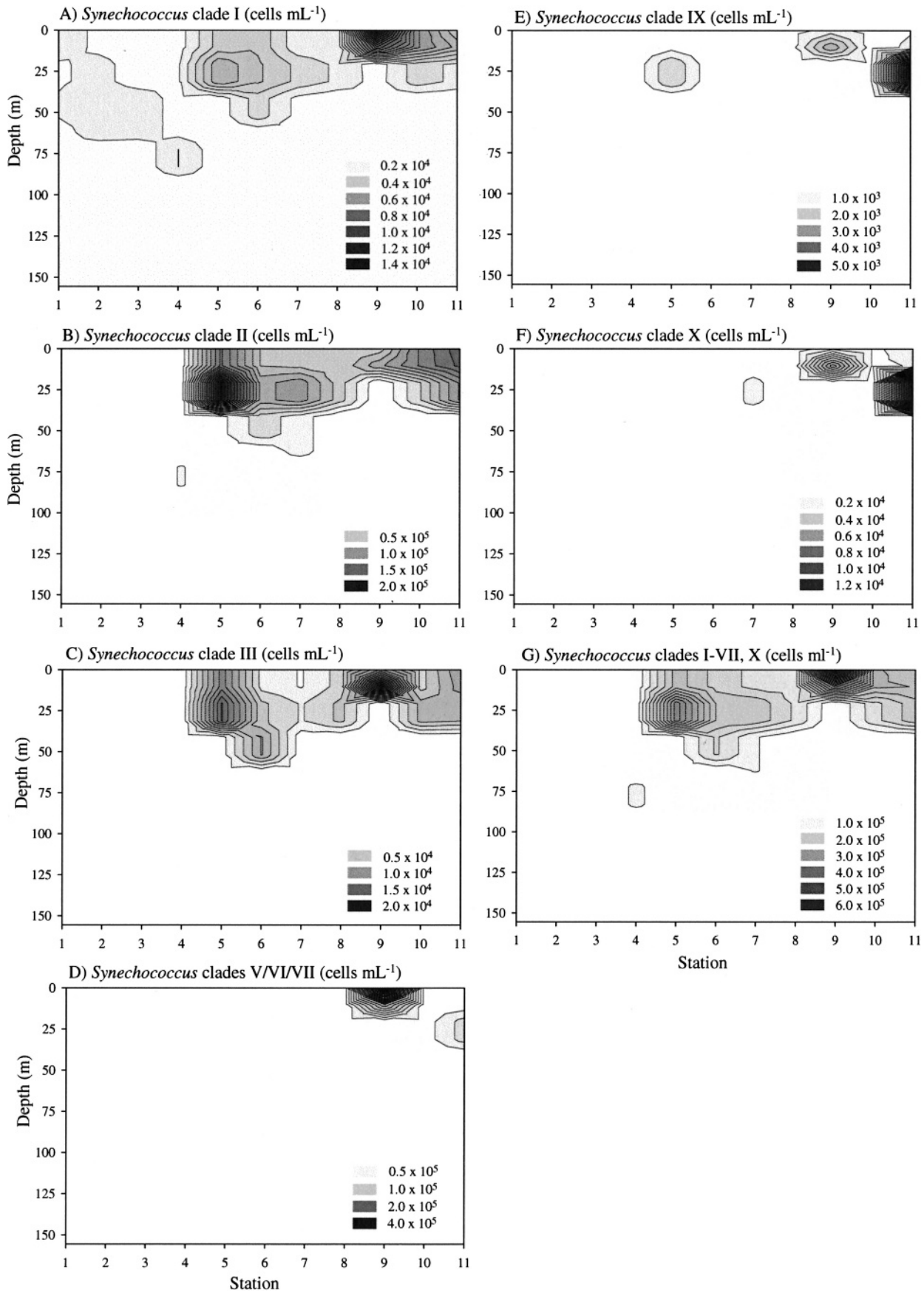
LL *Prochlorococcus* ecotype population structure along the transect showed a distribution typical of stratified water column conditions, a result that has been reported in several other oceanic environments (West and Scanlan 1999; Fuller et al. 2005; Zinser et al. 2006), being confined to deeper waters toward the base of the euphotic zone, particularly in the southern stations. This partitioning of LL (and HL) ecotypes is now recognized as a fundamental

