

# Vertical structure of the phytoplankton community associated with a coastal plume in the Gulf of Mexico

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**ABSTRACT:** Low salinity plumes of coastal origin are occasionally found far offshore, where they display a distinct color signature detectable by satellites. The impact of such plumes on carbon fixation and phytoplankton community structure in vertical profiles and on basin wide scales is poorly understood. On a research cruise in June 1999, ocean-color satellite-images (Sea-viewing Wide Field-of-view Sensor, SeaWiFS) were used in locating a Mississippi River plume in the eastern Gulf of Mexico. Profiles sampled within and outside of the plume were analyzed using flow cytometry, HPLC pigment analysis and primary production using <sup>14</sup>C incorporation. Additionally, RubisCO large subunit (*rbcL*) gene expression was measured by hybridization of extracted RNA using 3 full-length RNA gene probes specific for individual phytoplankton clades. We also used a combination of RT-PCR/PCR and TA cloning in order to generate cDNA and DNA *rbcL* clone libraries from samples taken in the plume. Primary productivity was greatest in the low salinity surface layer of the plume. The plume was also associated with high *Synechococcus* counts and a strong peak in Form IA *rbcL* expression. Form IB *rbcL* (green algal) mRNA was abundant at the subsurface chlorophyll maximum (SCM), whereas Form ID *rbcL* (chromophytic) expression showed little vertical structure. Phylogenetic analysis of cDNA libraries demonstrated the presence of Form IA *rbcL* *Synechococcus* phylogenotypes in the plume. Below the plume, 2 spatially separated and genetically distinct *rbcL* clades of *Prochlorococcus* were observed. This indicated the presence of the high- and low-light adapted clades of *Prochlorococcus*. A large and very diverse clade of Prymnesiophytes was distributed throughout the water column, whereas a clade of closely related prasinophytes may have dominated at the SCM. These data indicate that the Mississippi river plume may dramatically alter the surface picoplankton composition of the Gulf of Mexico, with *Synechococcus* displacing *Prochlorococcus* in the surface waters.

**KEY WORDS:** RubisCO · Gene expression · Gulf of Mexico · *Prochlorococcus* · *Synechococcus* · Coastal plume · Recycled production · Microdiversity

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## INTRODUCTION

One of the key processes influencing the global carbon cycle and the balance between the organic and inorganic pools of carbon in the atmosphere and ocean is the rate of oceanic carbon sequestration. Carbon sequestration is the removal of carbon from the immediate carbon cycle resulting from transport and burial of organic and inorganic particulate carbon into sedi-

ments. Biotic and abiotic carbonate precipitation as well as photoautotrophic carbon fixation by phytoplankton have been implicated in this process (Karl et al. 1998, Legendre & Michaud 1998). Carbon burial is thought to be particularly important along ocean margins (Jahnke 1996). New primary production along coastlines, which is primarily the result of the availability of NO<sub>3</sub><sup>-</sup>, accounts for as much as 27 to 57% of total oceanic new primary production (Wollast 1993,

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Chavez & Toggweiler 1995). Large phytoplankton species with high sinking rates, particularly diatoms, are thought to be the most significant new producers and to mediate the sequestration of carbon into coastal sediments. In contrast, production in the oligotrophic ocean is thought to be mostly recycled through microbial loop-like processes. Diatoms are less numerous and picocyanobacteria, such as *Synechococcus* and *Prochlorococcus*, dominate the phytoplankton community, presumably because of their greater affinity for low concentrations of reduced and recycled forms of nitrogen (mainly ammonium).

The dominant source of nutrients to the coastal ocean is river discharge. The most important source of river-water in the Gulf of Mexico is the Mississippi River, which contributes as much as 90% of the total freshwater input in this environment. Nearshore nutrient laden plume waters are areas of intense new production, supporting large populations of diatoms and are virtually devoid of *Prochlorococcus* (Liu et al. 1999). Little is known however about the autotrophic picoplankton community associated with these highly productive offshore plume waters. In this paper, we have used molecular and traditional techniques to describe the diversity and activity of the photoautotrophic picoplankton community in the offshore Mississippi River plume, in order to gain insight into the biological processes at work in this environment.

The molecular techniques we have used focus on the diversity and expression of genes involved in photosynthetic carbon fixation (Pichard et al. 1993, 1997a,b, Paul et al. 2000a,b). The key enzyme in the Calvin-Benson-Bassham (CBB) reductive pentose phosphate pathway of photosynthetic carbon fixation in phytoplankton is ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO; Raven 1995, Tabita 1999). RubisCO is found in several forms distinguished by subunit assembly and biochemical properties (Tabita 1988, Watson & Tabita 1996, Watson et al. 1999). All eukaryotic algae (with the exception of Dinoflagellates), cyanobacteria and photosynthetic as well as chemoautotrophic proteobacteria have a Form I type enzyme (Tabita 1999). Form II of RubisCO is expressed by certain photosynthetic bacteria and some dinoflagellates, while Forms III and IV are found in Archaea and certain bacteria (Tabita 1999). Phylogenetic analysis of sequence information for the large subunit of RubisCO (*rbcL*) from Form I-containing organisms has revealed that there are 4 distinct lineages referred to as Forms IA, IB, IC and ID (Tabita 1995), respectively. Form IA *rbcL* is commonly found in nitrifying and sulfur oxidizing chemoautotrophic bacteria as well as some *Synechococcus* and all *Prochlorococcus* strains. Form IB is carried by most cyanobacteria and all green algae. Form IC is expressed by some photosynthetic

bacteria as well as hydrogen oxidizers, and Form ID *rbcL* is produced by a diverse group of algae including essentially all chromophytic eukaryotes (phaeophytes, rhodophytes, diatoms, prymnesiophytes, pelagophytes and several other groups).

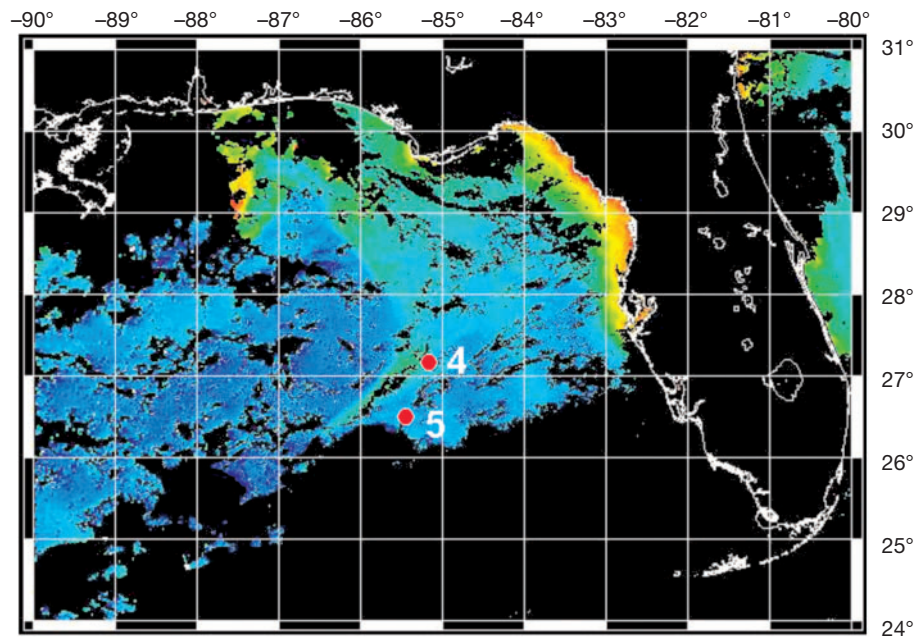
We have previously described a low salinity surface plume feature in the NE Gulf of Mexico in 1997 that we attributed to riverine input from coastal waters (i.e. the 'Green River'; Paul et al. 2000a). This feature showed a steep vertical stratification in the distribution of Form IA *rbcL* mRNA, but no other *rbcL* forms. To characterize a similar feature in detail, we used satellite imagery to locate a surface plume originating from the Mississippi River that had traversed far into the Gulf of Mexico. The purpose of this study was to compare the vertical structure of the phytoplankton community within and outside this plume using flow cytometry and *rbcL* gene expression. A second goal was to describe the diversity transcriptionally active *rbcL* phylotypes present in a profile of the plume site to determine the identity of the photosynthetically active members of the phytoplankton community.

## MATERIALS AND METHODS

**Sampling.** Sampling was performed aboard the RV 'Pelican' in the SE Gulf of Mexico on June 14 and 15, 1999 (Stn 4: 27° 7.92' N, 85° 2.55' W; Stn 5: 26° 32.00' N, 85° 30.00' W) (Fig. 1). The sampling site was chosen by following the movement of a high chlorophyll plume resulting from Mississippi River discharge between June 8 and 13 using SeaWiFS satellite images. The low salinity feature associated with the surface absorbance (Fig. 1) was located using the onboard MIDAS continuous sampling system. Seawater was sampled during early morning hours using a rosette of Niskin bottles attached to a Seabird CTD. Samples from greater than 40 m of depth were protected from light by wrapping carboys in black plastic bags during filling and sample processing. Sampling depths were as follows: 3, 10, 40, 60, 78, 86, 100 and 130 m below surface.

