Limnol. Oceanogr., 44(1), 1999, 12–23
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Evidence for a clade-specific temporal and spatial separation in ribulose bisphosphate carboxylase gene expression in phytoplankton populations off Cape Hatteras and Bermuda

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Abstract

The factors affecting the regulation of photosynthetic carbon fixation in diverse phytoplankton populations are not yet understood. To this end, we have measured the expression of the gene (*rbcL*) for the major carbon fixation enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase, in coastal phytoplankton populations off Cape Hatteras and in oligotrophic oceanic picoplankton near Bermuda. Using gene probes specific for the cyanobacterial/chlorophytic clade and the chromophytic clade (diatoms, chrysophytes, prymnesiophytes, and others) of Form I *rbcL* genes (''cyano'' and ''chromo'' probes, respectively), we have measured *rbcL* messenger ribonucleic acid (mRNA) levels in size-fractionated coastal waters, in a decktop diel incubator and a Lagrangian drifter study, and in vertical profiles in stratified, oligotrophic ocean water. In coastal waters influenced by estuarine plumes, an equal distribution of carbon fixation between the picoplankton and the micro/nannoplankton occurred, with cyano *rbcL* mRNA coinciding with *Synechococcus* counts in the <1-μm fraction, with the majority of the chromo *rbcL* mRNA expression occurring in the larger sized phytoplankton fraction. In profiles of oligotrophic oceanic waters, the cyano *rbcL* mRNA was found in the upper water column (~50-m depth) and coincided with peaks in *Synechococcus* counts. The chromo *rbcL* mRNA was concentrated at the subsurface chlorophyll *a* (Chl *a*) maximum (~85 m) and corresponded to red-fluorescing cell counts, thought to be picoeucaryotes and diatoms. Photosynthetic carbon fixation and RUBISCO enzyme activity encompassed both cyano and chromo *rbcL* mRNA peaks, suggesting a near equal contribution to carbon fixation in the water column by these two phytoplankton clades. Both decktop diel incubator studies and a Lagrangian drifter study in coastal waters indicated cyano *rbcL* transcription in the morning and chromophytic *rbcL* transcription in the late afternoon/early evening. Thus, the two major clades of RUBISCO-containing phytoplankton occupy separate niches in time, space, and cell size in the waters off Cape Hatteras. The factors determining such clade-specific niches may include efficiency of nutrient utilization, differences in relative carboxylase/oxygenase activity (τ values) of cyano (Form IB) and chromophytic (Form ID) RUBISCOs, and differences in pigment composition/adaptation to light regimes. Additionally, we propose that chromo *rbcL* mRNA may be indicative of new production, whereas cyano *rbcL* mRNA correlates with recycled production in stratified, oligotrophic oceanic environments.

Although photosynthetic carbon fixation is one of the most important biological processes occurring in the ocean, little is known concerning the molecular regulation of this process in natural phytoplankton assemblages. The enzyme responsible for the vast majority of photosynthetic carbon fixation by phytoplankton is ribulose bisphosphate carboxylase/oxygenase (RUBISCO) (Raven 1993). There is evidence for β-carboxylation (Descolas-Gros and Fontugne 1985; Colman 1989; Collos et al. 1992) occurring in natural populations, but the quantitative significance of this process is minimal under most conditions.

A diversity of phytoplankton taxa participate in photosynthetic carbon fixation in mesotrophic coastal environments and oligotrophic offshore communities. Coastal phytoplankton populations are characterized by chain-forming diatoms and phytoflagellates (Marshall and Nesius 1996; Webber and Roff 1996), with picocyanobacteria contributing significantly to primary production, usually in the summer (Malone et al. 1991). Primary production in offshore waters is dominated by autotrophic picoplankton (Waterbury et al. 1979; Li et al. 1983), which are comprised of three major groups: *Synechococcus* (Johnson and Sieburth 1979; Itturiaga and Mitchell 1986; Stockner 1988), *Prochlorococcus* (Chisholm et al. 1988; Campbell et al. 1994), and picoeucaryotes (Campbell and Vaulot 1994; Li 1994). How these diverse groups regulate the expression of their carbon fixation genes has become a topic of major interest to us.

The RUBISCO enzyme exists in nature as two forms, Form I and Form II (Tabita 1988). The vast majority of the phytoplankton contain Form I RUBISCOs (Pichard et al. 1997*b;* Watson and Tabita 1997), which are comprised of eight large and small subunits each (L_8S_8) (Tabita 1988). The Form II enzyme, believed to be an evolutionary precursor of the Form I enzyme, consists of a large subunit dimer (L_2). The Form II enzyme was thought to be limited to nonsulfur bacteria and chemotrophs but has recently been shown to occur in peridinin-containing marine dinoflagellates (Morse

Acknowledgments
This work was supported by NSF grants OCE 9216937 and BIR 9512122 and DOE grant DE-FG05-93ER6199 to J.H.P. and a Knight Ocean Research Fellowship to S.L.P.

