Vol. 65: 207–219, 2012
doi: 10.3354/ame01546

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online February 1

OPEN ACCESS

Phylogenetic composition of *Prochlorococcus* and *Synechococcus* in cold eddies of the South China Sea

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ABSTRACT: Two mesoscale cyclonic eddies (CE I and CE II) occurred along the Vietnam coast in the South China Sea during summer 2007; CE I was in the decaying stage and CE II was in the intensifying stage. CE II was also influenced by the Mekong River plume. The upwelling effect of the 2 eddies on the phylogenetic diversity of *Prochlorococcus* and *Synechococcus* was investigated by clone library construction based on sequences of the internal transcribed spacer (ITS) region, with station SEATS in the oligotrophic basin as a comparison. Phylogenetic analysis revealed the presence of sequences affiliated with 3 *Prochlorococcus* clades (HL I, HL II & LL IV) and 7 *Synechococcus* clades (II, III, VII, X, XV, XVI, and sub-cluster 5.2) from the various water bodies, with HL II *Prochlorococcus* and clade II *Synechococcus* being the dominant phylotypes. Multivariate analysis demonstrated that spatial distribution of the 2 genera was mostly explained by temperature, but also affected by salinity and chlorophyll *a* concentration. Upwelling of the nutrient-rich subsurface water at the center of the cold eddy resulted in a significant increase in the abundance of *Synechococcus* in the young and intensifying CE II, whereas *Prochlorococcus* dominated in the old and decaying CE I and the surrounding oligotrophic waters. It is intriguing that *Synechococcus* clades III, XV and XVI, which are known to contain chromatically adapting members, were most numerous in eddy and river plume influenced waters, where light quality changes might be expected, compared to the oligotrophic basin waters. The disparity in cyanobacterial phylogenetic composition between the 2 eddies clearly reflected the differing hydrographic dynamics and developmental stages of these eddy systems.

KEY WORDS: *Prochlorococcus* · *Synechococcus* · Cold eddy · South China Sea

INTRODUCTION

Unicellular picocyanobacteria, *Prochlorococcus* and *Synechococcus*, the most abundant photosynthetic microorganisms in the global oceans, occupy a key position in the marine microbial food web and contribute significantly to global primary production (Partensky et al. 1999, Jardillier et al. 2010). *Prochlorococcus* are generally more abundant than *Synechococcus* by 1 to 2 orders of magnitude except in coastal and upwelling regions (Partensky et al. 1999). Although the 2 genera often co-occur, they have different geographical distributions. *Prochlorococcus* are numerically dominant in the warm oligotrophic oceans (Campbell et al. 1994), whereas *Synechococcus* predominate in coastal and more temperate/mesotrophic open oceans with wider global distribution (Partensky et al. 1999).

Despite the well documented importance of *Prochlorococcus* and *Synechococcus* in marine ecosystems, fine-scale discrimination of closely related groups or species within these 2 genera has been made possible only recently, based on the genetic information of the 16S/23S rRNA internal transcribed

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spacer (ITS) region (Rocap et al. 2002, Johnson et al. 2006, Zinser et al. 2006, Martiny et al. 2009, Malmstrom et al. 2010). *Prochlorococcus* is composed of multiple genetically and physiologically differentiated subgroups, including at least 6 closely related light-adapted subgroups which are distributed at different water depths (Rocap et al. 2002). High-light-adapted (HL) *Prochlorococcus* dominate in the surface mixed layer and can be further subdivided into HL I and HL II subgroups; whilst the low-light-adapted (LL) *Prochlorococcus* dominate in the deeper layer of the euphotic zone and comprise at least 4 ecotypes LL I, LL II, LL III and LL IV (Rocap et al. 2002). As for *Synechococcus*, at least 10 distinct lineages have been found so far (Rocap et al. 2002, Fuller et al. 2003), and even greater diversities were reported based on *rpoC1*, *ntcA* and *petB* genes (Mühling et al. 2005, Lindell et al. 2005, Mazard et al. in press). In addition, the distinct biogeographic distributions of the most common *Synechococcus* clades, I, II and IV, throughout the world's oceans have been documented (Zwirglmaier et al. 2008): clades I and IV dominate in temperate, coastal environments (Brown & Fuhrman, 2005, Zwirglmaier et al. 2008) and clade II proliferates in subtropical/tropical environments (Fuller et al. 2006, Zwirglmaier et al. 2008) and off-shore, oligotrophic environments (Toledo & Palenik 2003).

Upwelling in mesoscale cyclonic eddies pumps nutrients into the euphotic zone and thus affects the activities and composition of different microbial groups, especially the phytoplankton community (Vaillancourt et al. 2003, Bibby et al. 2008). During each summer, an offshore current is formed eastwards near the coast of Vietnam between 11° N and 14° N and induces cold-core eddies and open-ocean upwelling in the South China Sea (SCS) (Xie et al. 2003). The SCS is a large semi-enclosed marginal sea in the tropical-subtropical western North Pacific. It is typically oligotrophic with shallow mixed layer and nutricline depths and low primary production (Liu et al. 2002). The Mekong River on the Indochina peninsula carries significant nutrient input into the southwestern SCS. *Cyanobacteria* are the major component of the picoplankton and hence key primary producers in the SCS (Jiao & Yang 2002, Liu et al. 2007, Chen et al. 2009), but little information is available on the phylogenetic composition of *Prochlorococcus* and *Synechococcus* or of the effects of eddies on picocyanobacterial population dynamics. Therefore, we constructed ITS clone libraries from water samples collected from 2 cold eddies formed in the SCS during summer to investigate the phylogenetic diversity and population dynamics of picocyanobacterial communities in various water masses and in response to cold eddy formation.

MATERIALS AND METHODS

Sample collection

Two cold-core cyclonic eddies, named cold eddy I (CE I) and cold eddy II (CE II), were formed in the western SCS during the GOE-2 cruise on board the RV 'Dongfanghong 2' from 10 August to 14 September 2007. These eddies are thought to occur as a result of the prevailing southwestern monsoon in summer (Hu et al. 2011). Satellite altimeter data were obtained from the Global Near Real-Time Sea Level Anomaly Data Viewer at the Colorado Center for Astrodynamics Research (CCAR, USA) to monitor the evolution of the eddies. Surface seawater was collected at 8 stations in the western SCS during the same cruise from 24 August to 7 September 2007 (Fig. 1). Seawater from the deep chlorophyll *a* (chl *a*) maximum (DCM) was also collected at Stns TS-1 and SEATS to examine the vertical distribution. Water samples were collected using a CTD-General Oceanic rosette sampler with Go-Flo bottles (SBE 9/17 plus, SeaBird). Between 1.2 and 2.4 l of seawater were filtered on board sequentially through 3 µm and 0.22 µm pore-size polycarbonate membranes (47 mm diameter, Millipore). The membranes were stored at −80°C until DNA extraction on land.