***rbcL* mRNA analysis.** Bulk mRNA was extracted from seawater using RNeasy spin columns (Qiagen) as described by Paul (2001). Eight hundred ml seawater samples were treated with 0.1% v/v DEPC (Di-Ethyl-Pyrocabonate; Sigma) and immediately filtered onto 25 mm, 0.45 µm HV polyvinylidene difluoride filters (Millipore Durapore). Filters were then transferred to 2.0 ml screw cap tubes containing 0.1 mm muffled glass beads (Biospec Products) and 750 µl RLT lysis buffer (Qiagen). Tubes were then frozen and stored in liquid nitrogen for processing in the lab. Cells were lysed by bead-beating and 550 µl lysate was then extracted following the RNeasy kit (Qiagen) protocol.

Fig. 1. Chlorophyll *a* (chl *a*) concentration patterns derived from the Sea-viewing Wide Field-of-view Sensor (SeaWiFS) over the eastern Gulf of Mexico in June 1999. Blue colors indicate low concentrations, and green, yellow and red indicate increasing concentrations. Concentrations near the Mississippi River Delta and in the river plume as well as in other coastal areas are suspect because of known effects by high concentrations of colored dissolved organic matter, suspended sediments or bottom reflectance, which can artificially raise chl *a* estimates. Land and clouds are colored black. Station locations are indicated



One third of the extract remained undigested on ice, while the other 2/3 were digested with DNase-free RNase or RQ1-DNase. Samples were then dot-blotted onto Zeta-probe charged nylon filters (Bio-Rad). The filters were dried and RNA was immobilized by UV-crosslinking. Duplicate samples were probed with Forms IA, IB and ID probes. The Form IA *rbcl* probe was derived from *Synechococcus* WH7803 (Watson & Tabita 1996). Form IB and ID *rbcl* probes were derived from *Synechococcus* PCC6301 and *Cylindrotheca* sp. N1, respectively (Paul et al. 1999). Radiolabeled probes were made by *in vitro* transcription of these genes using  $^{35}\text{S}$ -UTP. Dot blots were quantified with a BioRad model GS363 molecular imager using standard curves made from *in vitro* transcripts generated by transcription of the same *rbcl* clones used to make the ribo-probes.

**Flow cytometry.** One ml samples were fixed with 20  $\mu\text{l}$  10% para-formaldehyde at room temperature for 10 min and frozen in liquid nitrogen on board until analyzed in the lab. *Prochlorococcus*, *Synechococcus* and pico-eucaryotic algal populations were then quantified using a Becton Dickinson FACSCalibur flow cytometer equipped with a 488 nm, 15 mW argon laser. Forward angle light scatter, right angle light scatter, green (530  $\pm$  30 nm), orange (585  $\pm$  30 nm) and red (650  $\pm$  30 nm) fluorescence parameters were collected for each event. Purple-yellow calibration beads (2.2  $\mu\text{m}$ , Spherotech) were added to each sample to permit normalization of all fluorescence signals. Data were collected using CellQuest<sup>TM</sup> software (V. 3, Becton Dickinson 1996), transferred to a PC and analyzed using CYTOWIN software (Vaultot 1989,

[www.sb-roscoff.fr/Phyto/cyto.html#cytowin](http://www.sb-roscoff.fr/Phyto/cyto.html#cytowin)). Event rates were recorded for each sample and abundances were corrected for volume analyzed and enumeration efficiency factor. The efficiency factor was derived from event rate and counts for series of known concentrations of calibration beads. For a brief review on flow cytometric methodology, see Campbell 2001.

**HPLC pigment analysis.** Four l seawater samples were filtered in duplicate through 2.5 cm Whatman GF/F glass fiber filters. The filters were folded in half, wrapped in aluminum foil, then immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . Extractions were performed using acetone and analyzed by B. Pedersen, Mote Marine Lab, using the method of Millie et al. (1993).

**Chlorophyll *a* analysis.** Samples for chlorophyll *a* (chl *a*) were filtered onto 25 mm Whatman GF/F glass fiber filters, frozen in liquid nitrogen and stored at  $-20^{\circ}\text{C}$  in the dark until extraction. Triplicate samples were extracted with methanol and the chl *a* concentration was determined fluorometrically (Holm-Hansen & Riemann 1978).

**$^{14}\text{C}$ -carbon fixation.**  $^{14}\text{C}$ -carbon fixation was measured as described by Carpenter & Liveley (1980) using sterile, acid cleaned 500 ml polycarbonate flasks and 325 ml water samples. Flasks were incubated under natural irradiance that was adjusted to resemble the intensity and spectral features of the underwater light field using neutral-density screening and colored acetate filters. Irradiance intensity as a function of depth was determined by use of a Li-Cor light meter equipped with Li-190SA and Li-192SA surface and underwater photosynthetically active radiation sen-

sors. Water column productivity was integrated for 1 m<sup>2</sup> of surface ocean as follows. The water column was divided into segments bounded by the surface, the half-way points between each pair of adjacent sampling depth and the depth of the bottom sample (Stn 4 intervals: 0–5, 5–25, 25–50, 50–69, 69–82, 82–93, 93–115 and 115–130 m; Stn 5 intervals: 0–10, 10–30, 30–50, 50–67.5, 67.5–78.5, 78.5–91, 91–115 and 115–130 m). Primary productivity measurements taken within each interval were then extrapolated to the volume of the segment and all estimates for the water column segments were then added.

**DNA sampling and extraction for PCR.** Eight hundred ml of seawater was filtered onto 25 mm, 0.45 µm polyvinylidene difluoride filters (Milipore Durapore) and stored in 2 ml screw cap tubes in 1 ml STE (0.1 M NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) in liquid nitrogen. Extraction was performed by boiling lysis in STE and 1% SDS as in Pichard et al. (1993). *Prochlorococcus* cells were provided by Dr. F. Partensky and Ms. F. Le Gall (Strains NATL1, NATL2B, SS120, TAKA9803-2 and SB) and Dr. L. Campbell (Strain PAC1) as frozen cell pellets. Pellets were resuspended in 1 ml STE and extracted as described above.

**RNA extraction for RT-PCR.** Samples of 800 ml seawater were filtered onto 25 mm, 0.45 µm polyvinylidene difluoride filters (Millipore Durapore) and extracted as indicated above. Samples were not treated with DEPC due to its interference with the RT-PCR reaction. Samples were DNase digested on the RNeasy columns for 15 min using the RNase-free DNase reagent set (Qiagen) according to the protocol provided by Qiagen in order to remove contaminating DNA from RNA preparations. To show that DNase-digested RNA was not contaminated with DNA, a digestion control was run. A crude lysate was split equally and individual aliquots were left undigested, DNase, RNase or RNase + DNase digested. Extracts were then RT-PCR amplified to show purity of extracted RNA.

**RT-PCR.** Reverse transcriptions were performed with freshly extracted RNA using random hexamers, 4.7 mM MgCl<sub>2</sub> and M-MLV reverse transcriptase (Promega) for 30 min at 37°C. Five µl of the reaction was then added to a PCR reaction such that a final volume of 100 µl was attained. The 2 primer sets used to amplify the *rbcL* fragments were the Form IA/B primer set (615 bp fragment; forward primer: TCIGCITGRAACTAYGGTCG, reverse primer: GGCA TRTGCCAIACRTGRAT) and Form ID set (554 bp fragment; forward primer: GATGATGARAAYATTA ACTC, reverse primer ATTTGDCACAGTGDATACCA). Primer sequences are stated using IUPAC degeneracies. The final primer concentration in all PCR reaction mixtures was 1 µM for both primers. The MgCl<sub>2</sub> final concentration was 1.5 mM and all nucleotides were added to a final concentration

of 2.5 mM each. Five U TAQ polymerase (Promega) was used per reaction tube. Cycle parameters were as follows: 3 min at 95°C; 40 cycles of 1 min at 95°C, 1 min at 52°C and 1 min 30 s at 72°C. Cycling was followed by a 15 min 72°C elongation step.

**PCR of *Synechococcus*-like clones.** During the course of these experiments, we discovered that our Form IA/B primers (Paul et al. 2000b) would not amplify *Synechococcus rbcL* sequences. No *Synechococcus*-like clones were obtained in any of our cDNA libraries, despite the high abundance of these organisms. Using recently deposited sequence information in GenBank, a new Form IA reverse primer was designed to detect *Synechococcus*-like genotypes: CTGAGIGGIAARAACTACGG. Using this new reverse primer, we obtained a 455 bp amplicon, which overlaps in its entirety with the amplicons from the other 2 previously described primer sets. DNA was extracted as described above and 5 µl DNA extract was added to 100 µl reaction mixture. RT-PCR was performed as in the 'RT-PCR' subsection.

**Cloning and screening of clone libraries.** *rbcL* mRNA was RT-PCR amplified from 8 different depths of Stn 4 using the Form ID and Form IA/B primer sets. Stn 4 surface water DNA was later amplified with the modified cyanobacterial reverse *rbcL* primer. *Prochlorococcus* DNA (Strains NATL1, NATL2B, SB, SS120, TAKA9803-2 and PAC1) was amplified with the Form IA/B primer set. Immediately after amplification, the amplicons were purified using the QIAquick PCR purification kit (Qiagen). Amplicons were then ligated into pCR<sup>®</sup> 2.1 vector using a TA cloning kit (Invitrogen) according to the protocol provided by the manufacturer. All white colonies were then picked and streaked onto individual 2 × YT plates containing 50 µg ml<sup>-1</sup> kanamycin and 50 µg ml<sup>-1</sup> ampicillin. To screen for the presence of clones with the correct insert size, clones were PCR amplified by touching a colony with a sterile loop and transferring this loop to a PCR reaction tube. PCR reaction mixture and parameters were as described above. Amplifications were then run on 1.5% agarose gels in 1 × TAE buffer stained with ethidium bromide. Amplifications with products of the correct size were purified using a QIAquick PCR purification kit (Qiagen) and taken up in 50 µl deionized water. Twenty µl purified product were then digested with 5 U *Sau3IA* (digests at ↓GATC↑) and 5 U *AluI* (digests at AG↓↑CT) in Buffer B (Promega) for 30 min at 37°C. Digestions were run on 2.5% low melting agarose gels (Fisher) at 13.5 V cm<sup>-1</sup> for 2 h and stained with ethidium bromide. Gels were photographed and clones with unique restriction patterns were later visually selected for sequencing. Individual colonies from plates of selected clones were then picked into 5 ml 2 × YT media containing 50 µg ml<sup>-1</sup> kanamycin and 50 µg ml<sup>-1</sup> ampicillin, and grown for



16 h. Plasmid DNA was then extracted using a Wizard<sup>®</sup> SV plasmid mini kit (Promega) and sequenced in an Applied Biosystems model 373 sequencer by the University of Florida core sequencing laboratory (University of Florida, Gainesville, FL).

