Form IA *rbcL* transcripts associated with a low salinity/high chlorophyll plume ('Green River') in the eastern Gulf of Mexico

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ABSTRACT: Coastal plumes of low salinity water that extend hundreds of kilometers offshore into oligotrophic waters are often found in the Gulf of Mexico. To characterize one such feature, a series of photoautotrophic activity and biomass parameters were measured at 2 stations in the eastern Gulf of Mexico, including pigments by high performance liquid chromatography (HPLC), autotrophic picoplankton abundance by flow cytometry, photoautotrophic ^{14}C-HCO_3^- fixation, and Ribulose-1,5-diphosphate carboxylase large subunit gene (*rbcL*) transcriptional activity. One sampling site (Stn 4) was in a 15 m deep, low salinity (29.8 ppt) plume 242 km west of Tampa Bay. This feature contained relatively high chlorophyll *a* (chl *a*) concentrations, carbon fixation rates, and *Synechococcus* cell abundance (8.6 × 10^4 cells ml^{-1}) at 3 m depth and a relatively shallow (45 m depth) subsurface chlorophyll *a* maximum (SCM). We also found a high level (1.1 ng l^{-1}) of Form IA *rbcL* mRNA in the surface water as determined by probing with a 1.1 kb *Synechococcus* WH7803 *rbcL* probe. Form IA *rbcL*s have been found to occur mainly in chemosynthetic autotrophic bacteria but have recently been described in *Synechococcus* WH7803 as well as in *Prochlorococcus* GP2. In contrast, a nearby station outside of the plume (Stn 7) had a SCM at 83 m, lower chl *a*, *Synechococcus* cell counts, and carbon fixation rates in the surface waters. The amount of Form IA *rbcL* was only about 3% of the concentration found in the surface waters of Stn 4. Both stations had an abundance of *Prochlorococcus* cells (>10^5 ml^{-1}) at intermediate depths (20 to 70 m). The picoeucaryote community occurred principally below the *Prochlorococcus* community, coinciding with the SCM, and was composed of diatoms, prymnesiophytes, and pelagophytes as determined by HPLC pigment analysis. This report represents the first description of Form IA *rbcL* transcriptional activity in the marine environment, and indicates that Form IA *rbcL*-containing picoplankton (like *Prochlorococcus* GP2 and *Synechococcus* WH7803) may be important in the primary production of low salinity, surface water plumes of the Gulf of Mexico.

KEY WORDS: RuBisCO · Gene expression · Phytoplankton · Gulf of Mexico

INTRODUCTION

The oceans are now known to be environments of abundant microbial diversity, in both the autotrophic and heterotrophic components of microbial assemblages (Giovanonni et al. 1990, DeLong 1992, Fuhrman et al. 1992, 1993). The photoautotrophic community in oligotrophic environments has been shown to be domi-

nated by the picoplankton, which include the cyanobacteria *Synechococcus* and *Prochlorococcus*, and small eucaryotic algae (picoeucaryotes). These groups have been shown to occupy their own respective niches in space and time (Stockner 1988, Landry et al. 1996, Campbell et al. 1997). In stratified water columns, the abundance maxima for each of these 3 major picoplanktonic groups are often vertically separated, yet the order of these groups varies with the environment sampled (Campbell et al. 1997, Paul et al. 1999).

Although *Synechococcus* has been termed a provisional taxonomic division which has been subdivided into 6 groups (Waterbury & Rippka 1989), analysis of several loci suggest a high degree of diversity even in the marine picoplanktonic strains (Urbach et al. 1998). For example, examination of the RNA polymerase genes (*rpo* loci) has indicated the existence of 2 major groups amongst 15 isolates and 3 minor groups within 1 major group (Toledo & Palenik 1997). Similarly, 2 deeply rooted branches of *Synechococcus* emerged upon study of the *petB*, *petD*, and *psbB* sequences (Urbach et al. 1998). *Prochlorococcus*, which was previously viewed as a homogeneous group (Chisholm et al. 1988, 1992), is now known to be an equally diverse group (Scanlan et al. 1996, Ferris & Palenik 1998, Moore et al. 1998, Urbach & Chisholm 1998).