In addition, total chl *a* concentrations were measured by a Turner Designs fluorometer (Model Trilogy 040) and the abundance of *Prochlorococcus, Synechococcus* and heterotrophic bacteria were enumerated using a Beckon-Dickinson FACSCalibur cytometer (Chen et al. 2009). Samples for inorganic nutrients (nitrate+nitrite, phosphate and silicate) were filtered through 0.45 µm cellulose acetate filters and measured immediately onboard using a low injection analyzer (Tri-223 autoanalyzer) and standard spectrophotometric methods (Pai et al. 1990).

DNA isolation and amplification

Total genomic DNAs were recovered from biomass collected with the 0.22 µm filters by phenol:chloroform:isoamyl alcohol (25:24:1) extraction at 60°C after lysis with CTAB buffer containing RNase A (10 mg ml^{-1}) and lysozyme (50 mg ml^{-1}). Extracted DNAs were precipitated with isopropanol and then stored at −80°C. The environmental DNA subsequently served

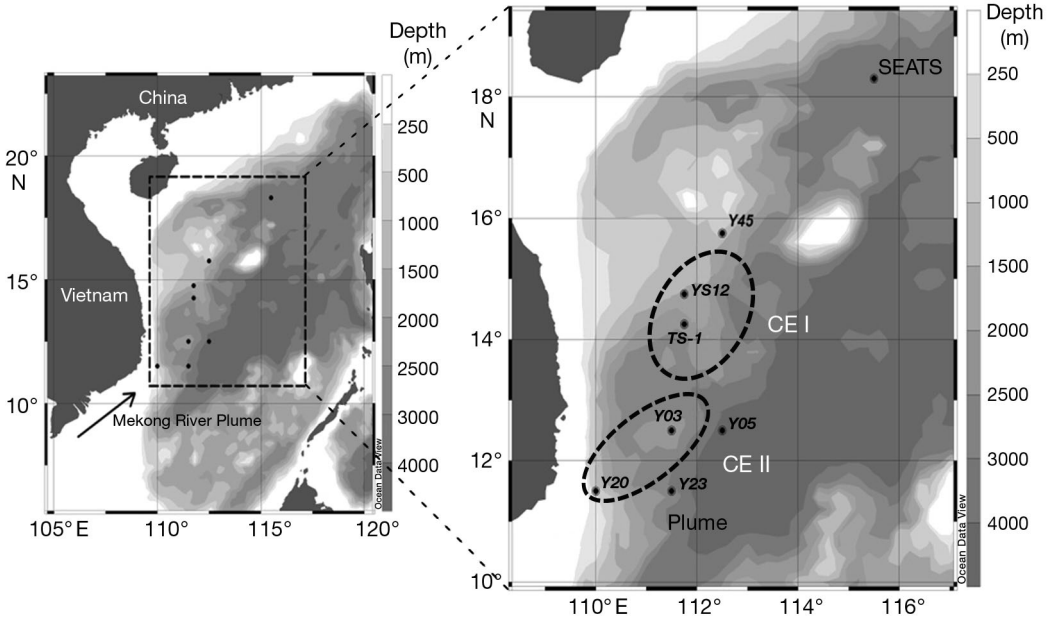

Fig. 1. Geographical location of the 2 cold eddies, occurring in the South China Sea off Vietnam during summer 2007. Sampling stations are shown, including those of cold eddy I (CEI) — In (TS-1), Edge (YS12) and Out (Y45) —, those of cold eddy II (CEII) — In (Y03), Edge (Y20) and Out (Y05) —, the Plume-affected station (Y23), and the control station of the Southeast Asia Time-Series Study (SEATS)

as template for amplification of a 1.5 kb DNA fragment encompassing the ITS region. This 1.5 kb fragment covered the distal portion of the 16S rRNA gene, the internal transcribed spacer region and the proximal region of the 23S rRNA gene. The picocyanobacterial specific primers used were 16S-1247F (5′-CGT ACT ACA ATG CTA CGG-3′) (Rocap et al. 2002) and 23S-495R (5′-ACG GTT TCA GGT TCT ATT TCA CTC-3′) (Jing et al. 2009). The PCR reaction was carried out with 50 µl master mix including 5 µl of 10× Buffer, 2 µl of $MgCl_2$ (25 mM), 4 µl of dNTPs (2.5 mM), 0.2 µl of *Taq* polymerase (5 U) and 1 µl of each primer (10 µM) with the following programme: 95°C for 3 min; 30 cycles of 95°C for 1 min, 55°C for 50 s, 72°C for 1 min; final extension at 72°C for 10 min. PCR products were stained with ethidium bromide and visualized on a 1% (w/v) agarose gel, and a strong single band within the range of 1300 to 1600 bp for each sample was revealed.

Clone library construction and RFLP screening

Independent PCR products from triplicate samples were pooled to reduce the chances of PCR artifacts and then purified using the PureLink™ Quick Gel Extraction Kit (Invitrogen). Purified amplicons were quantified with a NanoDrop ND-1000 spectrophotometer prior to cloning into the pCR4.0 vector using the TOPO TA cloning kit (Invitrogen). In total, 10 clone libraries were constructed. From each library 50 white colonies were randomly picked. The correct insertion was identified by direct amplification of the inserted DNA fragment with the original PCR primer set. Positive colonies were digested by 3 restriction enzymes, *Eco*RI, *Hae*III and *Hin*dIII, at 37°C for 3 h in 10× Buffer M (Amersham Biosciences). The digestion products were separated by electrophoresis on a 3% (w/v) agarose gel. The restriction fragment length polymorphism (RFLP) patterns were visualized under UV radiation using a Universal Hood digital imaging system (Bio-Rad) of universal hood and were normalized against a 1 kb Plus DNA Ladder (Invitrogen).