**Determination of PCR error.** Two clones (P99FH2 and P99HH25) were grown in 500 ml 2 × YT media for 16 h and plasmid was extracted using a Qiagen plasmid kit. One ng plasmid DNA for both clones was then added to individual PCR reactions and the *rbcL* insert was amplified. Amplified DNA was then purified using a QiaQuick (Qiagen) PCR purification kit. Amplicons were then ligated into the pCR<sup>®</sup> 2.1 vector using a TA cloning kit (Invitrogen) according to the protocol provided by the manufacturer. From FH2 and HH25 plates, 10 and 12 colonies were then picked, respectively, the plasmid was extracted and the insert sequenced. Sequences obtained were then aligned using Omega 1.1 (Oxford Molecular Group) and PCR and sequencing errors were counted as deletions, insertions or differences in sequences from the original clones.

**Phylogenetic analysis.** Deduced amino acid sequences were aligned with a representative sample of *rbcL* sequences obtained from GenBank, using Omega 1.1, which uses Clustal W, a pairwise, weighted alignment method. Amino acid sequences were used as third codon positions were saturated, and also in order to avoid potential biases introduced by codon usage and GC content. Alignments were then corrected manually for obvious misalignments and exported to Mega 2.0 beta (Kumar et al. 1993). Phylogenetic analysis was then performed using parsimony and the neighbor-joining method using a gamma distribution (gamma parameter = 2.0) to correct for rate heterogeneity across sites. Accession numbers for the clones obtained here are as follows: AF381648 to AF381753 and AY042086 to AY042090.

**Area estimates.** To estimate the area of the plume, a 7 d composite of level 3 SeaWiFS images was used. The June 8 composite was imported into a graphics editor and the plume was first outlined by hand and then shaded in white. All non-plume pixels were eliminated by shading them black. All white pixels were then counted by importing the image into Matlab (Mathworks). Because level 3 SeaWiFS images are mapped to a resolution of 1 × 1.1 km, an area estimate can be derived. In a similar fashion, an area estimate for the total oligotrophic Gulf of Mexico (oGOM) as well as the eastern portion of the oGOM was obtained. The Gulf of Mexico was bounded by a line from the tip of Yucatan to Key West and all pixels south of this line were excluded from area estimates. For the eastern portion of the oGOM, a second line was drawn from Yucatan to the Mississippi delta and the portion of the Gulf to the west of this line was excluded. In both

cases, the desired area was then shaded in white, excluding high chlorophyll coastal regions and the total number of white pixels was counted in Matlab.

## RESULTS

A SeaWiFS ocean color satellite image of the northern Gulf of Mexico taken on the day of sampling is shown in Fig. 1. The image depicts a high chl *a* plume originating at the Mississippi delta. This plume was estimated to extend over approximately 26 800 km<sup>2</sup> covering ca. 2% of the total area of the oligotrophic Gulf of Mexico and ca. 6% of the eastern portion. Fig. 2 shows salinity, carbon fixation and *in situ* fluorescence traces of a station within (Stn 4, Fig. 2A) and outside the plume (Stn 5, Fig. 2B). The salinity at Stn 4 varied between 36 and 36.5 ppt throughout most of the euphotic zone. In the uppermost 10 m, however, we observed a well defined low salinity feature (33.5 ppt; Fig. 2A) resulting from the Mississippi plume. The most prominent feature of the CTD trace at Stn 4 (Fig. 2A) is the bimodal distribution of *in situ* fluorescence. The subsurface chlorophyll maximum (SCM) occurred between 80 and 100 m of depth and an equally prominent peak in fluorescence occurred in the low salinity surface layer. No such increased surface *in situ* fluorescence was observed at Stn 5 (Fig. 2B).

Photosynthetic carbon fixation at Stn 4 was also bimodal, with a surface maximum of 3.39 μg C l<sup>-1</sup> h<sup>-1</sup> and a second, much smaller peak at the SCM (86 m sample) of 0.59 μg C l<sup>-1</sup> h<sup>-1</sup> (Fig. 2A). There was no similar surface peak in primary production at Stn 5 and highest primary productivity of 2.05 μg C l<sup>-1</sup> h<sup>-1</sup> occurred in the 82 m, i.e. the SCM sample (Fig. 2B). Integrated water column primary productivity was estimated at ca. 63.1 mg C h<sup>-1</sup> m<sup>2</sup> for Stn 4 and ca. 83.7 mg C h<sup>-1</sup> m<sup>2</sup> for Stn 5. Light intensity measurements indicated that the ratio of subsurface to surface light intensity ( $I_z/I_0$ ) was greater at Stn 5 than 4 below 10 m, the average difference ranging from 13.6 to 33.9% (data not shown). Thus, the plume may have shaded the lower water column, decreasing its rate of carbon fixation.

Flow cytometry data for Stns 4 and 5 are shown in Fig. 3A and B, respectively. *Prochlorococcus* was completely absent in the surface plume of Stn 4, but was more than 10-fold more abundant than *Synechococcus* in surface samples from Stn 5. At Stn 4, *Prochlorococcus* was most abundant between 10 and 80 m of depth with a maximum cell count of 1.7 × 10<sup>5</sup> cells ml<sup>-1</sup> in the sample from 40 m. *Prochlorococcus* counts below 20 m of depth (below the low salinity plume) were of similar magnitude for both stations and their profiles correlated well to each other by paired *t*-test analysis (*t* =

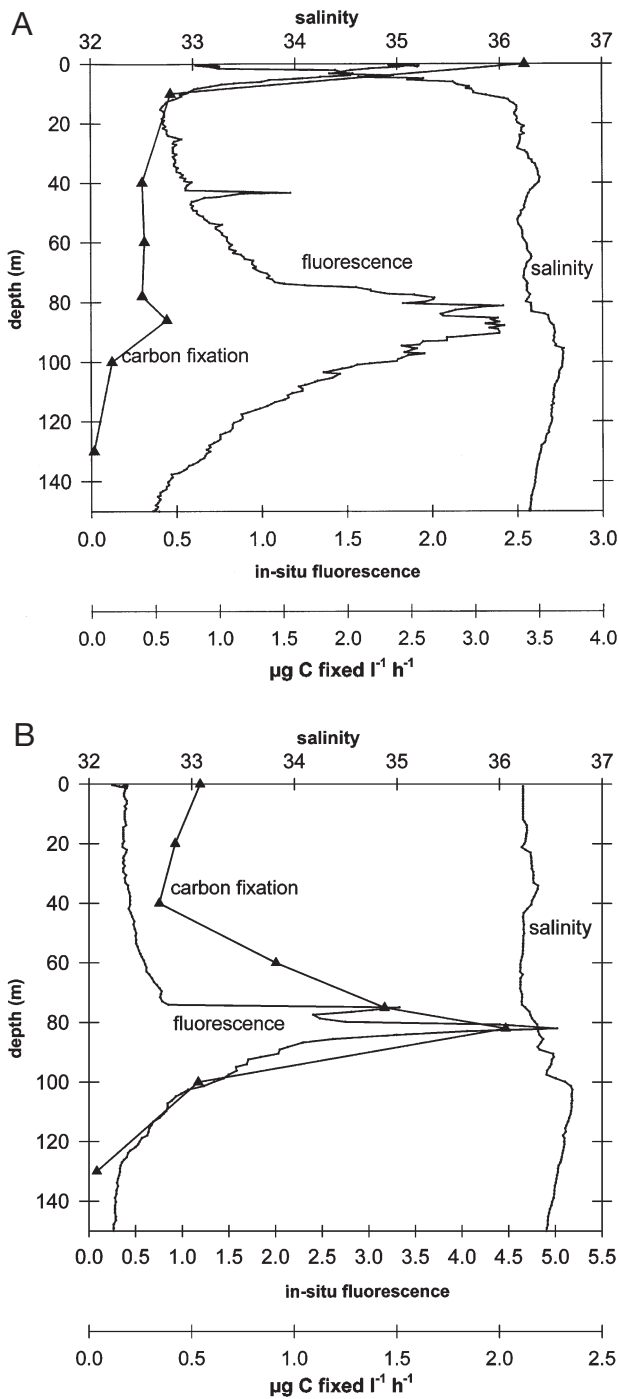


Fig. 2. CTD trace of (A) Stns 4 and (B) 5. Shown are carbon fixation (▲), in situ fluorescence and salinity

-0.20,  $p = 0.85$ ). *Synechococcus* counts in surface plume samples from Stn 4 were ca. 5-fold higher than counts obtained from Stn 5 surface samples. In the lower water column, *Synechococcus* counts reached a second maximum just above the SCM at both stations and then decreased. *Synechococcus* profiles of the 2