Over the years, we have been interested in the molecular regulation of photosynthetic carbon fixation, focusing on the picoplankton (Pichard et al. 1993, 1997a,b, Paul et al. 1999). Ribulose-1,5-diphosphate carboxylase/oxygenase (RuBisCO) is believed to be responsible for 95% of the carbon fixation in oceanic phytoplankton (Raven 1995). Our approach to this problem has been to investigate the transcriptional regulation of the gene for the large subunit of RuBisCO, *rbcL*. Although there is some conservation in sequence, the diversity of this gene enables its use for identification of taxa (Paul et al. 2000, in this issue). There are 3 major evolutionary divisions in this gene which encode for Form I, Form II, and Form III/IV RuBisCOs (Tabita 1988, Watson & Tabita 1996, 1999). The primary type found in oceanic phytoplankton and land plants is the Form I. Form II is found in several photosynthetic bacteria and some dinoflagellates (Morse et al. 1995) and a third and fourth form have recently been described in Archaea, although no active enzyme has been found in these (Watson & Tabita 1999).

The important phytoplankton clades of the Form I enzyme include Form IB, which encompasses cyanobacteria and green algae, and Form ID, which is found in diatoms, prymnesiophytes, cryptophytes, pelagophytes, brown and red algae (Douglas et al. 1990, Newman & Cattocolico 1990, Valentin & Zetsche 1990, Hwang & Tabita 1991, Watson & Tabita 1997). We have previously used gene probes derived from partial transcripts from both a Form IB and a Form ID RuBisCO as a means to measure transcriptional regulation of RuBisCO in oceanic phytoplankton (Pichard et al. 1997a). We have demonstrated a temporal and spatial separation in the expression of Form IB and ID *rbcL*s, with Form IB occurring in the upper water column and coincident with *Synechococcus* abundance maxima, and Form ID primarily at the subsurface chlorophyll *a* maximum (SCM), correlating with picoeucaryote counts (Paul et al. 1999).

Recently, *rbcL* sequences from *Synechococcus* WH-7803 and *Prochlorococcus* GP2 have placed these organisms in the Form IA group, thought to contain only β- and γ-purple (chemosynthetic sulfur oxidizing and other) bacteria (Watson & Tabita 1996). In this report we have employed a Form IA *rbcL* gene probe derived from *Synechococcus* WH7803 to determine if transcriptional activity was occurring from this group of organisms in the Gulf of Mexico.

MATERIALS AND METHODS

Sampling sites. Sampling was performed aboard the RV 'Pelican' in the SE Gulf of Mexico (see Fig. 1A) on 14 August 1997 (Stn 4) and 10 August 1997 (Stn 7). Sampling was performed using a rosette of Niskin bottles attached to a Seabird CTD between 08:00 and 10:00 h each day. Samples from depths greater than 40 m were protected from light by wrapping carboys in black plastic garbage bags.

rbcL mRNA analysis. Samples for mRNA analysis (usually 500 to 800 ml) were treated with 0.1% (final concentration) diethylpyrocarbonate (DEPC; Sigma Chemical Corp.) immediately prior to filtration through 25 mm, 0.45 μm polyvinylidene difluoride filters (Millipore Durapore). Use of the 0.22 μm pore-sized filters resulted in no recovery of RNA from the samples. Filters were added to 2.2 ml bead-beater tubes (Microspecs, Bartlesville, OK) containing guanidinium isothiocyanate extraction reagent (Pichard et al. 1993), immediately frozen in liquid nitrogen, and stored at −80°C until extraction (within 1 mo of sampling). RNA was extracted by bead-beating, followed by dot-blotting and molecular probing (Pichard et al. 1993, 1997a). This process has been shown to result in ~80% recovery of cellular RNA (Pichard et al. 1993). Duplicate samples were probed with the Form IB and Form ID probes derived from *Synechococcus* PCC6301 and *Cylindrotheca* sp. N1 as previously described (Pichard et al. 1997a). A third set of duplicate samples was probed with a Form IA probe derived from *Synechococcus* WH7803 (Watson & Tabita 1996). This probe was constructed by subcloning an 1196 Sal/Sac restriction fragment of the *Synechococcus* WH7803 *rbcL* gene into the pGEM3Z vector (Promega, Madison, WI). Radiolabeled probes were made by *in vitro* transcription of the genes using ^{35}S-UTP. Dot blots were quantitated with a BioRad Model GS363 Molecular Imager using standard curves made from *rbcL* genes from *Synechococcus* PCC6301, *Cylindrotheca* sp N1, and *Synechococcus* WH7803.