Sequencing and phylogenetic analysis

For groups of colonies showing the same RFLP pattern, representative clones were randomly chosen for subsequent sequencing. Plasmid DNAs of all colonies showing distinct RFLP fingerprints were extracted, purified and then sequenced by an Applied Biosystems 3730 genetic analyzer using the BigDye Terminator Cycle Sequencing kit v3.1 (Applied Biosystems) with the QM13 primers.

BLAST searches of the GenBank database (www.ncbi.nlm.nih.gov) were performed to identify closely related sequences. We used 98% sequence

similarity as a cutoff value. Sequences were also checked for chimeric properties by using Chimera_CHECK from the RDP-II (Ribosomal Database Project II). Maximum Likelihood (ML) trees were generated using the Phylip 3.63 programme (http://evolution.genetics.washington.edu/phylip.html) for all sequences, which included selected representative sequences from GenBank following alignment using Clustal X 1.80. Bootstrap values were obtained with 1000 resamplings and clades with greater than 50% bootstrap value are shown on the nodes of branches.

Statistical analysis

Genetic diversity was assessed by the following indices: (1) number of clones examined; (2) number of different operational taxonomic units (OTUs); (3) Coverage, derived from the equation Coverage = 1 – (N/total no. of clones), N being the number of clones occurring only once (Kemp & Aller 2004); (4) Shannon-Weaver index (H') and Simpson's Index of diversity ($1 - D$) (Krebs 1989). OTU was defined based on RFLP pattern and also the 98% cutoff value of ITS sequence similarity. Firstly, all clones showing the same RFLP pattern were considered as the same OTU. Then, representative clones of each RFLP pattern were sequenced, and all sequences obtained were multiple aligned for similarity comparison. If the sequence similarity of different RFLP patterns was ≥98%, those RFLP patterns were grouped as the same OTU.

Detrended correspondence analysis (DCA) was carried out using CANOCO V4.5 (Biometrics-Plant Research International) to determine whether linear or unimodal species distribution models were more suitable for our data. In DCA, the correspondence analysis axes are divided into segments and the sample scores of the second axis are reassigned to be centered on the centroid to remove distortion. The length of the first DCA axis is 2.066 for the picocyanobacterial population. Therefore, canonical correspondence analysis (CCA), which assumes unimodal distributions of OTUs along environmental gradients, was performed in order to reveal the relationships between picocyanobacterial phylogenetic groups and environmental variables. Abiotic and biotic factors (see Table 1) were included as explanatory variables in the CCA and biplot scaling was used. The effects of high collinearity among those factors were removed by eliminating variables with variance inflation factor (VIF) >20, one at a time beginning with the variable with the highest VIF. Forward selection was then used to determine the minimum set of environmental variables that could explain the largest amount of variance in the community. The statistical significance of an explanatory variable added in the course of forward selection was tested with the Monte Carlo permutation test (999 permutations, $p \leq 0.05$). In addition, correspondence analysis (CA) was carried out with CANOCO V4.5 to visualize the relationship of different stations with varied picocyanobacterial compositions.

Nucleotide sequence accession numbers

ITS sequences of all the clones obtained from this study were deposited in GenBank under accession numbers HQ849909 to HQ849928 for *Prochlorococcus* phylotypes and HQ849929 to HQ849994 for *Synechococcus* phylotypes.

RESULTS

Physical and biological characteristics of the 2 eddies

The cores of the 2 cold-core eddies (CE I and CE II) were located at ~111.75° E, 14.25° N (TS-1) and 111.5° E, 12.5° N (Y03), respectively (Fig. 1). Based on the satellite altimetry, CE I was in the decaying stage and CE II was in the intensifying stage (Hu et al. 2011). The locations of sampling stations relating to the 2 eddies are indicated in Fig. 1 as well as in Table 1. In addition, Y23 is influenced by the Mekong River plume (referred to as 'Plume' thereafter) and the Southeast Asia Time-Series Study (SEATS) station located at the deep central basin serves as an oligotrophic gyre control station.

The studied area was generally oligotrophic, with inorganic nitrogen (nitrate + nitrite) consistently below the detection limit (0.2 µmol l^{-1}) (Table 1). The 2 eddies were characterized by low temperature, high salinity and high nutrient concentrations compared with the SEATS station, and the DCM in the core of CE I was apparently shallower than that in the SEATS. Much higher phosphate and chl *a* concentrations were associated with the core of the 2 CEs, whilst phosphate concentration was comparable between Plume and surface water of SEATS. Salinity in CE II was lower than in CE I, because the former is located geographically closer to the Mekong River plume.

Prochlorococcus cell densities ranged from 0.68×10^5 to 4.16×10^5 cells ml^{-1} with higher abundance in CE I than CE II, and also higher abundance in the

Table 1. Physico-chemical characteristics of different water masses during the summer cruise in 2007 in the South China Sea. CE: cold eddy; Plume: Mekong River plume; SEATS: Southeast Asia Time-Series Study; Location: In = core of eddy, DCM = deep chlorophyll *a* maximum, Edge = margin of eddy, Out = outside eddy. Stations are shown in Fig. 1

Parameter	CE I				CE II			Plume	SEATS	
	In TS-1	DCM TS-1-DCM	Edge YS12	Out Y45	In Y03	Edge Y20	Out Y05	Y23	Surface	DCM
Latitude (°N)	14.25	14.25	14.75	15.75	12.50	11.50	12.50	11.50	18.30	18.30
Longitude (°E)	111.75	111.75	111.75	112.5	111.5	110	112.5	111.5	115.5	115.5
Sampling depth (m)	5	45	5	5	5	5	5	5	5	65
Temperature (°C)	27.60	20.27	28.90	29.40	28.40	28.40	29.30	32.50	29.60	22.58
Salinity (PSU)	34.10	34.48	34.10	33.40	33.30	33.90	32.80	32.50	33.8	34.45
Chl *a* (μg l^{-1})	0.17	0.46	0.15	0.07	0.17	0.12	0.10	0.14	0.10	0.32
SiO_3^{2-} (μmol l^{-1})	2.20	8.63	2.44	2.40	1.35	2.86	2.60	1.46	2.07	3.60
Phosphate (nmol l^{-1})	24	408	14	11	20	13	10	8	9	145
Prochlorococcus ($\times 10^5$ cells ml^{-1})	1.78	4.16	1.67	1.18	0.09	1.01	1.10	0.68	1.02	3.67
Synechococcus ($\times 10^4$ cells ml^{-1})	1.24	6.86	2.38	1.86	12.00	1.09	2.24	3.56	1.14	0.03

DCM layer compared to surface waters (Table 1). *Synechococcus* was generally less abundant than *Prochlorococcus* but with substantially higher variability in cell numbers between stations and with the highest abundance appearing in the core of CE II. The plume was characterized by high *Synechococcus* and low *Prochlorococcus* cell abundance, whilst very low *Synechococcus* cell numbers (300 cells ml^{-1}) were detected at the DCM (65 m) of the SEATS station. The total abundance of picocyanobacteria was much higher in the core of CE I than CE II, and the lowest appeared in the Plume.