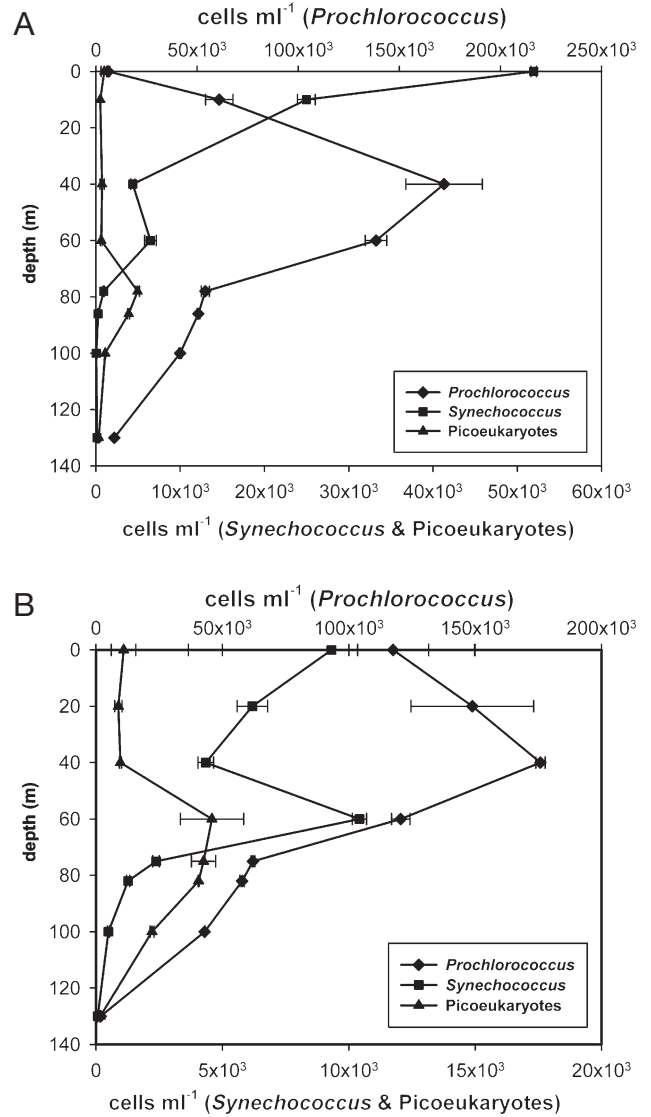


Fig. 3. Flow cytometry of (A) Stns 4 and (B) 5. *Synechococcus* (■), *Prochlorococcus* (◆), Picoeukaryotes (▲). *Synechococcus* is abundant in the plume surface feature, while *Prochlorococcus* is found below

sites did not correlate well to each other using paired  $t$ -test analysis ( $t = 1.8$ ,  $p = 0.13$ ). Picoeukaryotes were the least numerous picoplankton fraction and reached their highest abundance at the SCM at both stations ( $4.9 \times 10^3$  and  $4.6 \times 10^3$  cells  $ml^{-1}$  at Stns 4 and 5, respectively).

HPLC pigment data for Stn 4 are shown in Fig. 4. Divinyl-chl *a*, a pigment diagnostic of *Prochlorococcus*, was absent from the low salinity surface plume corroborating the observation made by flow cytometry that *Prochlorococcus* was absent there. Below the plume, the divinyl-chl *a* concentration increased with depth and reached a maximum just above the SCM. Highest cell counts of *Prochlorococcus* were observed at slightly shall-

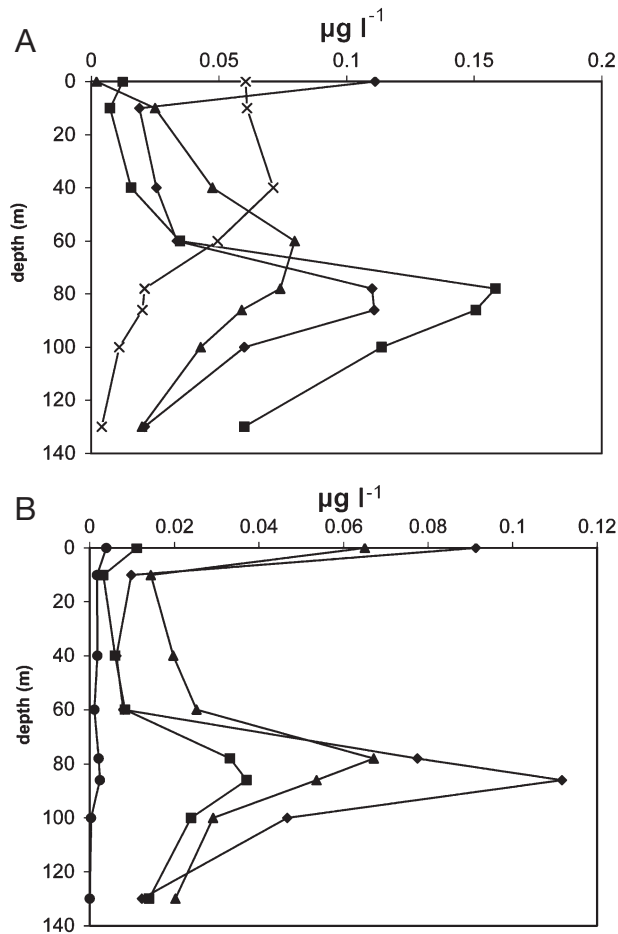


Fig. 4. Pigment data from Stn 4. (A) Divinyl-chlorophyll  $a$  ( $\mu\text{g l}^{-1}$ ,  $\blacktriangle$ ), chl  $b$  ( $\mu\text{g l}^{-1}$ ,  $\blacksquare$ ), prasinophytes, chlorophytes, euglenophytes), zeaxanthin ( $\mu\text{g l}^{-1}$ ,  $\times$ , *Synechococcus*, *Prochlorococcus*), chls  $c_1$  and  $c_2$  ( $\mu\text{g l}^{-1}$ ,  $\blacklozenge$ , diatoms, prymnesiophytes, dinoflagellates). (B) Violaxanthin ( $\mu\text{g l}^{-1}$ ,  $\bullet$ , eustigmatophytes), fucoxanthin ( $\mu\text{g l}^{-1}$ ,  $\blacklozenge$ , diatoms and prymnesiophytes), 19'-butanoyloxyfucoxanthin ( $\mu\text{g l}^{-1}$ ,  $\blacksquare$ , prymnesiophytes and chrysophytes), 19'-hexanoylfucoxanthin ( $\mu\text{g l}^{-1}$ ,  $\blacktriangle$ , prymnesiophytes only)

lower depth (40 m) than the divinyl-chl  $a$  maximum (60 m). If the divinyl-chl  $a$  concentrations are divided by *Prochlorococcus* cell counts (Table 1, Column 3), cells between 78 and 120 m of depth are observed to contain significantly more ( $t = 3.86$ ,  $p = 0.008$ ) divinyl-chl  $a$  than cells from shallower depth, explaining this discrepancy. While this observation could potentially be the consequence of photoacclimation by *Prochlorococcus*, *rbcl* sequence data (see below) indicate the presence of 2 ecotypes of *Prochlorococcus*, 1 adapted to high light intensities and 1 adapted to lower light flux.

Zeaxanthin is found in both *Synechococcus* and *Prochlorococcus* and was found in surface to mid-depth samples, but decreased where divinyl-chl  $a$  reached its maximum. Zeaxanthin was almost absent at and below the SCM. Chl  $b$ , an indicator of chlorophytes (particularly Prasinophytes), as well as chls  $c_1$  and  $c_2$ , which are found in many chromophytic algae, were bimodally distributed, displaying one maximum at the surface and the second at the SCM (Fig. 4A). Fucoxanthin, produced by diatoms and prymnesiophytes, as well as 19'-hexanoylfucoxanthin, produced by both prymnesiophytes and chrysophytes, exhibited a similar bimodal distribution (Fig. 4B). At Stn 5 (data not shown), no such bimodal distributions were observed. Chl  $b$ , chls  $c_1$  and  $c_2$ , divinyl-chl  $a$ , 19'-hexanoylfucoxanthin as well as 19'-butanoyloxyfucoxanthin only displayed 1 peak at the SCM. Zeaxanthin was very similarly distributed at both stations.

Profiles of Forms IA, B and D *rbcl* mRNA were quantified at Stns 4 and 5 (Fig. 5A and B, respectively). Concentrations of Form IA *rbcl* mRNA were bimodally distributed with depth at both stations. However, while the shallow and deep maxima at Station 5 were of similar magnitude, surface Form IA *rbcl* concentrations at Stn 4 were twice as high as concentrations observed at the deeper maximum and almost 10-fold higher than both Form IB or ID concentrations. At Stn 4, Form ID *rbcl* mRNA was present at all depths (Fig. 5A) and its highest abundance was observed at the SCM. At Stn 5, Form ID transcript could only be detected at and below the SCM (Fig. 5B). The Form IB *rbcl* transcript profile at Stn 4 was bimodal and concentrations were highest in the surface and SCM samples. Form IB *rbcl* was not detected at 60 m, which coincided with the highest concentrations of *Prochlorococcus* observed. At Stn 5, Form IB *rbcl* mRNA was detected at all depths with notable minimum at the SCM.