Flow cytometry. One ml samples were fixed with 20 μl 10% paraformaldehyde at room temperature for 10 min and quickly frozen in liquid nitrogen. *Prochloro-*

coccus, Synechococcus, and picoeucaryotic algal populations were quantified using a Becton Dickinson FACSCalibur (San Jose, CA) Flow cytometer equipped with a 488 nm, 15 mW Argon laser. Forward angle light scatter, right angle light scatter, green (530 ± 30 nm), orange (585 ± 30 nm), and red (650 ± 30 nm) fluorescence parameters were collected for each event. Purple-Yellow calibration beads (2.2 μm, Spherotech Inc., IL) were added to each sample to permit normalization of all fluorescence signals. Data were collected using CellQuest™ software (Ver. 3, Becton Dickinson 1996), transferred to a personal computer (PC), and analyzed using CYTOWIN software (Vaulot 1989, http://www.sb-roscoff.fr/Phyto/cyto.html#cytowin). Event rates were recorded for each sample as it was run and abundances were corrected for volume analyzed and an enumeration efficiency factor. The efficiency factor was derived from event rate and counts for a series of known concentrations of calibration beads.

HPLC pigment analysis. Two to 4 l of sample water were filtered through 2.5 cm Whatman GF/F glass fiber filters in duplicate. The filters were folded in half, wrapped in aluminum foil, frozen immediately in liquid nitrogen, and stored at −80°C. The filters were extracted with 100% acetone and extracts analyzed by (HPLC) by B. Pedersen, Mote Marine Lab, using the method of Millie et al. (1993).

Chlorophyll a analysis. Samples for chl *a* were collected by filtration onto 2.5 cm Whatman GF/F glass fiber filters in triplicate and stored frozen until extraction. Filters were extracted in methanol and the chl *a* determined fluorometrically (Holm-Hansen & Riemann 1978).

¹⁴C-carbon fixation. ¹⁴C-carbon fixation studies were performed essentially as described in Pichard et al. (1993, 1997a) using sterile, acid-cleaned 500 ml poly-carbonate flasks and 325 ml water samples. On-deck incubations were performed with 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 sensors.

RESULTS

At Stn 4, the water column was highly stratified as indicated by the steep thermocline and the low salinity surface water (Fig. 1B). The surface water salinity was ~29 ppt and increased to 36 ppt at 25 m, where it remained constant to the base of the euphotic zone. The *in vivo* fluorescence was highest in the low salinity 'lens' near the surface (0 to 10 m). The SCM was relatively shallow (45 m) compared to other stations in this area and the depth of the 1% light intensity was approximately 60 m (Fig. 2A).

Photosynthetic carbon fixation showed a maximum in the surface waters (Fig. 2A) and decreased to 20 m, remaining near constant to 60 m (through the SCM). Below 60 m carbon fixation decreased dramatically. Chl *a* followed the *in vivo* fluorescence with maxima at the surface and at the SCM (45 m).

Fig. 2B shows the flow cytometry data for Stn 4. The highest numbers of *Synechococcus* occurred in the surface waters (8.6 × 10⁴ cells ml⁻¹) with a secondary peak at 20 m (4.3 × 10⁴ cells ml⁻¹). *Prochlorococcus* cell counts were undetectable in the surface waters but peaked at 40 m (1.4 × 10⁵ cells ml⁻¹).