Diversity and composition of ITS clone libraries

Ten independent ITS clone libraries based on water samples collected from different water masses were constructed and each library exhibited distinct picocyanobacterial community structures after RFLP screening (Table 2). Generally, the cores of each eddy showed the lowest diversity and highest coverage, compared with other water masses of the same eddy. Within CE I, the diversity increased from the core to edge and reached the highest at the Out station. In contrast, the highest diversity appeared at the Edge station in the CE II eddy. In terms of the numbers of OTUs and diversity, these indices were higher in the surface waters compared to those in the DCM at the SEATS station; the opposite pattern was found in CE I.

A total of 500 positive clones were examined from all ITS clone libraries, and 117 clones representing the distinct RFLP patterns were subjected to phylogenetic analysis. All ITS sequences recovered from this study were identified as either *Prochlorococcus* or *Synechococcus* after being subjected to a BLAST search of the GenBank database. Overall, *Prochlorococcus* (52% of total clones) were slightly more abundant than *Synechococcus* (48% of total clones).

Phylogenetic composition of picocyanobacteria

Maximum likelihood trees were constructed to reveal the detailed relationship between the obtained sequences and previously deposited sequences from the GenBank database. All of our phylotypes fell into known marine *Prochlorococcus* and *Synechococcus* clades (Figs. 2 & 3). Both high-light- and low-light-

Table 2. Composition and diversity analysis of all the clone libraries based on the sequences of the internal transcribed spacer (ITS). Operational taxonomic units (OTUs) were defined based on the restriction fragment length polymorphism pattern and also the 98% cutoff value of ITS sequence similarity. *H′*: Shannon-Weaver index; 1–*D*: Simpson's Index. See Table 1 for other abbreviations

Station	No. of clones examined	No. of clones sequenced	No. of OTUs	Coverage (%)	*H′*	1–*D*
CE I-In (TS-1)	50	10	7	86	0.46	0.14
CE I-DCM (TS-1-DCM)	50	10	9	82	0.97	0.18
CE I-Edge (YS12)	50	14	13	74	1.03	0.26
CE I-Out (Y45)	50	16	16	68	1.11	0.32
CE II-In (Y03)	50	6	3	94	0.44	0.06
CE II-Edge (Y20)	50	16	18	64	1.10	0.36
CE II-Out (Y05)	50	9	10	80	0.57	0.20
Plume (Y23)	50	12	12	76	0.96	0.24
SEATS-Surface	50	17	15	70	1.12	0.30
SEATS-DCM	50	7	6	88	0.80	0.12

adapted *Prochlorococcus* were recovered from our study falling into 3 ecotypes: HLI, HLII and LLIV (Fig. 2). The majority of our *Prochlorococcus* OTUs, 44 out of 51, belonged to the HLII ecotype; 2 OTUs recovered from In and Out of CEI clustered with HLI ecotype; 3 OTUs obtained from the DCM of SEATS and CEI and 2 from the surface of Stn Out of CEI grouped with the low-light-adapted *Prochlorococcus* strains MIT9313 and MIT9303 (LLIV).

Synechococcus exhibited more complex composition in this study. Clones were distributed into 6 clades of sub-cluster 5.1 (II, III, VII, X, XV and XVI) and each clade comprised OTUs recovered from both surface waters and the DCM (Fig. 3). In addition, 1 OTU from surface waters of the core of CEI grouped with *Synechococcus* WH8007 and fell into sub-cluster 5.2. The *Synechococcus* clade II was the major group, accounting for 39.4% of the total 66 sequences; 13 OTUs were closely related to clade XV, 10 fell into clade VII, and the rest were distributed between clades III, X and XVI.