Table 1. Cellular pigment content at Stn 4. Bulk chlorophyll  $a$  (chl  $a$ ;  $\mu\text{g l}^{-1}$ ) and cellular pigment content ( $\text{fg cell}^{-1}$ ) of divinyl-chlorophyll  $a$  (Div-chl  $a$ ) in *Prochlorococcus* (Pro), fucoxanthin (Fuco) plus 19'-hexanoylfucoxanthin (Hex) in picoeukaryotes (Picoeu), 19'-butanoyloxyfucoxanthin (19'-but) in picoeukaryotes and Zeaxanthin in *Synechococcus* (Syn)

Depth (m)	Bulk chl $a$ ( $\mu\text{g l}^{-1}$ )	Div-chl $a$ ( $\text{fg Pro}^{-1}$ )	Fuco + Hex ( $\text{fg Picoeu}^{-1}$ )	Zeaxanthin ( $\text{fg Syn}^{-1}$ )	19'-but ( $\text{fg Picoeu}^{-1}$ )
0	0.254	0.33	157.48	1.16	11.23
10	0.105	0.41	46.53	2.45	6.26
40	0.119	0.28	35.66	16.27	8.22
60	0.200	0.58	52.28	7.67	13.06
78	0.296	1.37	29.22	22.47	6.7
86	0.401	1.16	42.54	75.52	9.55
100	0.306	1.03	68.56	196.62	21.68
130	0.097	2.19	96.8	31.2	41.88

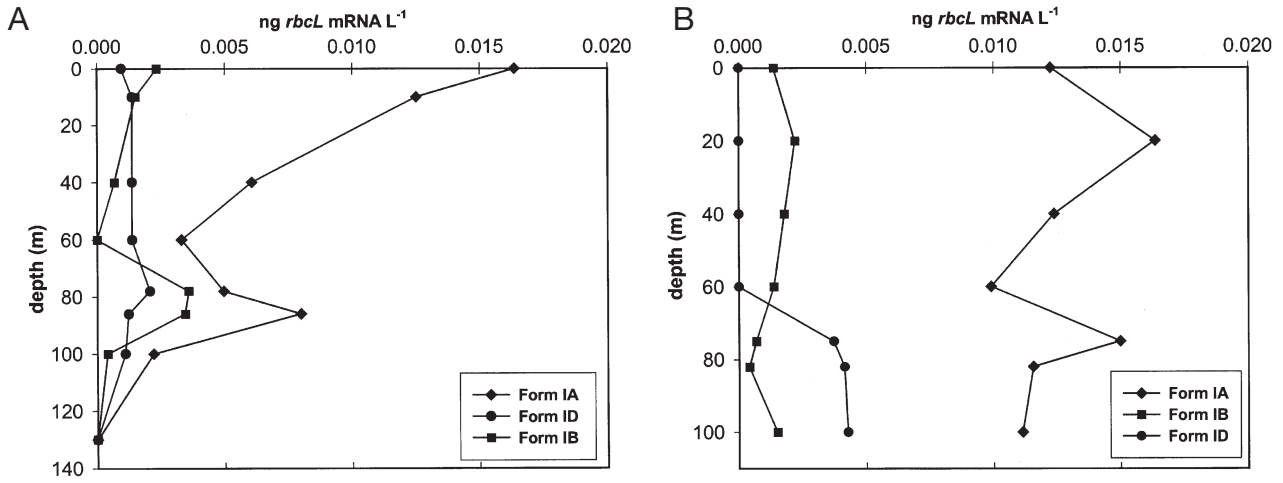


Fig. 5. *rbcL* mRNA expression at Stns (A) 4 and (B) 5. Form IA (ng l<sup>-1</sup>, —◆—, *Prochlorococcus* and *Synechococcus*), Form IB (ng l<sup>-1</sup>, —■—, green algae), Form ID (ng l<sup>-1</sup>, —●—, chromophytes)

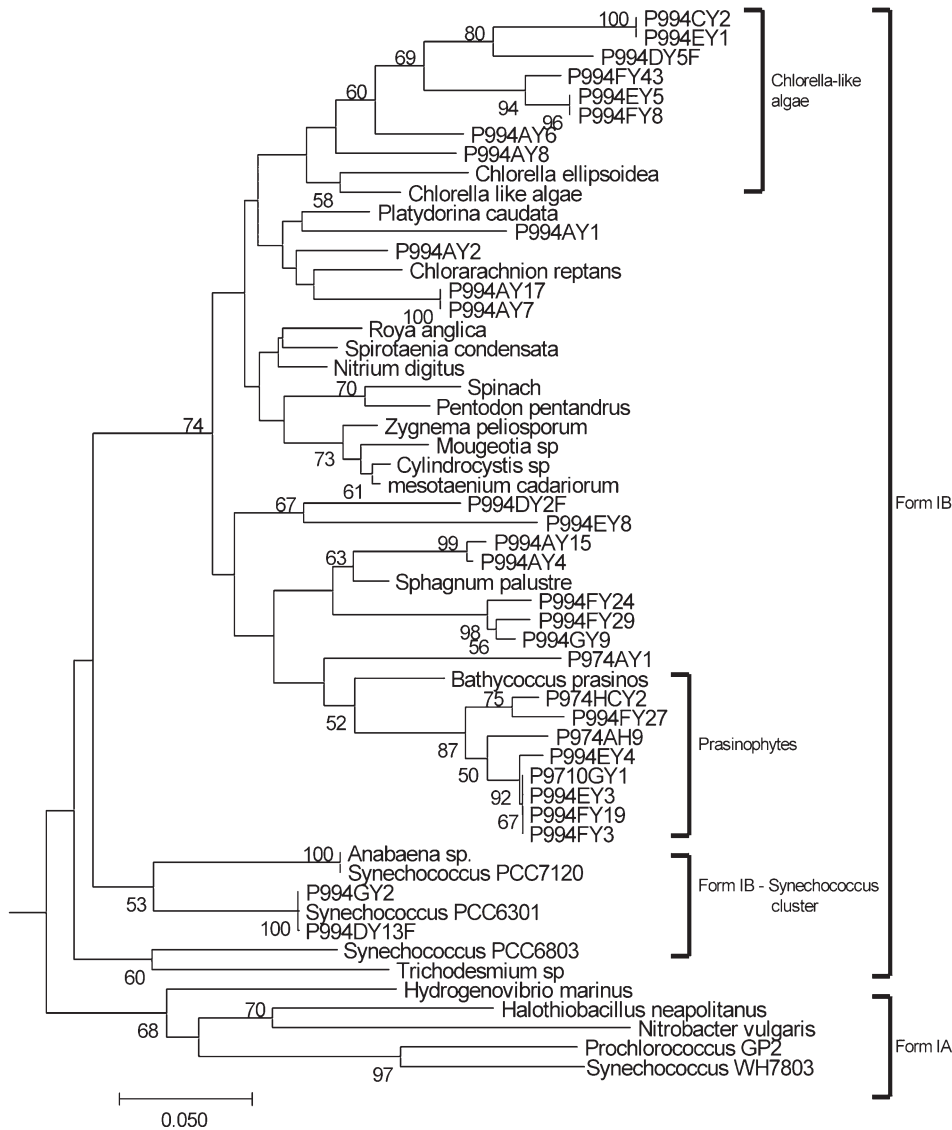


Fig. 6. Consensus tree obtained from neighbor-joining analysis of a 133 amino acid long alignment of sequences deduced from cloned cDNA fragments from Stn 4. This figure shows the diversity of Form IB *rbcL* phylotypes recovered, together with their closest relatives found in GenBank. Shown are 27 novel and 3 previously published operational taxonomic units (OTUs) (Paul et al. 2000b). Outgroup to the shown clade were several Form ID and 1 Form II (*Gonyaulax polyedra*) sequences. Numbers at internal nodes indicate bootstrap values obtained from 500 bootstrap replicates