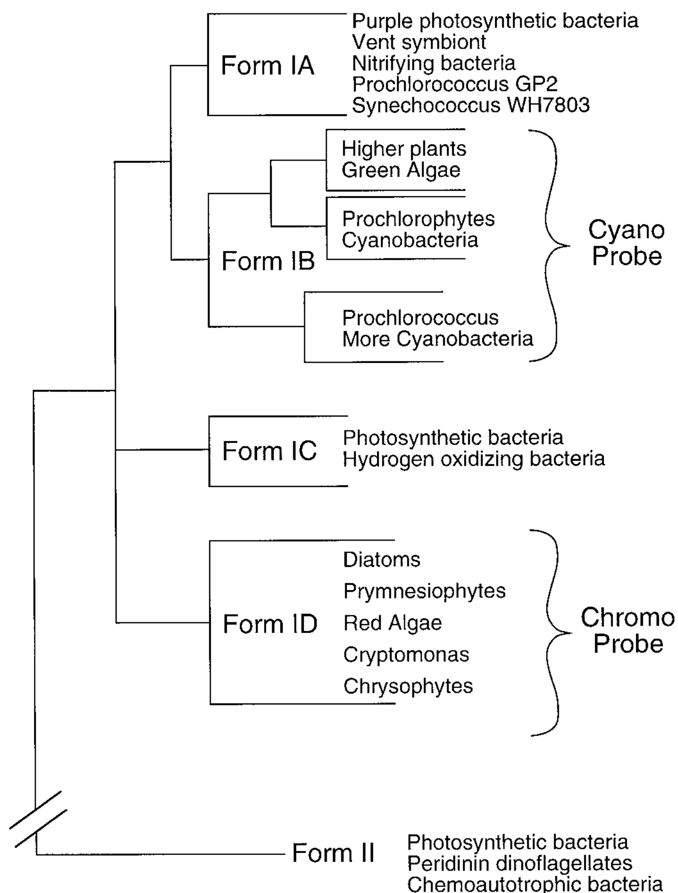

Fig. 1. Representation of the phylogenetic relationships of *rbcL* genes of major phytoplankton and other microbial groups, with indication of hybridization to the cyano and chromo *rbcL* gene probes.

et al. 1995; Rowan et al. 1996). The small subunits of the Form I enzymes are divergent in sequence, whereas the large (catalytic) subunits have been conserved within the major groups of autotrophs (Tabita 1988).

The large subunit types of Form I RUBISCOs found in phytoplankton are further divided between the cyanobacterial/green algal line of plant evolution (Form IB) and the chromophytic algal (including diatoms, prymnesiophytes, pelagophytes, and chrysophytes) line of evolution (Form ID) (Tabita 1995; Pichard et al. 1997*b;* Watson and Tabita 1997; Fig. 1). Form IA is found primarily in sulfur chemotrophic and photosynthetic bacteria (Tabita 1995) but was also recently found in *Synechococcus* WH7803 (Watson and Tabita 1996). The Form IC group of RUBISCO is comprised primarily of enzymes from nonsulfur purple and select autotrophic bacteria, with no known members in the phytoplankton.

There are a myriad of regulatory mechanisms for RUBISCO, including activation or inhibition by small molecular weight compounds and molecular mechanisms such as transcription, translation, and posttranslational modification of the enzyme (Falkowski and Raven 1997; Paul and Pichard 1998). Activation of the enzyme by CO_2 (termed ''carbamylation'') is central to this regulation (Hartman and Harpel 1994). Phosphorylated sugars or sugar alcohols can bind to the carbamylated or uncarbamylated enzyme to activate or inactivate the enzyme, respectively, at least in higher plants (Paul and Pichard 1998). The enzyme RUBISCO activase also plays a role in removing sugar bisphosphates from the active and inactivated enzyme (Portis 1995). Transcriptional regulation has been shown to be a major mechanism for regulating RUBISCO activity in a myriad of cyanobacteria, algae, and higher plants (*see* Paul and Pichard [1998] for review).