The picoeucaryote cell maximum (2.3 × 10³ cells ml⁻¹) occurred below the *Prochlorococcus* population at the
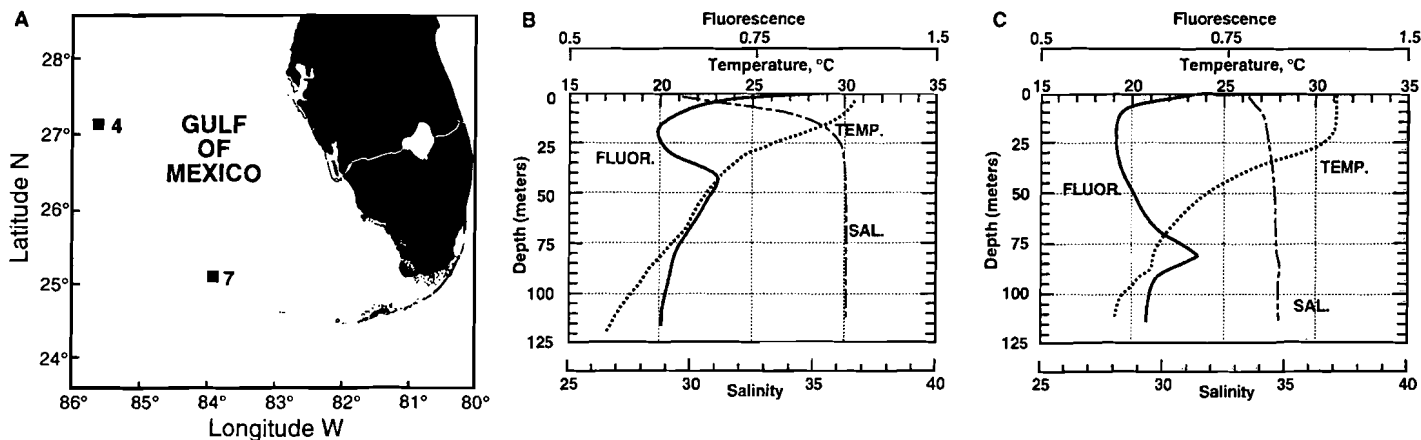

Fig. 1. (A) Location of stations sampled in this study. (B & C) CTD traces for Stns 4 and 7, respectively. Notice the low salinity surface feature at Stn 4 (B), and approximate position of the SCMs as indicated by *in situ* fluorescence trace (labeled 'FLUOR.')
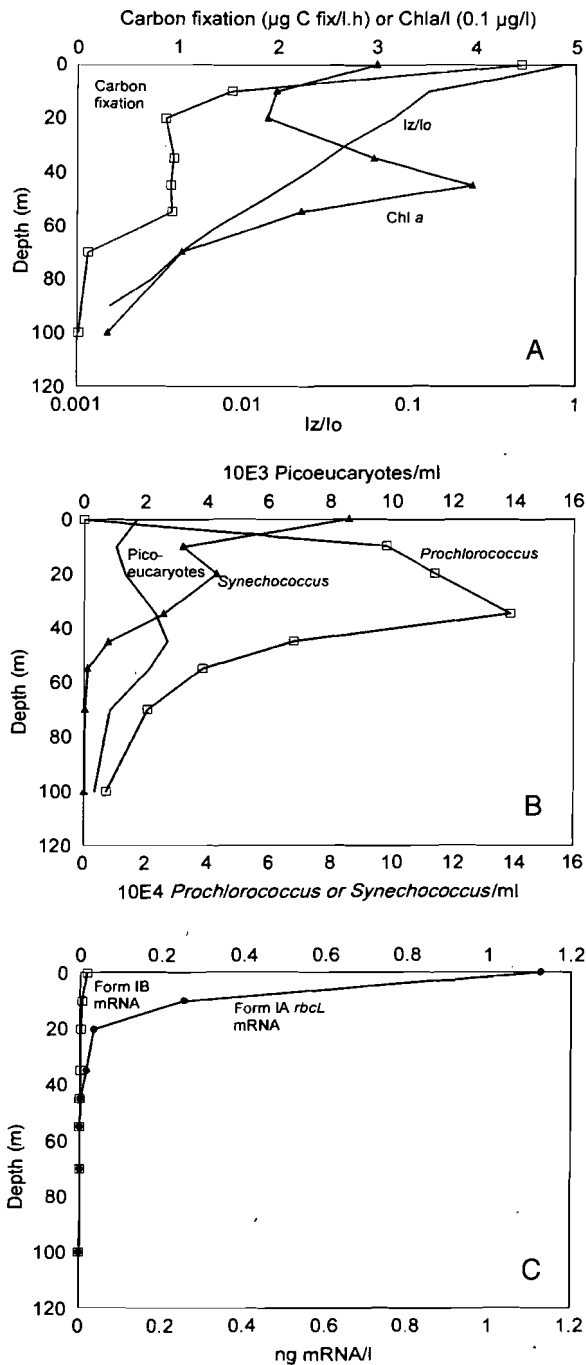
Fig. 2. (A) Relative light intensity (Iz/Io, ——), photosynthetic carbon fixation (Carbon fixation, □), and chl a (chl a, ▲) at Stn 4. (B) Flow cytometry data from Stn 4, including Prochlorococcus (□), Synechococcus (▲), and picoeucaryote (——) cell abundance. (C) rbcL mRNA levels for Stn 4, including Form IA (●) and Form IB (□)

SCM. Thus, these 3 picoplankton groups were vertically stratified, with the Synechococcus maximum at the surface, followed by Prochlorococcus at 40 m, and the picoeucaryotes at the SCM.