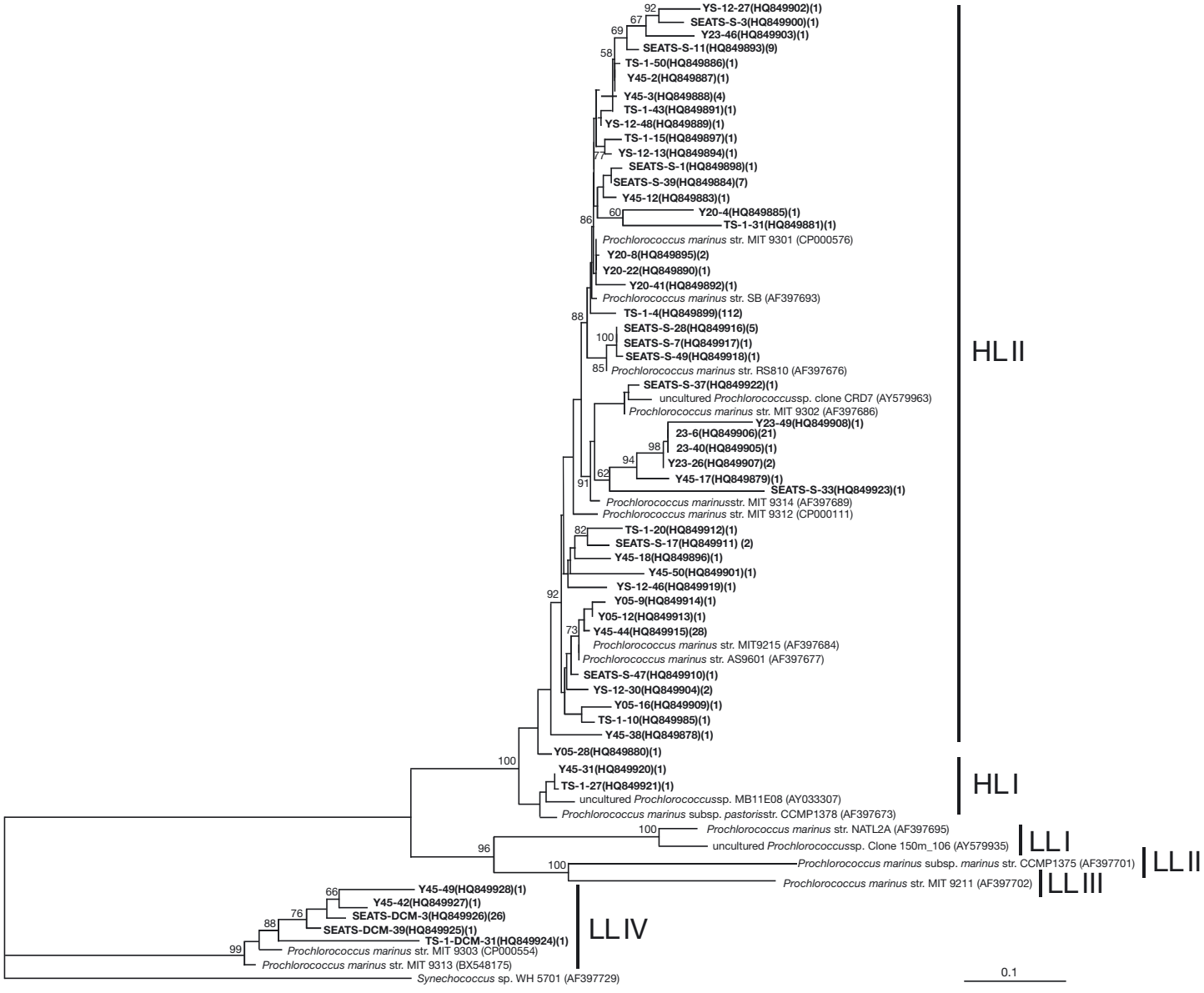

Fig. 2. Phylogenetic relationships among marine *Prochlorococcus* spp. based on maximum likelihood analysis of internal transcribed spacer (ITS) sequence data (ca. 1000 positions). Sequence codes in **bold** are derived from our study, and their abundance is indicated in parentheses; the prefixes denote stations where the sequences were obtained. The scale bar represents 0.1 nucleotide changes per position. High-light-adapted (HL) and low-light-adapted (LL) *Prochlorococcus* ecotypes are indicated

Distribution of picocyanobacteria in different water masses

A distinct composition of *Prochlorococcus* and *Synechococcus* communities was observed in the different water masses. HL II *Prochlorococcus* was the most abundant *Prochlorococcus* group in all samples with the exception of the DCM at the SEATS station, which was composed exclusively of LL IV ecotypes (Fig. 4a). However, whilst HL II OTUs dominated exclusively in the surface of both eddies, HL I and LL IV OTUs were also found in CE I, albeit in low proportions.

Synechococcus communities exhibited a more complex distribution pattern than *Prochlorococcus*. *Synechococcus* clade II was the only clade present in all water samples and was more abundant in the surface samples than in samples from the DCM (Fig. 4b).

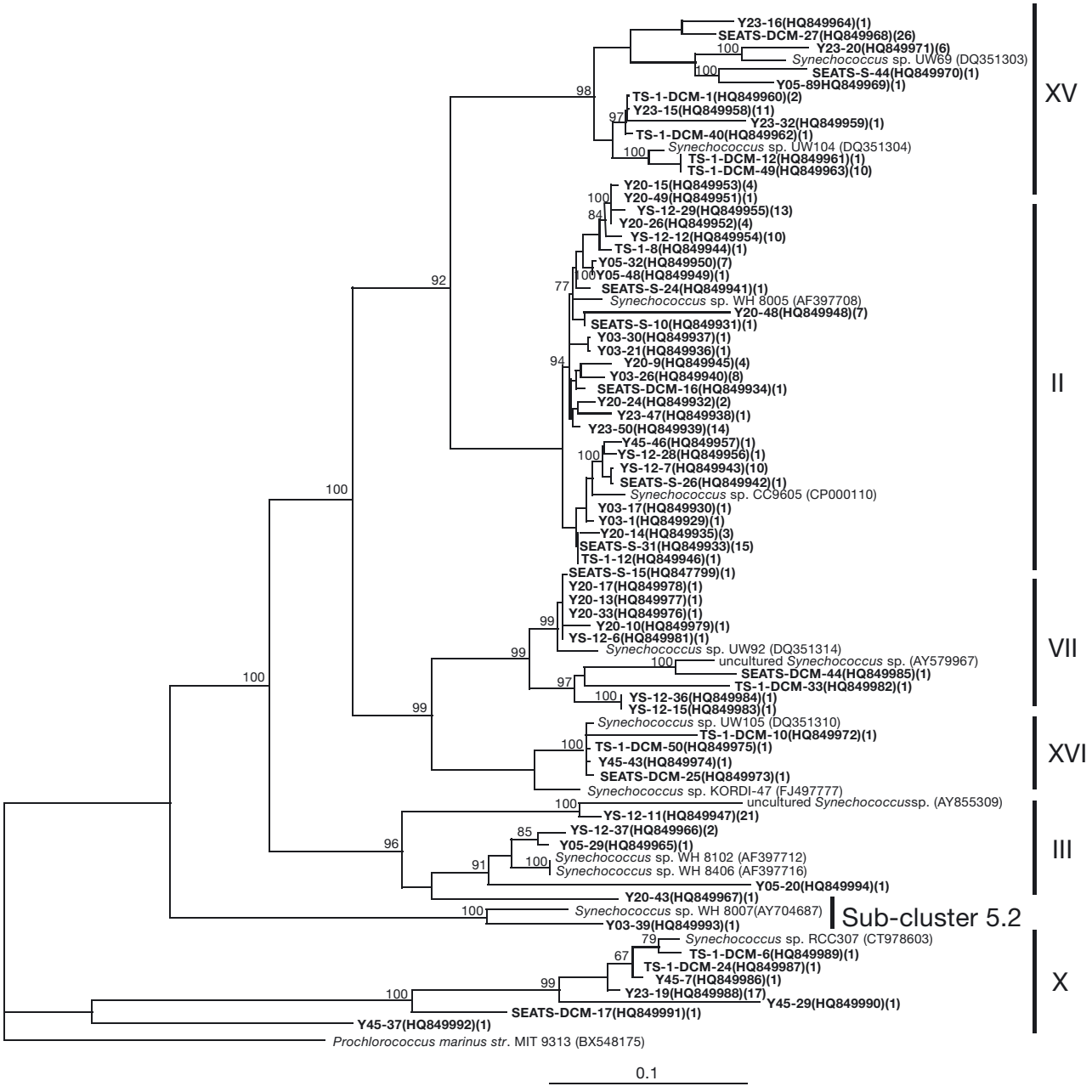

Fig. 3. Phylogenetic relationships among marine *Synechococcus* spp. based on maximum likelihood analysis of internal transcribed Spacer (ITS) sequence data (ca. 1160 positions). Roman numerals mark clades of *Synechococcus* sub-cluster 5.1. See Fig. 2 for further details