The regulation of RUBISCO gene expression in natural phytoplankton populations has recently been studied by mRNA analysis (Pichard et al. 1993, 1996, 1997*a*). The detection of transcriptionally active, specific components of the phytoplankton community can yield information on which of these are contributing to primary production. Additionally, elucidating the environmental cues that control this process in the various components of the phytoplankton will enable a better understanding of carbon fixation in general. The development of clade-specific RUBISCO gene probes has enabled us to begin deciphering the transcriptionally active components of the phytoplankton.

We have developed hybridization probes specific for the Form IB (cyanobacterial, green algal line—termed ''cyano'' probe) and the Form ID (chromophytic algae—termed ''chromo'' probe) RUBISCO large subunit genes (*rbcL*) (Pichard et al. 1997*a*). We have demonstrated the hybridization specificity of these probes with cultures of representative phytoplankton and have used them to study the expression of the *rbcL* genes in natural phytoplankton populations of the SE Gulf of Mexico. We extend the use of these probes to detect RUBISCO gene expression in the nearshore waters of the South Atlantic Bight near Cape Hatteras and oligotrophic waters near Bermuda.

Methods

Study sites—The locations of sampling sites appear in Fig. 2A. Samples were taken during cruises on the R/V *Cape Hatteras* (cruise CH-0995) from 18 July 1995 to 26 July 1995 and the R/V *Seward Johnson* (cruise SJ9607) from 22 July 1996 to 26 July 1996.

Sampling—During the Cape Hatteras cruise, surface water samples (3-m depth) were collected into acid-cleaned carboys by use of a submersible pump, while all subsurface samples were collected by use of a rosette sampler equipped with a Seabird conductivity-temperature-depth (CTD) with a polarographic O_2 sensor and blue light fluorometer. For the decktop incubator study, a 400-liter acid-cleaned polyethylene tank (Nalge) was filled by lowering a submersible pump over the side of the ship to a depth of 3 m. The tank was covered with two layers of neutral-density screening to simulate light intensity at 3 m, while temperature was maintained with a refrigerated cooling recirculator. Samples were collected every 4 h by siphoning into 20-liter acid-cleaned polycarbonate carboys. For the Seward Johnson cruise, all samples were taken with a rosette sampler at the times indicated in Fig. 7. For the Lagrangian study, two Brightwater drifters equipped with global positioning systems (GPSs), ARGOS telemetry ashore, and very high frequency radio