Fig. 2C shows the distribution of rbcL mRNA in the water column of Stn 4. The greatest amount of Form IA rbcL mRNA occurred in the surface waters, and rapidly decreased to undetectable levels at 45 m. The Form IB rbcL mRNA, an abundant form in the Gulf of Mexico (Pichard et al. 1997a, Paul et al. 1999), was also maximal in the surface waters but was approximately 70-fold less abundant than the Form IA rbcL mRNA. No Form ID rbcL was found in these samples.

Fig. 3 displays the results of HPLC pigment analysis for the waters of Stn 4. Divinyl chl a and divinyl chl b, diagnostic pigments for Prochlorococcus, were maximal at the SCM, with no detectable pigment levels in the surface waters. These data paralleled the flow cytometry data. Zeaxanthin, a pigment diagnostic for cyanobacteria, was highest in the surface waters, with a secondary peak occurring at 35 m. Chl c1 and c2, components of all chromophytic algae, exhibited a peak in the surface waters and then a large peak at the SCM. Fucoxanthin, found in diatoms and prymnesiophytes, showed a peak at the surface and also at the SCM, mirroring the chl c1 and c2 data. Thus, the surface plume was comprised of chromophytic eucaryotes as well as Synechococcus. Both 19'-hexanoyl-oxyfucoxanthin, a pigment contained primarily in prymnesiophytes, and 19'-butanoyl-oxyfucoxanthin, a pigment found in pelagophytes, were maximal at the SCM.

Stn 7 was more typical of the oligotrophic Gulf of Mexico than Stn 4. The depth of the mixed layer was ~25 m, after which a steep thermocline occurred (Fig. 1C). Unlike Stn 4, the SCM occurred at approximately 83 m. Salinity was relatively constant over the entire 110 m euphotic zone, ranging from 35.8 to 36.4 ppt.

Fig. 4A shows the distribution of carbon fixation, light intensity, and chl a as a function of depth at Stn 7. The depth at which illumination was 1% of that at the surface was approximately 80 m, and coincided with the depth of the SCM. Unlike Stn 4, there was no dramatic maxima in carbon fixation or chl a in the surface waters. The carbon fixation peak covered a broad expanse of depths, from 20 to 80 m.

Fig. 4B shows the results of flow cytometry data for Stn 7. Prochlorococcus was the most numerically abundant picoplankter, with a maximal abundance of 1.83×10^5 cells ml^{-1} at the 50 m depth. As with Stn 4, the Synechococcus peak was in the surface waters (2.22×10^4 cells ml^{-1}) and the picoeucaryotic peak occurred at the SCM (2.76×10^3 cells ml^{-1}).

Fig. 4C shows the results of the rbcL mRNA extraction and hybridization studies. As with Stn 4, the highest level of Form IA rbcL mRNA occurred in the surface water, but this was only approximately 2.8% of the signal observed in the surface waters of Stn 4. A second subsurface peak occurred at 50 m depth, coin-
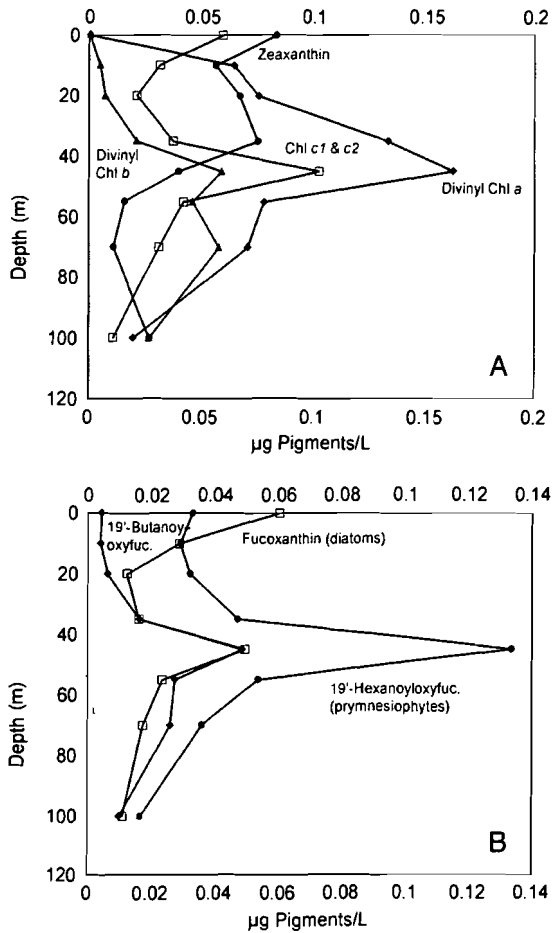
Fig. 3. Pigment data for Stn 4, including divinyl chlorophylls, chl c1 and c2, and (A) zeaxanthin, and (B) fucoxanthins