mechanism by which this genus populates the entire water column, and as indicated in the data set presented here, it can be seen that such a structure can span large oceanic regions. Comparison of relative hybridization and relative abundance data for the LL- and MIT9303-specific probes used in this study demonstrated that the MIT9303 lineage is partitioned beneath other genotypes recognized by the LL probe (compare Figs. 2B, 3B with Figs. 2C, 3C) at Sta. 1–4. In contrast, in the less stratified waters at Sta. 8, MIT9303 genotypes comprised the majority of the LL signal, but here they resided higher in the water column, just below the DCM. Such differences highlight that *Prochlorococcus* population structure can change both vertically and horizontally; this change is presumably largely a function of the mixing history of a given water mass. The evidence that LL lineages can themselves partition in stratified water columns indicates that differences in LL ecotype physiology (Moore et al. 1998, 2002, 2005) are important ecologically. A greater understanding of the underlying factors that allow, for example, MIT9303 genotypes to partition beneath other LL genotypes (e.g., NATL2A, PAC1) will require further work, though (e.g., genomic analysis of other LL genotypes as well as competition experiments between genotypes to assess subtle differences in photophysiology or nutrient acquisition potential with regard to fitness).

In this article we have presented our genetic population structure data sets not only as % relative hybridization (an indication of the proportion of each ecotype as compared to the total number of oxygenic phototroph amplicons), as in previous studies (West and Scanlan 1999; Fuller et al. 2005), but also as relative abundance values. We believe the latter data set gives clarity to the actual numerical contribution of each genotype/ecotype/clade at each location. Thus, relative hybridization data showed relatively high signals of LL ecotypes at 150 m from Sta. 1 to 3 (Fig. 2B,C), while relative abundance data (Fig. 3B,C) reveal that the actual number of cells at this depth is relatively low. Similarly, several *Synechococcus* clades show high relative hybridization signals at Sta. 5, 150 m, whereas relative abundance values are low here. It should be borne in mind, though, that the accuracy of this relative abundance value is to some extent complicated by rDNA copy number in individual *Prochlorococcus* and *Synechococcus* strains, while it also assumes that the suite of marine *Synechococcus* 16S rRNA gene probes used here encompass the majority of the phycoerythrin (PE)-containing *Synechococcus* cells detected by flow cytometry. This is relevant given that other PE-containing marine *Synechococcus* have been isolated from this region (see Wingard et al. 2002), although their relative abundance in situ is unknown.

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Fig. 4. Contour plots of the Arabian Sea transect, from Sta. 1 in the south to Sta. 11 in the north, of *Synechococcus* clades, as % relative hybridization (as a proportion of all amplified by primers OXY107F and OXY1313R). (A) Clade I, (B) clade II, (C) clade III, (D) clade V/VI/VII, (E) clade IX, (F) clade X, and (G) clades I–VII and X.