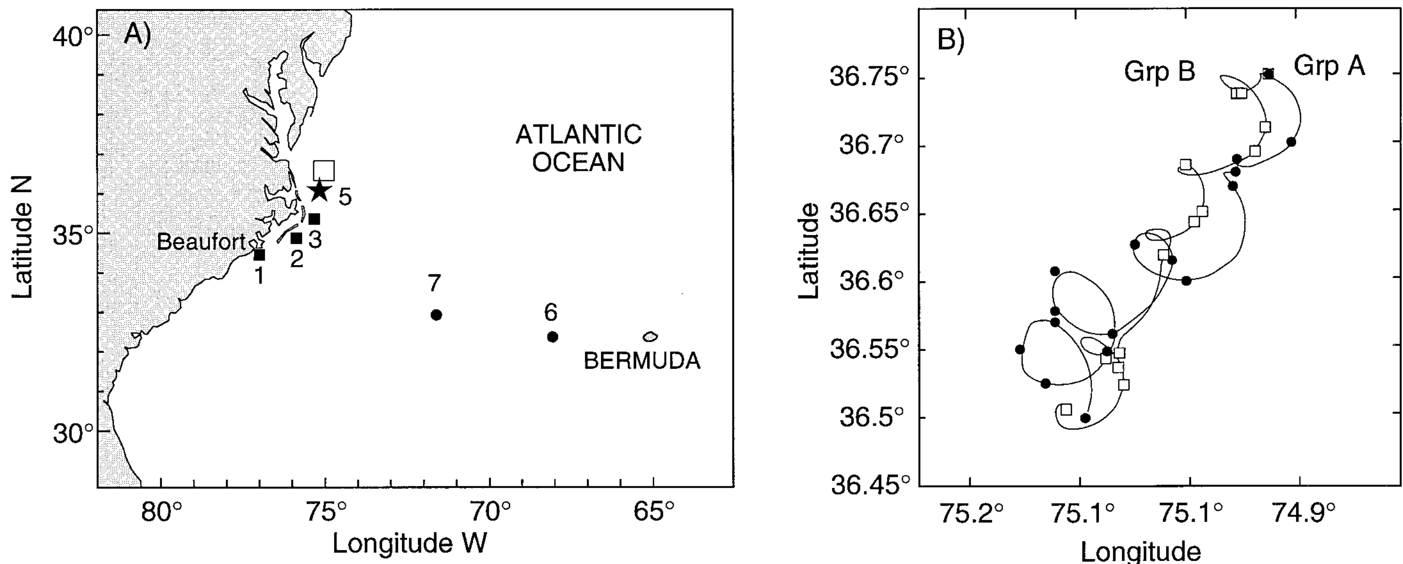

Fig. 2. (A) Location of stations sampled during the R/V *Hatteras* cruise. Squares and star indicate location of samplings for size-fractionation studies. Star also indicates location of decktop incubator diel study. Box is the location of the Lagrangian drifter study, as displayed in Panel B. (B) Telemetry location of surface (Group A, ●) and subsurface (Group B, □) drifters during the Lagrangian study. Symbols indicate location of samplings.

telemetry to the ship were employed. One drifter (Group A) was deployed in the upper layer between 4 and 10 m, and the other (Group B) was deployed in the lower layer between 18 and 24 m (Fig. 2B). Sampling occurred approximately every 12 h, around 0900 h and 2100 h.

rbcL *mRNA analysis*—Five hundred-milliliter to 1-liter samples were amended with diethyl pyrocarbonate (DEPC; Sigma Chemical) to 0.1% and filtered in duplicate on 25-mm, 0.45-μm Durapore polyvinylidene difluoride membranes (Millipore). One milliliter of guanidinium isothiocyanate reagent (Pichard et al. 1993; Paul and Pichard 1995) was added, and the RNA was extracted by bead beating (0.1–0.15-mm glass beads) shipboard (R/V *Hatteras* cruise), or the samples were frozen in liquid nitrogen (R/V *Seward Johnson* cruise) until extraction (between 10 and 20 d after collection). RNA extraction, dotting, and probing were performed as previously described (Pichard et al. 1993; Paul and Pichard 1995). Duplicate samples were probed with both the cyano probe (derived originally from *Synechococcus* PCC 6301) and the chromo probe (derived from *Cylindrotheca* sp.) (Pichard et al. 1997*a*). These probes have been shown to specifically hybridize to phytoplankton containing Form IB RUBISCO (cyano probe) or Form ID RUBISCO (chromo probe) (Pichard et al. 1997*a*).

Chl a *and cell counts*—Samples for Chl *a* were collected by filtration onto 25-mm Whatman GF/F filters and stored frozen until analysis. Chl *a* concentrations were determined fluorometrically (Holm-Hansen and Riemann 1978). Autofluorescent cell counts (phycoerythrin [PE]-containing cyanobacteria as *Synechococcus* and red-fluorescing cells, believed primarily to be picoeucaryotes and some larger eucaryotic phytoplankton; Pichard et al. 1997*a*) were performed as described by Vernet et al. (1990). Diatom and dinoflagellate counts were performed as described by Sournia (1978).

^{14}C carbon fixation—All ^{14}C-carbon fixation studies were performed essentially as described in Pichard et al. (1993, 1997*a*), using sterile, acid-cleaned 500-ml or 1-liter polycarbonate flasks. For diel and size-fractionation studies in coastal waters, incubations were performed in an incubator equipped with cool-white fluorescent bulbs at 75–89 μE s^{-1} m^{-2}. Samples were size fractionated at the end of the incubation period by filtration through 5- or 1-μm, 47-mm Nuclepore filters prior to filtration though 0.2-μm, 25-mm Millipore GS filters. For profiles, on-deck incubations were performed with natural irradiance that was adjusted to resemble the intensity and spectral features of the underwater light field through the use of neutral-density screening and colored acetate filters. Irradiance intensity as a function of depth was determined by use of a Li-Cor underwater light meter equipped with Li-190SA and Li-192SA surface- and underwater photosynthetically active radiation sensors.

RUBISCO enzyme activity—Samples (20-liter) were concentrated to 50 ml by vortex flow filtration using a Benchmark rotary biofiltration device (Jiang et al. 1992). Ten-milliliter aliquots of the concentrated retentate were further concentrated by centrifugation at 10,000 $\times$ g at 4°C. The pellets were resuspended in 300–500 μl seawater, then combined and centrifuged in a microcentrifuge. The pellets were frozen in liquid nitrogen until analysis. RUBISCO activity was essentially determined by the method of Read and Tabita (1992).

Results

Size distribution of rbcL *expression in coastal environments*—Table 1 shows the temperature, salinity, and bacte-

Table 1. Temperature, salinity, bacterial direct counts, and RUBISCO activity of coastal stations used in size-fractionation studies.