DISCUSSION

A high abundance of Form IA *rbcL* transcripts were found associated with a low salinity/high chl surface feature in the eastern third of the Gulf of Mexico. This is the first report of Form IA *rbcL* transcripts or DNA in general in surface waters. We have previously reported Form IB *rbcL* transcripts in the surface and mid-depth water off Bermuda, that closely followed the
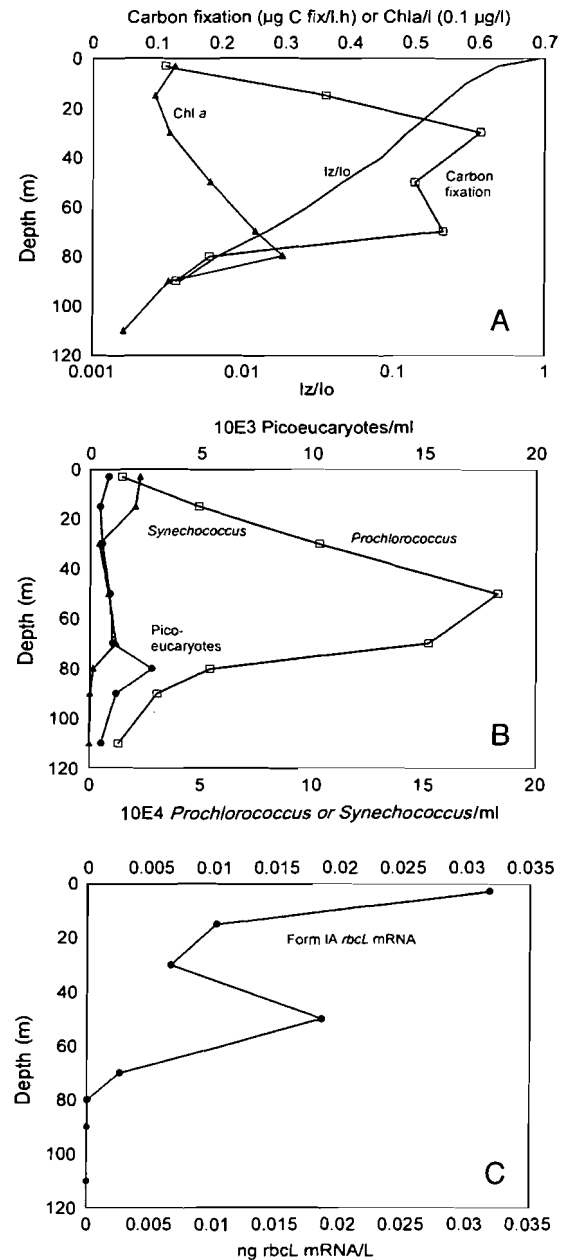
cident with the *Prochlorococcus* peak in cell abundance. There were no detectable levels of Form IB or Form ID *rbcL* mRNA at any depth.

Fig. 5A,B shows pigment data for Stn 7. As with Stn 4, zeaxanthin or the diagnostic cyanobacterial pigment was greatest in the surface water, while a secondary peak was found at 50 m. Divinyl chl *a* had a peak at 70 m while divinyl chl *b* had peaks at 70 and 90 m, which overlapped or were slightly below the *Prochlorococcus* peak as determined by flow cytometry. We interpret this to mean that the deeper portion of the *Prochlorococcus* population (those occurring at 70 and 83 m) had more cellular pigment content than those at 60 m. Chl c1 and c2, the signature pigments of chromophytic picoeucaryotes, were maximal at the SCM (83 m), which coincided with the picoeucaryote peak as determined by flow cytometry. The signature pigments for prymnesiophytes and pelagophytes (19'-hexanoyl-oxyfucoxanthin, and 19'-butanoyloxyfucoxanthin, respectively) all were maximal at the SCM, as found for Stn 4.