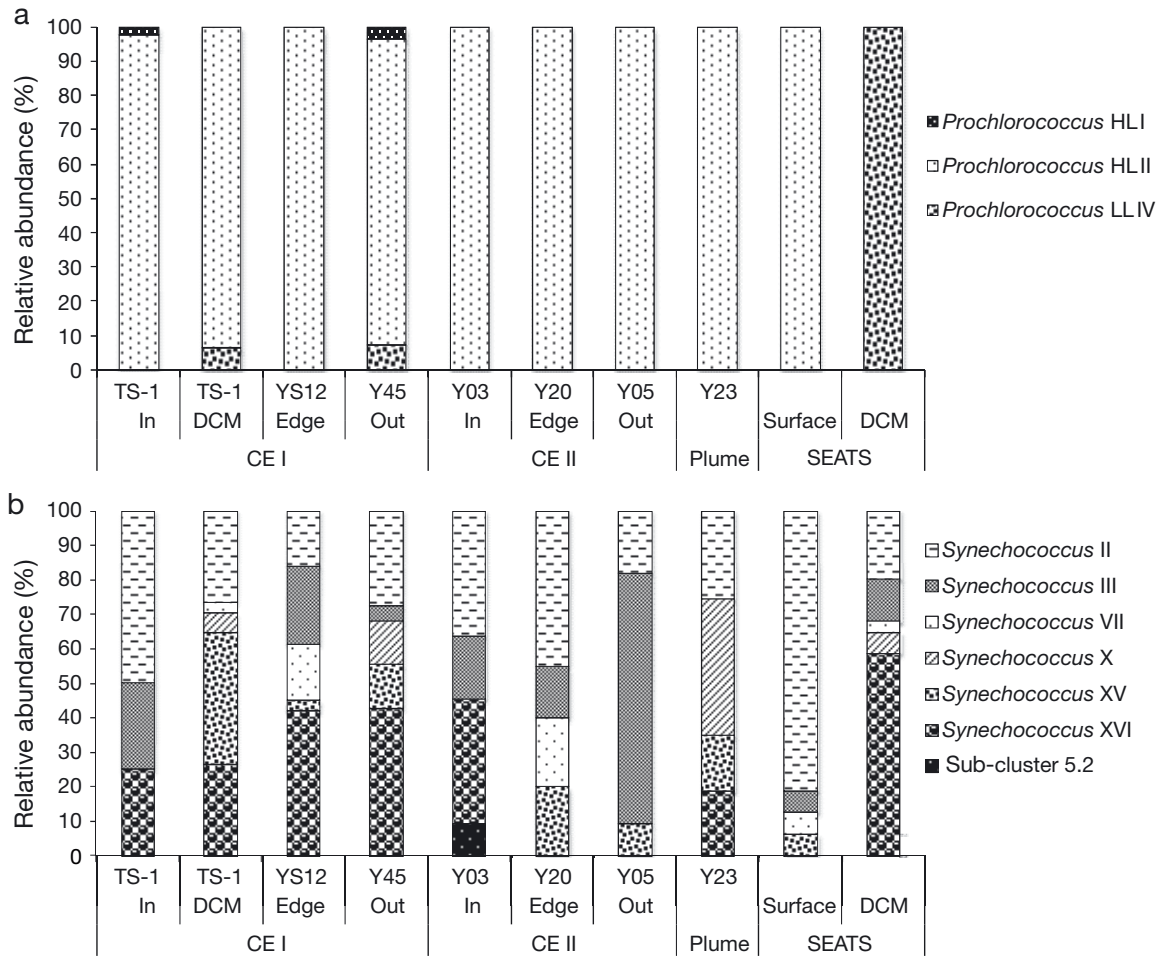

Fig. 4. Relative abundance of different phylogenetic groups of (a) *Prochlorococcus* and (b) *Synechococcus* at each station, reflected by the number of clones belonging to the 3 *Prochlorococcus* ecotypes and 7 *Synechococcus* clades in each clone library. CE: cold eddy; DCM: deep chlorophyll *a* maximum; SEATS: Southeast Asia Time-Series Study; HL: high-light-adapted; LL: low-light-adapted. See Table 1 for other abbreviations

The surface and DCM of the SEATS station was dominated by clade II and clade XVI OTUs, respectively. In contrast, clade XV was the major group at the DCM of CE I. Clade X was most abundant in water affected by the Mekong River plume and also occurred in low relative abundance in the 2 DCM samples. In the core of CE I, clade II had the highest relative abundance, whilst clade XVI became the most abundant phylotype in both the Edge and Out waters. Clades II and XVI were also the major groups in the core of CE II, whilst a significant increase in relative abundance of clade III was found outside CE II. OTUs of sub-cluster 5.2 were only detected in the Out waters of CE I and In waters of CE II.