Station	Sample depth (m)	Temp. (°C)	Sal. (ppt)	Bacterial direct counts (10^6 ml^{-1})	RUBISCO activity (pmol min^{-1} liter^{-1})
1	3	28.2	34.8	1.5±0.16	3.9
2	3	27.6	35.6	0.45±0.8	3.7
3	3	27.8	33.9	1.58±0.21	30.3
5S	3	26.4	31.8	0.33±0.38	134.9
5B	25	16	33.2	1.3±0.24	25.2

rial direct counts for coastal Sta. 1–5 (Fig. 2). Carbon fixation and Chl *a* are parameters common to all members of the active phytoplankton community, and the size distribution for these appears in Fig. 3A,B. In the surface waters of Sta. 1, 3, and 5S, ~40% of the Chl *a* and carbon fixation was in the >5-μm fraction, indicative of eucaryotes and chain-forming diatoms. Approximately 20–30% of the carbon fixation and Chl *a* occurred in particles <1 μm in size (picoplankton) at the same stations. Thus, Sta. 1, 3, and 5 showed an approximately equal distribution in carbon fixation and biomass between larger phytoplankton and ultra- and picoplankton. These stations were in estuarine plumes, as indicated by surface-water salinity (31–34 ppt; Table 1). Sta. 2, apparently influenced by oceanic water (salinity = 35.6 ppt), had the lowest Chl *a* and carbon fixation values of all four stations, with the picoplankton-sized fraction ac-