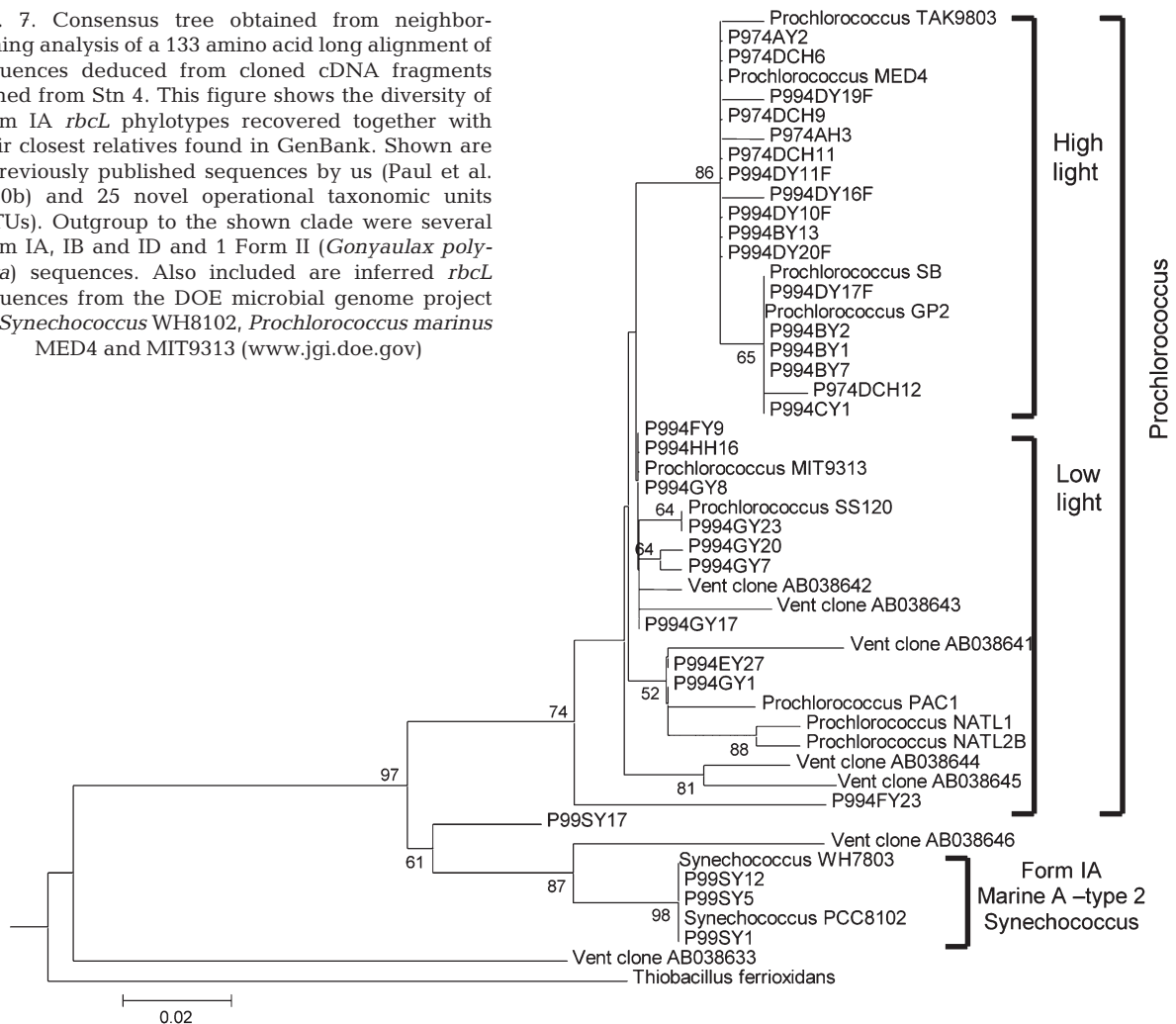


By the PCR restriction analysis, 489 *rbcL*-containing clones were screened as described in the 'Materials and methods'. From Stn 4, 105 unique *rbcL* sequences were recovered. Clones were designated by cruise date (P99), Stn (4), depth (A to H) and primers used for PCR amplification ('Y' for Form IA/B, 'H' for Form D primers and 'SY' for the cyanobacterial reverse primer). A rich assembly of *rbcL* phylotypes related to green algae was recovered from our clone libraries (Fig. 6). Predominant among these was a group of sequences closely related to *Chlorella* and a group of operational taxonomic units (OTUs) forming a micro-diverse clade closely resembling *Bathycoccus prasinos*. Nucleotide similarity within the prasinophyte clade was between 96 and 98% identity. Several more deeply rooted green algal sequences formed 3 clades. These were not closely related to any *rbcL* sequence in the database, but were distantly related to *Sphagnum palustre*. Among the Form IB sequences were also 2 sequences, which were almost identical to the *rbcL* of the freshwater *Synechococcus* PCC6301 (formerly

*Anacystis nidulans*), indicating the presence of Form IB cyanobacteria.

Fig. 7 shows the diversity of Form IA sequences recovered from Stn 4. Most notable is the presence of 2 clades of sequences closely resembling *Prochlorococcus*. One group of these sequences was exclusively isolated from samples between 10 and 60 m depth and shared between 89 and 98% DNA sequence identity. Shallow depth *Prochlorococcus*-like sequences formed a tightly conjoined clade with *Prochlorococcus* GP2 and the *rbcL* sequences amplified from DNA samples of *Prochlorococcus marinus* strains MED4, TAKA9803-2 and SB. These cultured strains have been described as high-light adapted *Prochlorococcus* isolates (Moore et al. 1995, Shimada & Miyachi 1996, Garczarek et al. 2000). The second group of *Prochlorococcus*-like sequences was recovered from water samples taken between 78 and 130 m of depth. These sequences shared between 82 to 94% sequence identity and appeared closely related to *Prochlorococcus* Strain MIT 9313, a low-light adapted *Prochlorococcus* isolate

Fig. 7. Consensus tree obtained from neighbor-joining analysis of a 133 amino acid long alignment of sequences deduced from cloned cDNA fragments cloned from Stn 4. This figure shows the diversity of Form IA *rbcL* phylotypes recovered together with their closest relatives found in GenBank. Shown are 6 previously published sequences by us (Paul et al. 2000b) and 25 novel operational taxonomic units (OTUs). Outgroup to the shown clade were several Form IA, IB and ID and 1 Form II (*Gonyaulax polyedra*) sequences. Also included are inferred *rbcL* sequences from the DOE microbial genome project for *Synechococcus* WH8102, *Prochlorococcus marinus* MED4 and MIT9313 ([www.jgi.doe.gov](http://www.jgi.doe.gov))



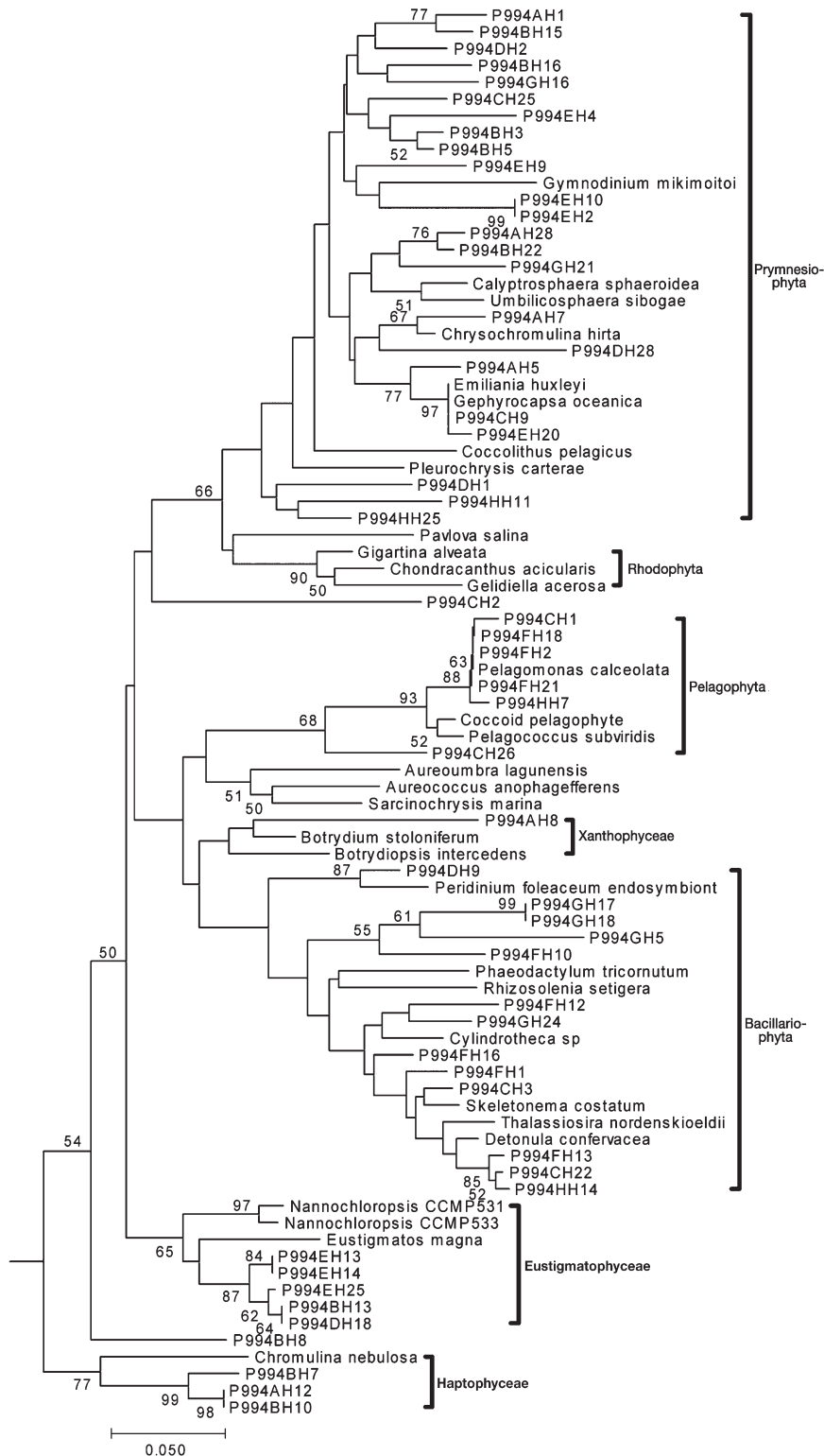


Fig. 8. Consensus tree obtained from neighbor-joining analysis of a 178 amino acid long alignment of sequences deduced from cloned cDNA fragments cloned from Stn 4. This figure shows the diversity of Form ID *rbcL* phylogenotypes recovered together with their closest relatives found in GenBank. Shown are 53 novel operational taxonomic units (OTUs). Outgroup to the shown clade were several Form IA and IB and 1 Form II (*Gonyaulax polyedra*) sequences

(Moore et al. 1998) and the *rbcL* sequences cloned from *Prochlorococcus* Strains NATL1 and NATL2B, both of which are also low-light adapted strains (Partensky et al. 1993). Interestingly, it was found that several *rbcL* sequences recently recovered from deep-sea thermal vent bacteria (Elsaied & Naganuma 2001; GenBank accession #AB038641 to AB038643) were closely associated with the clade of low-light adapted *Prochlorococcus* sequences. Between group comparison (high-light vs low-light) of *Prochlorococcus*-like sequences revealed that the 2 clades were between 76 and 81% identical at the DNA level supporting the hypothesis that the 2 clades are phylogenetically distinct. To further test this assumption, individual or several branches were moved from the high-light into the low-light clade or vice versa, and the tree topology was analyzed using Templeton (Templeton 1983) statistical analysis. No significant ( $p < 0.05$ ) alternate topology was found if the division between these 2 groups was not preserved.