Ordination analysis was carried out using the OTU matrix recovered from each station and the environmental variables listed in Table 1. After excluding factors with VIF > 20, 3 variables — chl *a*, temperature and salinity — were used for CCA. Temperature has close negative correlations with chl *a* (r = −0.9147) and salinity (r = −0.7790). According to the values of λ_A, which represent the variance that each variable explains in the model during forward selection, temperature (λ_A = 0.21) and chl *a* (λ_A = 0.15) explained more of the total variance than salinity (λ_A = 0.03) and were closely associated with the first (r = 0.8016) and the second (r = 0.8475) axes, respectively. The sum of all eigenvalues indicated an overall variance of 0.980 in the data set and the total variance that could be explained by environmental variation accounted for 0.689, as indicated by the sum of all canonical eigenvalues. The first axis represents 23.9% of the variance explained by the environmental variables. Species–environmental correlations were high, indicating a significant relationship between picocyanobacterial communities

and environmental variables. Biplot scaling of CCA based on the canonical axes 1 and 2 demonstrated the relationship between picocyanobacterial phylogenetic groups and environmental factors (Fig. 5): the 2 high-light-adapted *Prochlorococcus* groups clustered together, while LL IV *Prochoorococcus* showed its association with high salinity; *Synechococcus* groups showed a broader distribution and were influenced more by temperature and chl *a* (nutrient condition), with clade X located far from any other clades. In addition, CA exhibited the inter-sample relationship (Fig. 6): the picocyanobacterial community in Plume (upper right of Fig. 6), which was influenced by the freshwater discharge, was very distinct from those in other water masses; water samples from the DCM with high chl *a* and salinity, were separated from those collected in the surface; samples from the cores and edges of both CEs were closely distributed, but they were distant from those of Out waters.

DISCUSSION

Phylogeny and distribution of picocyanobacteria

Phylogenetic diversity amongst the 2 genera obtained from our study was similar to a previous report from the SCS based on *rpoC1* gene sequences, which showed that picocyanobacteria in both surface waters and at 80 m depth were composed of HL II *Prochlorococcus* and clade II *Synechococcus* (Ma et al. 2004). However, the considerable phylogenetic diversity within the 2 predominant clades, HL II *Prochlorococcus* and clade II *Synechococcus*, was augmented by the presence of HL I and LL IV *Prochlorococcus* genotypes and *Synechococcus* clades III, XV and XVI genotypes. These enhanced inter- and intra-clade diversities in response to eddy perturbations could enhance resistance or resilience to external perturbation (Hughes & Stachowicz 2004).

HL II *Prochlorococcus* was the predominate group in all our subtropical water samples with the exception of the DCM at SEATS, which was composed exclusively of the LL IV ecotype. This is consistent with the fact that HL II dominates subtropical and tropical regions whilst HL I is more abundant at temperate latitudes (Zwirglmaier et al. 2008). Unlike that of SEATS, the DCM of CE I, which was shallower (45 m) than that of SEATS (65 m) was dominated by HL II *Prochlorococcus*, presumably reflecting significant water mixing caused by the upwelling of the eddy. Similar mixing likely explains the presence of HL II *Prochlorococcus* genotypes extending their distribution to the base of the euphotic zone in the Red Sea (West et al. 2001, Zwirglmaier et al. 2008). Similarly, HL II *Prochlorococcus* was the only lineage

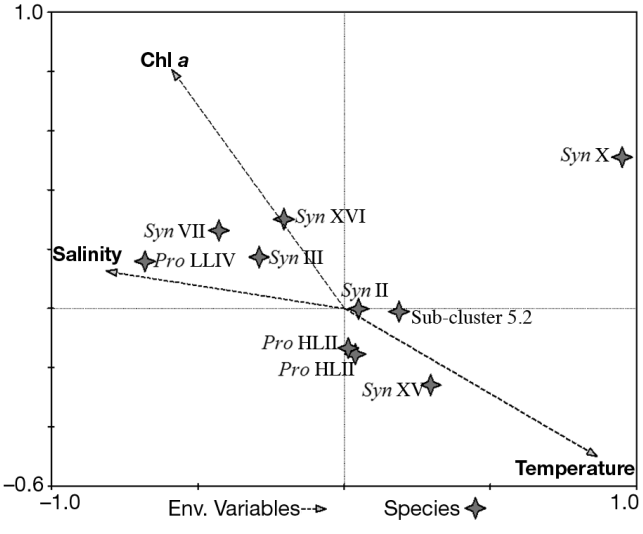

Fig. 5. Canonical correspondence analysis (CCA) biplot showing the relationship between different picocyanobacterial phylogenetic groups and environmental (env.) factors with the abiotic data set as explanatory variables. *Syn*: *Synechococcus*; *Pro*: *Prochlorococcus*; HL: high-light-adapted; LL: low-light-adapted; roman numerals identify clades of *Synechococcus*

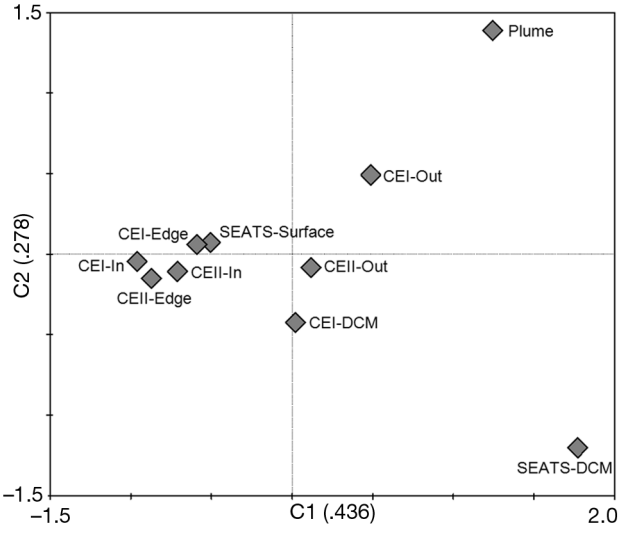

Fig. 6. Correspondence analysis (CA) biplot showing the distribution pattern of different sampling stations. Distances between station points give an indication of the similarity of picocyanobacterial taxonomic compositions in different water masses. CE: cold eddy; DCM: deep chlorophyll *a* maximum; SEATS: Southeast Asia Time-Series Study. See Table 1 for abbreviations

detected in both surface waters and at 80 m depth in the SCS in winter (Ma et al. 2004).

Synechococcus exhibited more dramatic population dynamics and diversity than *Prochlorococcus* in our study. Most of our sequences belong to *Synechococcus* sub-cluster 5.1, with only one sequence falling into sub-cluster 5.2. The latter contains mostly halotolerent strains isolated from coastal waters that possess phycocyanin but lack phycoerythrin; whilst the former is the dominant group within the euphotic zone of both open-ocean and coastal waters (Olson et al. 1990, Ferris & Palenik 1998) and have an elevated salt requirement for growth with phycoerythrin as their major light-harvesting pigment (Waterbury et al. 1986, Rocap et al. 2002).