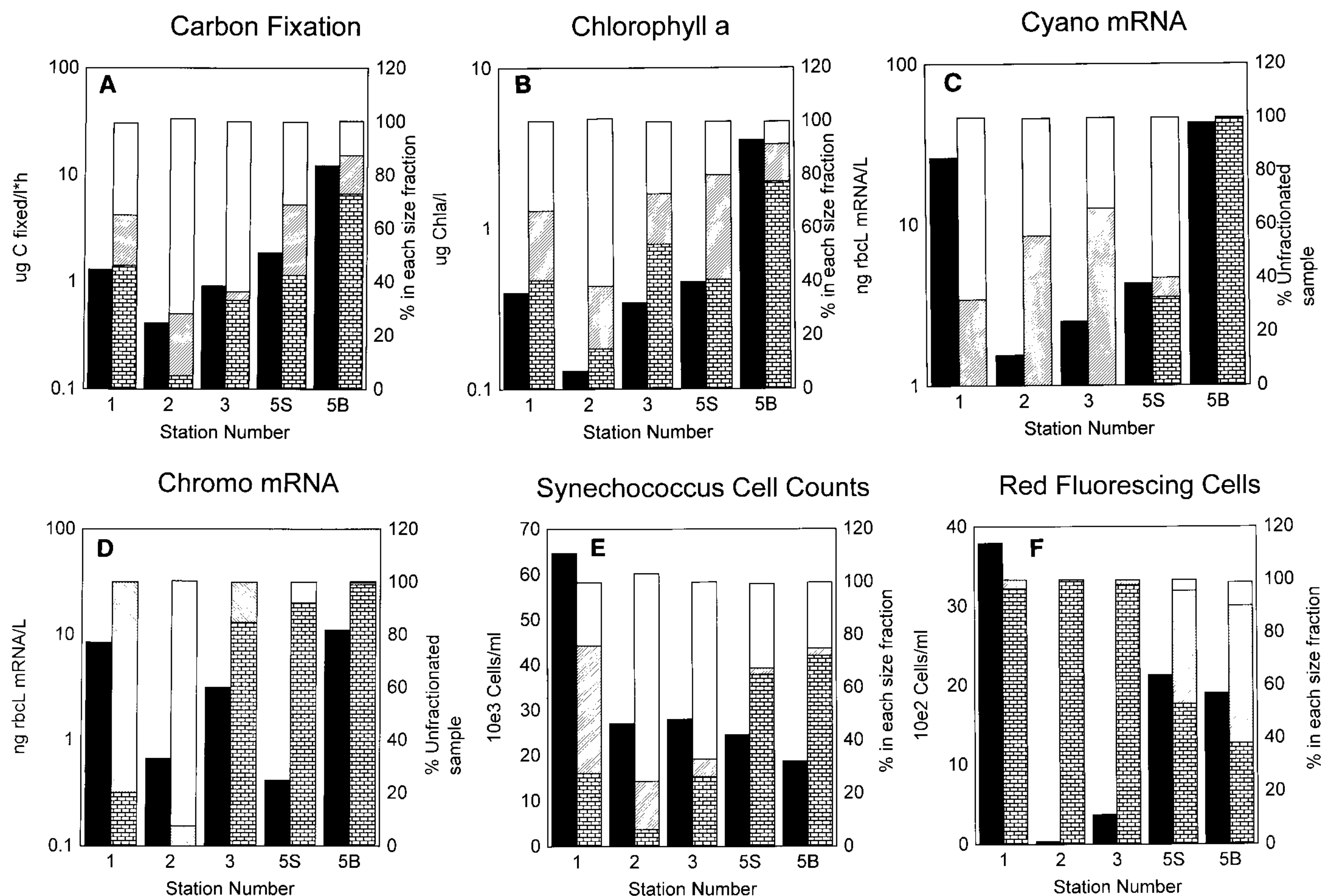

Fig. 3. Size distribution of carbon fixation (Panel A), Chl *a* (Panel B), cyano *rbcL* mRNA (Panel C), chromo *rbcL* mRNA (Panel D), *Synechococcus* cell counts (Panel E), and red-fluorescing cells (Panel F). The solid bars are the values for unfractionated samples and are given in values of concentration or abundance/volume seawater in the left Y axis. The other bars are the result of size fractionation and are given as percent in each size fraction. The open bars indicate the 0.2- to 1-μm–sized fraction, the cross-hatched bar indicates the 1- to 5-μm–size fraction, and the brick pattern designates the >5-μm size fraction.

counting for 62 and 72% of the Chl *a* and carbon fixation, respectively. At Sta. 5B, sampled near the bottom (at 25 m of 30-m total depth), the phytoplankton was dominated by larger organisms, with >70% of the Chl *a* and carbon fixation present in the >5-μm size fraction.

Figure 3C,D shows the size distributions of the cyano and chromo *rbcL* mRNA-containing particles. The cyano mRNA was primarily associated with the smaller sized particles in the surface waters of all the stations, with 34–68% <1 μm and 67–100% <5 μm in size. The exception to this pattern was again in the bottom water of Sta. 5, of which 99% of the *rbcL* mRNA-containing particles were >5 μm. Because this was near the bottom, there may have been a large number of phytoplankton cells attached to resuspended particles. The size distribution of cyano *rbcL* mRNA closely resembled that of the PE-containing cyanobacterial counts (orange-fluorescing *Synechococcus* counts; Fig. 3E). As with cyano *rbcL* mRNA, very high concentrations of *Synechococcus*-like cells were found at Sta. 1 near the mouth of the Pamlico Sound estuary. The *Synechococcus* cell counts were primarily associated with the <1- and 1–5-μm fractions, as found for the cyano *rbcL* mRNA.

The chromo *rbcL* mRNA was primarily associated with the >5-μm fraction (Sta. 3, 5) or the 1–5-μm fraction (Sta. 1), indicating that the chromophytic *rbcL*-containing organisms were larger than the cyano *rbcL*-containing organisms. The exception to this was again found for Sta. 2, where even the chromophytic *rbcL* mRNA was associated with small particles (>93% were <1 μm). Thus, both cyanobacterial and chromophytic organisms comprised the picoplankton at this station. Figure 3F shows the size distribution of red-fluorescing cells. These organisms are primarily larger eucaryotes and were associated with the largest size fraction. Sta. 2 had a very low number of red-fluorescing cells, although they were all (>97%) >5 μm in size. These data further illustrate the dominance of picoplankton at this inshore station.

The overall size distribution of carbon fixation (Fig. 3A) most closely resembled the size distribution of PE-containing *Synechococcus* cells (Fig. 3E) and that of cyano *rbcL* mRNA, indicating that these organisms were perhaps the major contributors to carbon fixation in these waters. The lack of cyano *rbcL* mRNA in the >5-μm fraction indicates the importance of chromophytic *rbcL* gene expression in determining carbon fixation for the large phytoplankton. The distribution of red-fluorescing cells (Fig. 3F) did not follow the size distribution of carbon fixation (Fig. 3A).