Fig. 4. (A) Relative light intensity (Iz/Io, ——), photosynthetic carbon fixation (□), and chl *a* (▲) as a function of depth at Stn 7. (B) Flow cytometry data for the vertical profile at Stn 7, including *Prochlorococcus* (□), *Synechococcus* (▲), and picoeucaryote (●) cell counts. (C) Form IA *rbcL* mRNA at Stn 7
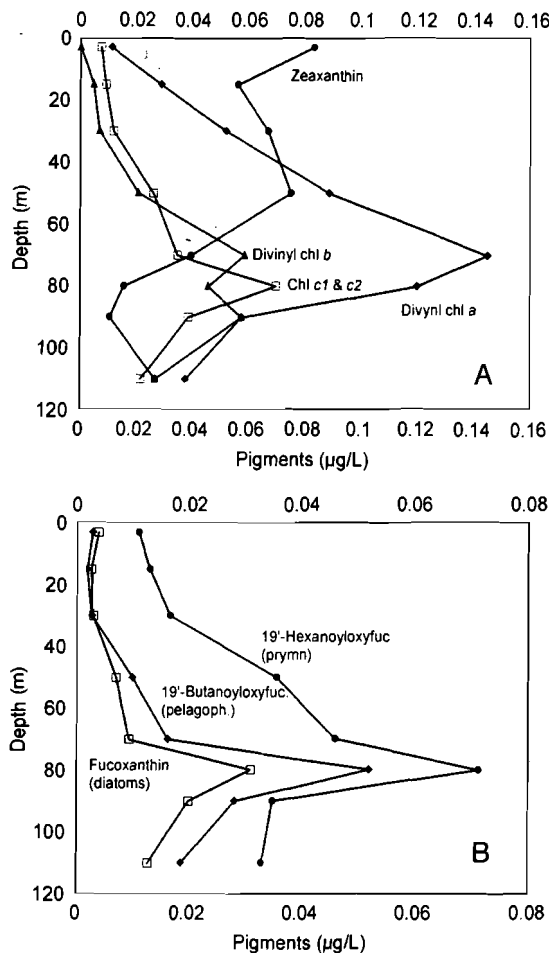
Fig. 5. Results of HPLC pigment analysis of Stn 7, including (A) divinyl chlorophylls, chl *c1* and *c2*, and zeaxanthin and (B) fucoxanthin and derivatives

Synechococcus cell abundance maxima (Paul et al. 1999). Also, Form ID transcripts were most abundant at the SCM, coinciding with 'red fluorescent cell counts', presumably picoeucaryotes.

In the present study, the Form IA *rbcL* coincided with a peak in *Synechococcus* abundance and the highest rates of carbon fixation at Stn 4. *Synechococcus* WH-7803 has been shown to contain a Form IA RuBisCO (Watson & Tabita 1996), as has *Prochlorococcus* GP2 (Shimada et al. 1995). These *rbcL* genes are divergent from other *Synechococcus* and *Prochlorococcus rbcL* sequences which are Form IB and more closely related to higher plants. It is thought that the Form IA *rbcL* in *Synechococcus* WH7803 is the result of a lateral transfer event, potentially from a β/γ purple bacterium (chemoautotroph or anoxygenic phototroph; Watson & Tabita 1996). The importance of this form of RuBisCO in the ocean has not been previously recognized. The following paper indicates that sequences obtained from these waters were most closely related to *Pro-*

chlorococcus GP2 and *Synechococcus* WH7803 and not other β/γ purple bacteria.

A second peak in *Synechococcus* abundance occurred at 20 m at Stn 4; yet little Form IA mRNA was found. This population may have been a population distinct from that found in the upper 10 m, as it occurred in higher salinity water, not influenced by the plume. This population possessed less than 20% of the carbon fixation rate of the surface plume population. It may be that transcript degradation had already occurred in this population, while RuBisCO enzyme activity was still intact, enabling carbon fixation.