The major *Synechococcus* clades detected in our study have been reported with distinct distributions by other investigators (Partensky et al 1999, Zwirglmaier et al 2008, Mazard et al 2011). Clade II is one of the most abundant and widely distributed clades that is found in greatest abundance in the upper euphotic zone of subtropical oceanic waters (Toledo & Palenik 2003, Zwirglmaier et al. 2008). This clade is always phylogenetically closely related to clade XV, which can use ammonium for growth (Ahlgren & Rocap 2006). The fact that clade II *Synechococcus* was the major component in all our samples is in agreement with its dominant distribution in subtropical/tropical latitudes. The presence of lineages with the capability of chromatic adaptation (clades III, XV and XVI), i.e. containing strains that are able to alter their relative ratio of accessory pigments, phycourobilin and phycoerythrobilin, according to the spectral quality of light they receive (Palenik 2001, Ahlgren & Rocap 2006), potentially reflects the highly dynamic environment induced by mesoscale eddies.

CCA analysis demonstrated that the community variations of both *Prochlorococcus* and *Synechococcus* in the different water masses in our study were related to temperature, salinity and chl *a*. This is consistent with the well-established finding that *Prochlorococcus* dominate in the subtropical oligotrophic oceans (Goericke & Welschmeyer 1993, Campbell et al. 1994), whilst *Synechococcus* are usually more abundant under intermediate nutrient conditions (Liu et al. 1997, 1998). Spatial distribution profiles of *Prochlorococcus* appear mostly influenced by physical parameters, such as light and temperature (Zwirglmaier et al. 2008, Jameson et al. 2010), but relationships to salinity have not been documented. The inclusion of a low-salinity plume and high-salinity upwelling waters in our study, forming a clear salinity gradient, suggests that salinity is an important environmental variable in this regard.

Effect of cyclonic eddies

Upwelling of cold-core eddies is very important in supplying nutrients to surface waters in oligotrophic oceans. The fact that chl *a* in the centers of both eddies was elevated above background levels indicates favorable *in situ* growth conditions for phytoplankton. Similar to what is found in the subtropical North Pacific gyre, *Prochlorococcus* are the most abundant autotrophic picoplankton in the South China Sea during summer (Liu et al. 2007). On the other hand, *Synechococcus*, which are able to respond quickly to nutrient pulses (Glover et al. 1988) and could increase their abundance along a nutrient gradient (Campbell et al. 1997, Cavender-Bares et al. 2001), were more abundant in the South China Sea during winter when the prevailing NE monsoon deepens the mixed layer depth (Liu et al. 2007). Our results show that the cores of CE I and CE II contained different abundances of *Prochlorococcus* and *Synechococcus*. This may reflect the fact that the 2 cold eddies were in different development stages; CE I was formed on 28 July and was in the decaying stage during our sampling period (24 to 28 August), whilst CE II was formed 1 mo later and was in the intensifying stage when we sampled there (3 to 7 September), according to the satellite altimeter data (Zhang et al. 2009, Hu et al. 2011). The biological responses are affected by a combination of the timing, magnitude and duration of nutrient inputs, which vary with eddy intensity and age (Benitez-Nelson & McGillicuddy 2008). The predominance of *Prochlorococcus* in CE I reflects the decaying stage of the eddy, while the dramatic increase of *Synechococcus* abundance in CE II indicates the acceleration of upward flux of inorganic nutrients from the deeper layer as a result of the formation of the eddy (Fig. 2). It is also possible that a parcel of water with traces of the river plume, as inferred by relatively low salinity, was wrapped in by CE II. The increased percentage of *Synechococcus* in the DCM layer relative to that in surface waters of CE I may be an indicator that the nutrient-rich subsurface water did not reach the surface as the eddy was in its decaying stage. On the other hand, the near-absence of *Synechococcus* in the DCM layer of SEATS can be simply explained by the fact that the depth of the layer was below the optimal growth depth of *Syne-*

chococcus. This depth is typically shallower than that of *Prochlorococcus* due to some major differences in their absorption of visible light resulting from their unique pigment composition — particularly in the enhanced ability to absorb green light by *Synechococcus* (Ting et al. 2002).

Although both eddy-induced upwelling and freshwater discharge injected nutrients to the surface mixed layer, they created distinct hydrographical conditions that can lead to distinct taxonomic composition of picocyanobacterial communities. The relatively higher percentage of *Synechococcus* in the plume-influenced water agrees with the general distribution pattern of high abundance of *Synechococcus* associated with coastal river plumes contrasting to *Prochlorococcus* dominated oligotrophic water (Paul et al. 2000, Liu et al. 2004). Higher proportions of potentially chromatically adapting genotypes within clades III, XV and XVI appeared in the CE I (50 to 68%), CE II (35 to 81%) and Plume (34%) compared to the oligotrophic basin station SEATS (12%). This supports the idea that chromatically adaptable strains could be better suited to the variable light environment and changing water chemistry inherent within the eddies.

Cyclonic eddies are biologically productive and can induce large variations in the biogeochemical characteristics of oligotrophic regions (McGillicuddy et al. 1998). The upwelling of deep nutrient-rich waters into the euphotic zone can trigger not only an increase in primary production but also a shift in microbial community structure. The absolute magnitude and rate of nutrient injection at any point within an eddy's life cycle may have a significant impact on the composition of the microbial community (Rii et al. 2008, Brown et al. 2008, Chen et al. 2011). Future studies should monitor the various developmental stages of eddies in order to elucidate upwelling effects on the water chemistry and any subsequent changes in microbial community structure.

Acknowledgements. The authors thank Xiamen University for providing the opportunity to attend the cruise. Thanks to Prof. Minhan Dai and Jianyu Hu for providing the hydrographic and nutrient data and Ms. Xihan Chen for collecting the DNA samples. Insightful comments and language editing provided by Dr. Eva Lindström and 2 anonymous reviewers greatly improved the quality of the paper. This work was supported by Hong Kong RGC (RGF grants 661809 and 661610) and the TUYF Charitable Trust (TUYF10SC08) to H.L. The support of the National Basic Research Program ("973" Program) of China through grant 2009CB421203 and the Natural Science Foundation of China through grant 40730846 is also acknowledged.

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Editorial responsibility: Eva Lindström, Uppsala, Sweden

Submitted: June 6, 2011; Accepted: November 9, 2011
Proofs received from author(s): January 17, 2012