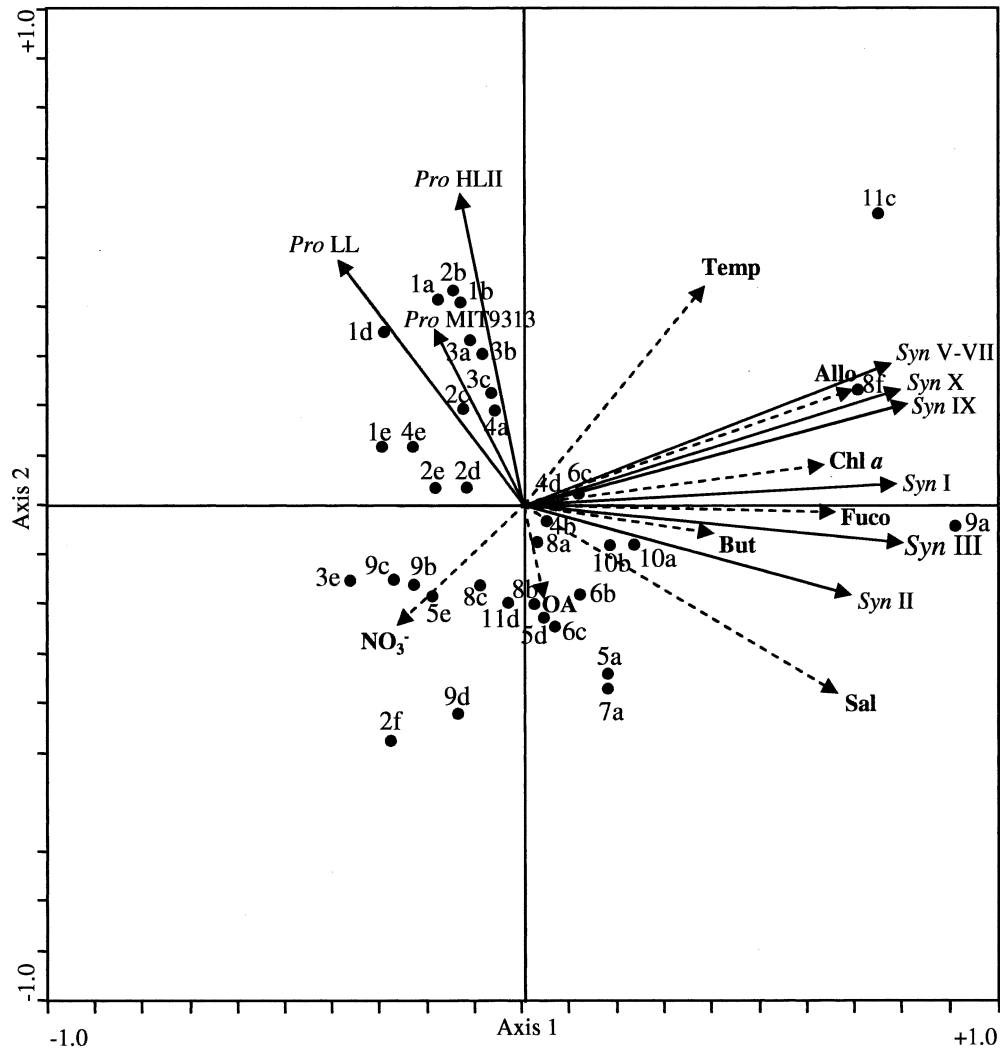


Fig. 6. RDA ordination diagram of *Synechococcus* and *Prochlorococcus* relative abundance data (solid lines) with environmental variables (dotted lines). Data points are labeled with the number according to the AMBITION cruise sampling station. The associated letters represent depths in the profile, with 'a' being the shallowest sample from a particular station.  $\text{NO}_3^-$  = nitrate concentration, Sal = salinity, Temp = temperature, Chl *a* = chlorophyll *a* concentration, Allo = alloxanthin, Fuco = fucoxanthin, But = 19'-butanoyloxyfucoxanthin. Arrows pointing in 'roughly' the same direction indicate a high positive correlation, arrows crossing at right angles indicate a near-zero correlation, and arrows pointing in the opposite direction have a high negative correlation.

Prior to this work little was known of the genetic structure of marine *Synechococcus* populations in any oceanic environment, particularly over horizontal scales. In a previous study we assessed *Synechococcus* population structure vertically over an annual cycle at a single site in the Gulf of Aqaba, Red Sea (Fuller et al. 2005). Here, though, we can see that in addition to the spatial dominance of either *Prochlorococcus* in southern or

*Synechococcus* in northern waters, there was also a distinct spatial separation of specific *Synechococcus* lineages horizontally along the transect. Thus, although over a large portion of the transect north of Sta. 4 it was *Synechococcus* clade II genotypes that dominated the water column, at the highly productive Sta. 9 (Morgan and Rees unpubl. data) and again at Sta. 11, it was genotypes of clades V/VI/VII that took over, particularly in surface waters. Clades I, IX,

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Fig. 5. Contour plots of the Arabian Sea transect, from Sta. 1 in the south to Sta. 11 in the north, of *Synechococcus* clades, as relative abundance. (A) Clade I, (B) clade II, (C) clade III, (D) clade V/VI/VII, (E) clade IX, (F) clade X, and (G) clades I–VII and X.

and X also showed a similar distribution to the latter lineages, albeit at lower abundance. This change in the genetic structure of the *Synechococcus* population appears to be a clear function of the change in nutrient load at these stations (see fig 3., Fuller et al. 2006), although at Sta. 10 and 11, surface water temperature is also higher than at other sites along the transect.