A third clade of Form IA sequences was amplified upon reexamination and redesign of our Form IA/B reverse primer. These sequences were closely related to the marine cyanobacterium WH7803, indicating the presence of Form IA *Synechococcus* in Stn 4 surface water.

The richness of Form ID *rbcL* phylogenotypes recovered from Stn 4 was staggering (Fig. 8). Fifty-three novel form ID OTUs were obtained. Of these, 23 were most closely related to Prymnesiophytes. Six Pelagophyte, 13 Diatom, 5 Eustigmatophyte, 3 Haptophyte-like and 3 more deeply rooted, unidentified sequences were also obtained.

Table 2 shows the distribution of clones we obtained and their phylogenetic affinity. Prymnesiophytes were observed throughout the water column, while other chromophytic algae were largely recovered from sub-surface samples. No diatoms were observed in the surface clone samples, whereas deeply rooted haptophytes were found in those samples. All pra-

Table 2. Distribution of *rbcL* phylotypes detected at individual depth in our *rbcL*-cDNA library. Pro: *Prochlorococcus*

Depth (m)	Number of clones screened	Number of unique clones obtained	Pelagophytes	Prymnesiophytes	Eutigmatophytes	Deeply rooted haplophytes	Diatoms	Pro high light	Pro low light	Prasinophytes	Other green algae
0	60	14	-	4	-	1	-	-	-	-	8
10	50	14	-	5	2	2	-	4	-	-	-
40	52	9	2	2	-	-	2	1	-	-	1
60	58	14	-	3	1	-	1	6	-	-	2
78	70	14	-	5	3	-	-	-	1	2	3
86	88	17	3	-	-	-	5	-	2	3	4
100	71	14	-	2	-	-	4	-	6	-	1
130	40	5	1	2	-	-	1	-	1	-	-

sinophyte-like sequences were cloned from water sampled at the SCM. *Prochlorococcus* low-light and high-light sequences are divided into shallower and deeper depth, respectively.

To determine PCR and sequencing error, a total of 11 264 bp were sequenced from 22 *rbcL* clones. Among these, 1 gap and 10 PCR or sequencing errors were found, indicating accuracy of better than 99.9% in the determination of sequence identity from PCR amplified *rbcL* sequences.

## DISCUSSION

A low salinity, high chlorophyll Mississippi River plume was tracked in the central NE Gulf of Mexico using SeaWiFS satellite images and subsequently sampled. Primary productivity was greatest in the low salinity surface layer, which contained large numbers of *Synechococcus* and an abundance of Form IA *rbcL* mRNA. A previous study of a plume-like feature in the Gulf of Mexico, yielded 23 unique *rbcL* clones, based upon amplification of extracted DNA (Paul et al. 2000b). It was not known if such sequences were derived from cells that were living or dead, or even from extracellular DNA. In the present study, 105 unique sequences were derived from 489 clones screened by RFLP analysis, all of which were derived from mRNA. Phylogenetic analysis of cDNA clone libraries from a profile at this station revealed a diverse group of *rbcL* phylotypes including *Synechococcus*, the high-light and low-light adapted clades of *Prochlorococcus* and several clades of eukaryotic algae. Among the eukaryotes, prymnesiophytes were most prevalent and were distributed throughout the water column. Diatoms were found in the lower portion of the water column and prasinophytes may have dominated picoeukaryotic phytoplankton at the SCM.

Oceanic picoplankton community structure has been studied using flow cytometry and epifluorescence microscopy (Binder et al. 1996, Blanchot & Rodier 1996, Blanchot et al. 2001). Based on these and many other studies, it is now believed that in tropical and subtropical environments, *Prochlorococcus* numerically dominates the picoplankton and usually exceeds other groups by 1 to 2 orders of magnitude (Campbell et al. 1994, 1997, Blanchot et al. 2001). Picoeukaryotes are usually distributed independently of light intensity and often represent the majority of the picoplankton at the SCM. *Prochlorococcus* occasionally dominates over picoeukaryotes at the SCM if the SCM is located above the nitracline (Blanchot & Rodier 1996). Both *Prochlorococcus* and picoeukaryotes generally outnumber *Synechococcus* at low light intensities and *Synechococcus* is often most abundant where *Prochlorococcus* is least numerous (Olsen et al. 1990, Partensky et al. 1996).

These generalizations held true for the non-plume Gulf of Mexico station described here (Stn 5). In contrast, plume at Stn 4 had a significantly altered picoplankton community structure. Picoeukaryotes were less abundant than at Stn 5 at all depths and *Prochlorococcus* was all but absent from the surface plume. The surface community at Stn 4 was instead dominated by *Synechococcus*, which was 5-fold more numerous than at Stn 5. Similar observations for high chlorophyll, low salinity plume features have previously been reported (Paul et al. 2000a), suggesting that large numbers of *Synechococcus* are a dominant feature of such plumes in the Gulf of Mexico. Paul et al. (2000a) also reported elevated Form IA *rbcL* gene expression associated with the plume reminiscent of the surface peak in Form IA *rbcL* described here.

The taxonomic group *Synechococcus* is an assembly of unicellular, coccoid cyanobacteria divided into 6 provisional genera. Two of these clusters are designated Marine A and B (for example Marine Cluster A contains *Synechococcus* Strains WH7805, WH8103 and WH7803, while Marine cluster B contains *Synechococcus* Strains WH8101 and WH8007), and express a Form IA *rbcL*. We consequently hypothesized that the elevated levels of Form IA *rbcL* observed in the plume were due to the presence and activity of the proliferating population of *Synechococcus* observed by flow cytometry. To support this hypothesis, we hoped to demonstrate the presence of *Synechococcus* Marine Clusters A- and B-like sequences by RT-PCR and cloning. Unfortunately, no such sequences were recovered from our cDNA libraries. Only 2 Form IB *Synechococcus*-like clones were obtained (Fig. 6) from the 60 and 100 m (just above and at the SCM) samples, both of which were closely related to *Synechococcus* Strain PCC6301. Upon reexamination of new *rbcL*

sequence information for marine *Synechococcus* more recently deposited to GenBank, we discovered that our cyanobacterial primer set did not match these newly submitted sequences and the lack of Form IA *Synechococcus rbcL* OTUs in our libraries might consequently be expected. Using a newly designed 3' primer, 4 unique OTUs most closely related to *Synechococcus* Strain WH7803 were recovered by PCR amplification of DNA extracted from Stn 4 surface samples (all RNA samples had been exhausted and only DNA samples were available at this point in the investigation). Unfortunately, sequence information obtained from DNA samples cannot be taken as evidence of transcriptionally active cells and can only demonstrate the presence of such genotypes in the plume.

Oligotrophic sites in the Gulf of Mexico typically exhibit a bimodal distribution of Form IA *rbcL* mRNA similar to Stn 5 (Fig. 5B). This distribution is expected due to the presence of large numbers of *Prochlorococcus*. *Prochlorococcus* isolates belong to 1 of 2 distinct chl *b/a<sub>2</sub>*-ratio phenotypes (Morel et al. 1993, Partensky et al. 1993, Moore et al. 1995). Cells with the low chl *b/a<sub>2</sub>* phenotype form a dimly fluorescing population in shallower, 'high-light' environments, while the deeper part of the euphotic zone harbors a more brightly fluorescing population with the high chl *b/a<sub>2</sub>* ('low-light') phenotype (Campbell & Vault 1993, Veldhuis & Kraay 1993, Blanchot & Rodier 1996, Partensky et al. 1996, Zubkov et al. 1998, Blanchot et al. 2001). *Prochlorococcus* GP2, a high-light adapted strain first isolated from the western Pacific Ocean (Shimada et al. 1996), was the first to be shown to encode a Form IA *rbcL* (Shimada et al. 1995). With the exception of 1 isolate, all *rbcL* sequences for *Prochlorococcus* available in GenBank and all strains screened in this study are also of the Form IA type. A bimodal distribution of Form IA *rbcL* mRNA is expected due to the presence of the 2 ecotypes of *Prochlorococcus*, which both express this form of *rbcL*. At Stn 4, elevated Form IA *rbcL* mRNA levels in the plume are superimposed on the expression levels typically found due to *Prochlorococcus* (Fig. 5A). Flow cytometry did in fact indicate that *Prochlorococcus* was absent from the surface plume and we recovered no *Prochlorococcus*-like sequences from our Stn 4 surface cDNA libraries. Why *Prochlorococcus* is absent from the plume remains unclear, but might be due to the lower salinity found there. *Prochlorococcus* is, for example, not found in low salinity estuarine waters (L. Campbell pers. comm.).