Vertical distribution of cyano and chromophytic rbcL *gene expression near Bermuda*—Two vertical profiles were performed in offshore waters near Bermuda, one each at Sta. 6 (east of Bermuda) and Sta. 7 (eastern edge of the Gulf Stream; Fig. 1). Similar results were obtained from both, and the results of the profile for Sta. 6 appear in Figs. 4, 5. Figure 4 shows the in situ fluorescence, salinity, and temperature data for the station, which indicated a broad subsurface chlorophyll maximum (SCM) from 80 to 100 m in depth. The mixed layer was approximately 25 m deep, with the thermocline spanning 25–40 m. Figure 5A shows photosynthetic carbon fixation, RUBISCO enzyme activity, Chl *a,* and % surface illumination (%I_o). The 1% surface illumination and the Chl *a* maximum occurred at approximately 85 m, indicating the oligotrophic nature of this station. Photosynthetic carbon fixation and RUBISCO activity followed a nearly identical trend, with a broad subsurface peak spanning 55–85 m deep. Figure 5B shows the distribution of cyano *rbcL* mRNA, *Synechococcus* counts, and dinoflagellate counts with depth. A similar pattern of abundance with depth was observed for the *Synechococcus* cell counts and cyano mRNA, with a peak in the surface water, a decrease at 25 m, and a subsurface peak at 55 m. This peak also corresponded to the depth of the greatest dinoflagellate abundance. The upper half (55 m) of the subsurface carbon fixation peak seems to be due to picocyanobacterial carbon fixation. The high level of cyano mRNA in the surface water did not correspond to a high level of carbon fixation, indicating that another factor besides transcriptional regulation was controlling RUBISCO activity there (i.e., perhaps some posttranslational modification). Alternatively, C fixation (and RUBISCO activity) could have been limited by some other factor (nutrients?) or by photoinhibition in the surface waters.

Figure 5C shows the distribution of chromophytic *rbcL* mRNA, diatom counts, and red-fluorescing cells. Chromo *rbcL* mRNA occurred at the depth of the Chl *a* maximum (85 m), where elevated levels of diatoms and red-fluorescing cells (presumably picoeucaryotes) were also found. Thus, the bottom half of the subsurface carbon fixation/RUBISCO activity peak was characterized by chromophytic carbon fixation, including diatoms and picoeucaryotes. A vertical separation between these two evolutionary clades of Form I RUBISCO-containing organisms occurred, with Form IB in the upper water column and Form ID in the lower water column.

Diel regulation of cyano and chromo rbcL *transcript abundance*—The results of a diel decktop incubator study with a surface-water sample from Sta. 5 appear in Fig. 6. Carbon fixation and *rbcL* mRNA appear in Fig. 6A, while the biomass parameters of Chl *a* and autofluorescent cell counts appear in Panel B. A diel pattern of carbon fixation at a fixed light intensity was observed, with the greatest carbon fixation occurring in the morning and the lowest occurring at the 2300- and 0300-h samplings. Strong evidence of diel regulation of cyano *rbcL* transcription was found, with *rbcL* abundance increasing in the morning and peaking at 1500 h. By 1900 h, there was no detectable cyano mRNA signal. The chromo *rbcL* mRNA also showed strong evidence of diel regulation of transcript abundance, but it peaked 4 h (1900 h) after the peak in the cyano *rbcL* expression. There was also evidence of diel variation in RUBISCO specific activity (units per milligram of protein), which closely followed variations in carbon fixation. However, when expressed as activity per milliliter of seawater, no obvious diel trend was observed (data not shown). There was little evidence of diel variation in autotrophic cell counts or Chl *a,* although a slight elevation in these parameters occurred at the 1500-h sampling. Thus, the diel changes noted in carbon fixation and *rbcL* mRNA were not caused by variation in autotrophic population size but were most likely caused by