Although *Prochlorococcus* was the most numerically abundant picoplankter at both stations throughout the water column, there were no detectable *Prochlorococcus* cells in the surface waters of Stn 4. This may have been caused by extremely low fluorescence of the surface populations of *Prochlorococcus*, preventing their detection by flow cytometry. However, divinyl chls were undetectable in the surface as well. If cellular pigment content was extremely low (as might be the case with surface populations), then it is possible that there was a surface *Prochlorococcus* population that was undetected by either pigment analysis or flow cytometry. In this case, given the low salinity plume, it appears that *Synechococcus* replaced *Prochlorococcus* in these surface waters.

The cellular levels of Form IA *rbcL* transcripts found in the surface waters of Stn 4 (0.059 fg per *Synechococcus* cell) are slightly lower than those found by us at other sites (0.075 to 0.184 fg cell^{-1}; Pichard et al. 1996). However, the latter data are for Form IB transcripts, and other organisms besides *Synechococcus* probably contributed to the hybridization signal.

The low salinity/high chl surface feature or plume has been observed previously, (Gilbes et al. 1996), and has been detected by Coastal Zone Color Scanner (CZCS) satellite imagery (Frank Muller Karger pers. comm.). At the time of sampling, there was no color satellite imagery available. Prior studies have termed a similar feature the 'Green River' for a low salinity plume extending out of Mobile Bay or Appalachicola Bay down the Eastern Gulf of Mexico/west Florida Shelf as far south as the Dry Tortugas (Gilbes et al. 1996). This feature is believed to be moved along in part by the Loop Current (Gilbes et al. 1996). It is not known if the *Synechococcus* cells found in this feature were oceanic species that bloomed in this nutrient rich coastal water or if this was a bloom of a coastal *Synechococcus* strain overlying oligotrophic Gulf of Mexico water.

The dominance of *Prochlorococcus* and the vertical separation of the 3 major picoplankton components (*Synechococcus, Prochlorococcus*, and picoeucaryotes) has been observed by others (Campbell et al. 1997).

We found the *Synechococcus* to be bimodally distributed, with a surface population and a 50 m population (i.e. Stn 7). Below this was the *Prochlorococcus* cell abundance maximum, followed by the picoeucaryotes at the SCM. In the North Pacific, picoeucaryotes were found to comprise the SCM, and were abundant when nitrate was abundant. *Prochlorococcus* was found to be abundant when the nitrate was <0.1 nM (Blanchot & Rodier 1996). Similarly, Landry et al. (1996) found *Prochlorococcus* and heterotrophic bacteria in the surface waters of the tropical Pacific, with *Synechococcus* and picoeucaryotes more abundant at depth. Analysis of a large data set suggests that *Synechococcus*, in most cases, is maximum in the upper portion of the water column (Partensky et al. 1999).

Chromophytic pigment analysis by HPLC indicated the presence of diatoms and other chromophytes (fucoxanthin and chl *c1* and *c2*) at the SCM of both stations and in the surface waters of Stn 4. The latter may have been the result of a coastal species entrained in the coastal plume encountered. Other chromophytic pigment signatures in our study included those of pelagophytes and prymnesiophytes, both occurring at the SCM. Prymnesiophytes have been shown to be important · in primary production in the northern Mediterranean (Bustillos-Guzman et al. 1995, Barlow et al. 1997), in the equatorial Pacific, in the polar fronts of the Antarctic (Peeken 1997) and the northeast Atlantic (Mejanelle et al. 1995). Unlike diatoms, prymnesiophyte production is often balanced by microzooplankton grazing (Verity et al. 1996, Latasa et al. 1997).

Despite the abundance of the chromophytic forms at the SCM and associated carbon fixation, there was no chromophytic *rbcL* mRNA measured. We have previously found Form 1D *rbcL* mRNA at the SCM off Bermuda and in the Gulf of Mexico (Pichard et al. 1997b, Paul et al. 1999). We suspect that the lack of Form ID *rbcL* mRNA may have been caused by the time of sampling. We sampled between 07:00 and 10:00 h at both Stns 4 and 7. We have previously found a temporal separation between the Form IB and Form ID *rbcL* mRNAs, with the latter produced between 12:00 and 19:00 h. The chromophytic *rbcL* regulation is apparently not controlled by transcription, but by post transcriptional/translational events (Paul et al. 1999). We have found a temporal shift in *rbcL* transcription in cultures of the Prymnesiophyte *Pavlova gyrans* when compared to *Synechococcus* PCC7002 (J.B.K. & J.H.P. unpubl. obs.). Alternatively, the lack of Form ID *rbcL* mRNA at these stations could have been caused by another form of *rbcL* yet to be described.

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