A large diversity of *Prochlorococcus*-like sequences was recovered from samples taken below the plume, where *Prochlorococcus* was very abundant. Phylogenetic analysis of these sequences revealed that *Prochlorococcus*-like phylotypes were found in 2 distinct clades. One group of sequences was recovered from samples taken between the plume and mid-

depth, and is most closely related to the high-light adapted *Prochlorococcus* isolates MED4, GP2, SB and TAKA9803-2. The second group sequences was exclusively isolated from deeper water samples, which included the SCM. These sequences were more closely related to the low light *Prochlorococcus* isolates MIT9313, NATL1, NATL2B and SS120. The tight linkage in phylogenetic analysis to *rbcL* sequences from cultured isolates lends strong support to the hypothesis that the 2 observed clades of *Prochlorococcus*-like *rbcL* phylotypes described here are indeed the high-light and low-light adapted *Prochlorococcus* ecotypes previously described. This surprising correlation between depth, pigment content and phylogenetic inference is remarkable, but has also been recognized for 16S rDNA sequence information from *Prochlorococcus* isolates (Urbach et al. 1998), the vertical distribution of petB/D genotypes depth profiles from the Sargasso Sea and the Gulf Stream (Urbach & Chisholm 1998) as well as genotype-specific gene probing in the North Atlantic Ocean (West & Scanlan, 1999). It is also interesting to note that several recently isolated deep-sea thermal vent bacterial Form IA *rbcL* sequences (Elsaied & Naganuma 2001) were found to group within the low-light *Prochlorococcus* clade, suggesting a gene transfer event from possibly extremophilic, chemotrophic bacteria to *Prochlorococcus* may have occurred.

Even though Form IA *rbcL* expressing phylotypes were apparently dominant in the plume, they were not found to the exclusion of other phytoplankton. We also found both Form IB and ID *rbcL* mRNAs in the plume. Form IB *rbcL* mRNA surface levels were in fact of similar magnitude as surface values recorded at Stn 5 and reached ca. 15% of Form IA levels, suggesting that Form IB-bearing phytoplankton may not have been affected strongly by the presence of the plume. The Form ID sequences found in the plume corroborate the pigment data indicating the presence of chromophytic algae, such as diatoms and prymnesiophytes. However, the co-occurrence of fucoxanthin and 19'-hexanoylfucoxanthin, the presence of prymnesiophyte clones, and the absence of diatoms in the surface clones strongly argues for the presence of prymnesiophytes over diatoms in the plume at this location.

Phylogenetic analysis of our cDNA libraries revealed 2 groups of Form IB clones. Several clones were most closely related to *Chlorella* and these were found both in surface and deeper samples (Table 2, Fig. 6). The other group of sequences was isolated from samples within the SCM and these sequences formed a microdiverse clade ingroup to *Bathycoccus prasinus* (Fig. 6) suggesting that prasinophytes may have been particularly important there. This hypothesis is substantiated by the presence of high concentrations of chl *b*, a green algal pigment (prasinophytes, chlorophytes and



euglenophytes) at the SCM, where this pigment reached a higher concentration than any other pigment except chl *a* (Fig. 4A). Interestingly, the SCM Form IB *rbcL* mRNA levels reached ca. 50% of Form IA levels. Because *Prochlorococcus* was more than 10-fold more abundant than picoeukaryotes at this depth (Fig. 3A), it appears that Form IB carrying phytoplankton, particularly prasinophytes, may have exhibited a 5-fold higher transcriptional activity of *rbcL* than Form IA containing picocyanobacteria.

Form ID *rbcL* mRNA was absent in the upper water column at Stn 5 (Fig. 5B) and only found at the SCM; yet at Stn 4, chromophytic *rbcL* was found in the entire water column (Fig. 5A). This suggests that the presence of the plume may have stimulated chromophytic *rbcL* expression above the SCM where other forms of *rbcL* (i.e. Form IB from picoeukaryotes and picocyanobacteria) may otherwise dominate. Depth integrated counts of picoeukaryotes at Stn 4 were not elevated over Stn 5, averaging ca. 87%, if similar depths are compared between the 2 stations. No elevated counts of picoeukaryotes were observed in the surface plume. This may mean that chromophytic picoeukaryotes (i.e. Prymnesiophytes) replaced Form IB-containing picoeukaryotes in the plume. A large diversity of Form ID phylotypes was observed at Stn 4 (Fig. 8) encompassing 4 major phytoplankton groups. Prymnesiophytes were by far the most diverse group of organisms and were found at all depth except the center of the SCM, although pigment data (Fig. 4B) suggests their abundance at this depth. We also detected the *rbcL* sequences of eustigmatophytes and pelagophytes at Stn 4. Unfortunately, there appears to be no clear pattern in the distribution of these clones. This lack in resolution is possibly the consequence of the insufficient scale of our clone libraries or potential drawbacks inherent in PCR based techniques. PCR suffers from many biases (for a review see Winzingerode 1997) as is illustrated by our initial failure to detect Form IA *Synechococcus* in the plume in 1997, as well as in the current sampling. Regardless of our ability to detect these organisms, pigment signatures suggest the presence of all 3 groups in the entire water column.

The observed phytoplankton diversity at Stn 4 is not surprising. It has already been put forward by Hutchinson (1961) that species diversity is often far greater than could be expected as a result of niche-partitioning based only on a few limiting resources. Besides the sheer number of species, 2 additional facets of diversity were observed in our data set. A very diverse and deeply rooted group of eukaryotic algae, including the chromophytes and *Chlorella*-like green alga, was observed. These organisms were found to be only distantly related and covered almost the entire phylogenetic spectrum of the known pelagic

eukaryotic phytoplankton. These phylotypes are likely representative of unique species. The greater genetic distances between these forms suggests that these evolved some time ago, or are under some genetic constraint in the evolution of the *rbcL* gene. On the other hand, prasinophytes and the high/low light *Prochlorococcus* clusters exhibited marked microdiversity reminiscent of microdiversity observed for *nosZ* (Scala & Kerkhof 1999), *nifH*-containing bacterial populations (Zehr et al. 1998) and *Prochlorococcus* in the plume sampled in 1997. The simultaneous occurrence of a large numbers of organisms with minute differences in sequence identity (less than 10% DNA dissimilarity) we believe indicates a rapidly evolving or recently evolved clade of organisms. This is not limited to prokaryotes because a cluster of eukaryotes, such as the group of prasinophytes, showed a similar microdiversity. If microbial microdiversity is a general phenomenon however, it would appear that most genetic diversity is found in small variations of functional genes; a fact most likely missed by recent microbial genome sequencing efforts.

It is interesting to speculate about the importance of the Mississippi River plume to primary productivity in the oligotrophic Gulf of Mexico. The Mississippi River discharges on average  $1.7 \times 10^4 \text{ m}^3 \text{ s}^{-1}$  fresh water onto the northern Gulf of Mexico shelf, most of which is carried westward along the Texas coastline (Müller-Karger et al. 1991). Eastward transport of the Mississippi plume is apparently sporadic and short lived, and occurs either as focused or diffuse dispersal along the coastline or as larger discolored water masses which are carried into the open Gulf. The plume described in this paper is an example of such a large discharge event. Müller-Karger et al. (1991) also investigated the frequency of occurrence of large offshore plumes by screening coastal zone color scanner (CZCS) images between November 1978 and May 1980. Two plume occurrences were observed in these images. This indicates that large eastward extending plumes are by no means frequent events, yet are also not rare. Image analysis indicates that the plume described here covered ca. 2% of the total oligotrophic Gulf of Mexico and ca. 6% of the eastern portion typically influenced by such plumes. Surface primary productivity at Stn 5 was measured at  $0.54 \mu\text{g C l}^{-1} \text{ h}^{-1}$ , while surface productivity at Stn 4 was estimated at  $3.39 \mu\text{g C l}^{-1} \text{ h}^{-1}$ . If these rate measurements are used as averages for the oligotrophic Gulf of Mexico and the plume, respectively, it can be estimated that in the upper 10 m of the water column the plume may account for as much as 11% of the total oligotrophic surface productivity of the Gulf. The plume may have also accounted for as much as 28% of oligotrophic surface productivity of the eastern portion of the Gulf. However, such esti-



mates of basin wide production based upon 2 points should be viewed with caution. Despite the positive impact on surface productivity, the reverse may have been true at the SCM. SCM primary productivity at Stn 4 was only 29% ( $0.59 \mu\text{g C l}^{-1} \text{h}^{-1}$ ) of the rate observed outside of the plume ( $2.03 \mu\text{g C l}^{-1} \text{h}^{-1}$ ). In fact, if primary productivity is integrated throughout the water column, it is found that Stn 4 only displayed ca. 75.4% of water column productivity observed at Stn 5. This is most likely due to the attenuated light intensity below the plume. These observations suggest that the plume may have significantly reduced productivity at a depth critical to the downward flux of carbon into the deep ocean via the biological pump and may have significantly impacted the rate of carbon sequestration in the Gulf of Mexico.

Collectively these data indicate that the presence of a low salinity, high chlorophyll plume derived from Mississippi River discharge may have significantly impacted the phytoplankton community structure of the Gulf of Mexico by allowing *Synechococcus* to replace *Prochlorococcus* in the low salinity surface layer and potentially stimulating the growth of larger, chromophytic phytoplankton in the upper portion of the water column. Using culture independent techniques, we detected 2 spatially separated clades of *Prochlorococcus* reminiscent of the high light and low light adapted *Prochlorococcus* ecotypes which numerically dominated below the plume. We also detected a clade of prasinophytes, which may have been important at the SCM. In combination with the altered picoplankton community of the plume, we observed that surface primary productivity of the oligotrophic Gulf of Mexico was enhanced by as much as 9.5%, while productivity may have been negatively impacted at the SCM by the presence of the plume. Despite this enhancement of surface productivity in the plume, integrated water column productivity may have in fact been reduced by as much as 25% by its presence.

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