The appearance of clade II genotypes as the dominant *Synechococcus* clade along the transect agrees well with data both from the Gulf of Aqaba, Red Sea (Fuller et al. 2003, 2005) and from 16S rDNA sequences derived from flow-sorted *Synechococcus* cells from the AMBITION cruise (Zubkov et al. 2003). Our own analysis of this latter data set showed 26 of 27 of these sequences to correspond to clade II (Fuller et al. 2005). More generally, Palenik and colleagues (Ferris and Palenik 1998; Toledo and Palenik 2003) have shown members of this clade to be present in oligotrophic waters offshore from California (via clone library construction and immunofluorescent studies). Hence, even the more global distribution of this clade still equates nicely to members of this lineage being most prevalent in relatively nutrient-poor offshore waters.

The high numerical abundance of members of clades V/VI/VII in the upwelling region mirrors the recent report of an abundance of clade VII marine *Synechococcus* genotypes (17 of 23 clones) in a clone library constructed from environmental DNA derived from surface waters of the Costa Rica upwelling dome (Saito et al. 2005), another highly productive region. The 16S rDNA oligonucleotide probe we have used here does not differentiate between the V, VI, and VII lineages, and, hence, it will require further clone library analysis to specify the clade(s) present in this Arabian Sea upwelling region. It is interesting, however, that in a previous study in the Arabian Sea using scanning fluorescence spectroscopy it was shown that a low-phycoerythrin (PUB) form of PE, spectrally similar to the major PE of the clade V strain *Synechococcus* sp. WH7803, tended to dominate upwelling-influenced surface waters, while PUB-lacking forms (=members of clade VI, the only clade known so far with members lacking PUB) were rarely detected (Wood et al. 1999). Should members of clade V indeed be present in this environment, then this observation would also tie together nitrogen physiology data for this strain. Thus, WH7803 appears to have an opportunistic strategy with respect to nitrogen depletion whereby its ability to utilize nitrate is maintained even after 2 d of depletion (Glibert et al. 1986), a scenario consistent with infrequent episodic pulsing of nitrate from water column mixing and with the data presented here.

Comparison of the relative hybridization data for the general *Synechococcus* probe (SYN1258) with the clade-specific probes does not reveal any peaks in hybridization that are not represented by lineages we have already probed. However, the sum of all clade-specific probes was less than the general SYN1258 in some cases—notably in surface samples among Sta. 5 through 10. This indicates the presence of other novel, apparently surface-localized *Synechococcus* clades in these waters. Such lineages might equate to lineages reported from recent *rpoC1*, *ntcA*, and 16S-23S rDNA internal transcribed spacer (ITS)

studies (Mühling et al. 2005; Saito et al. 2005; Penno et al. 2006) and for which the molecular evidence indicates they do not correspond to known 16S rDNA clades.

Multivariate statistical analysis of the molecular community structure (relative abundance) and environmental parameter data sets (Fig. 6) indicated that specific *Synechococcus/Prochlorococcus* clades responded somewhat differently to the dominant environmental gradients of salinity and temperature. Thus, in this oceanic environment, *Prochlorococcus* ecotypes were negatively correlated with salinity, while *Synechococcus* clade II and III genotype abundance correlated positively. Other *Synechococcus* lineages showed no correlation with salinity but correlated positively with temperature. The positive correlation between the distribution of several of the *Synechococcus* lineages and various pigments likely relates to the co-occurrence of *Synechococcus* and photosynthetic picoeukaryote populations, also indicated by flow cytometry data (Fig. 1). The parallel distribution of these groups is most likely a function of their preference for similar environmental conditions. However, the fact that picocyanobacteria have now been reported to be commonly associated with nonphotosynthetic eukaryotes as symbionts (Foster et al. 2006) opens up the possibility that there might even be biological interconnections (e.g., consortial interactions) between photosynthetic assemblages.

In summary, this study has shown that contrasting environmental conditions (e.g., provided by thermal stratification or physical mixing events), which give rise to change both in horizontal or vertical 'space,' result in specific spatial compartmentalization of both *Prochlorococcus* ecotypes and *Synechococcus* clades. This is likely due to a particular 'patch' of water providing a 'unique,' or at least fairly specific, combination of light, nutrients, and temperature that allows proliferation of the most competitive genotypes. Further analysis of picocyanobacterial community structure in different oceanic basins will allow for a more global analysis of the spatial compartmentalization of individual lineages, which in turn will provide a strong molecular ecological basis for explaining how these genera dominate picophytoplankton communities worldwide.

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