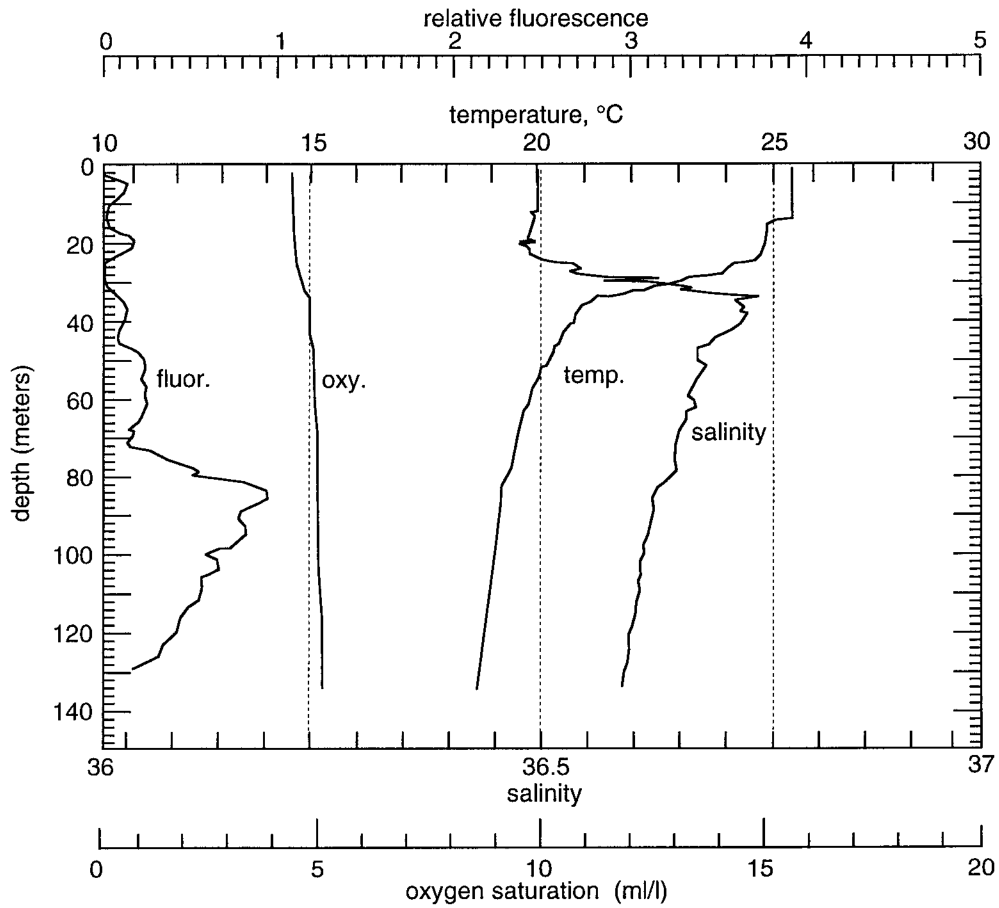

Fig. 4. In vivo fluorescence, salinity, dissolved oxygen, and temperature data obtained from CTD profile at Sta. 6.

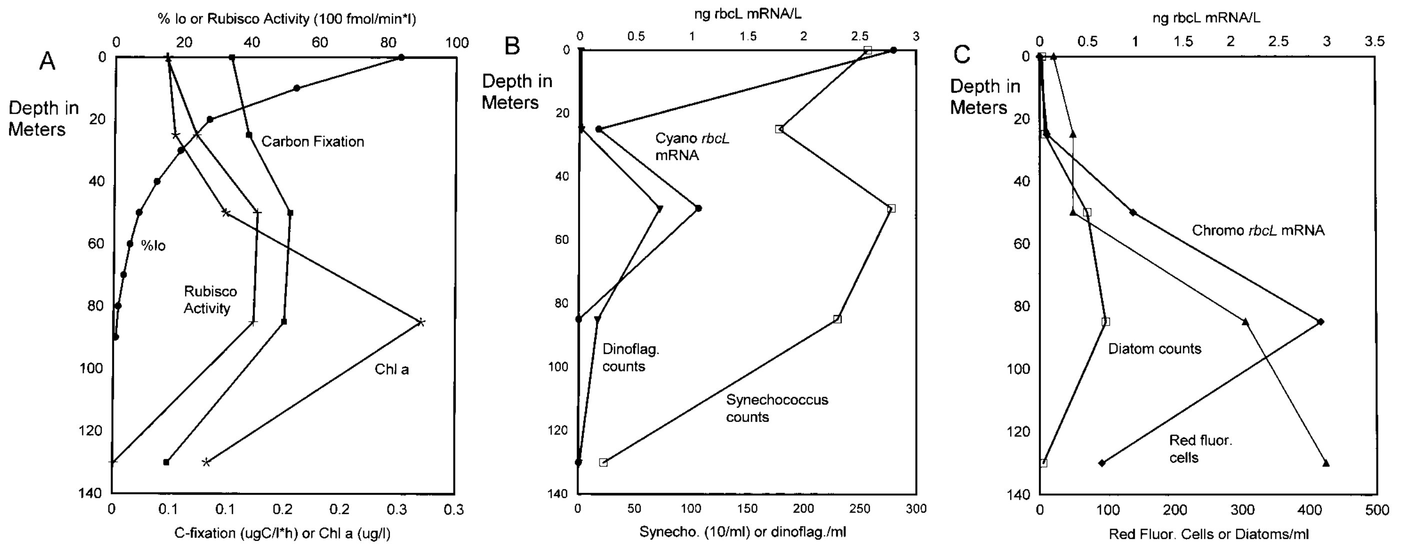

Fig. 5. Profile of parameters measured at Sta. 6 near Bermuda. Panel A: % surface irradiance (I_o, circles), carbon fixation (squares), RUBISCO activity (crosses), and Chl *a* (asterisks). Panel B: cyano *rbcL* mRNA (circles/dashed line), PE-containing picocyanobacteria (*Synechococcus* counts, squares), and dinoflagellate counts (inverted triangles). Panel C: chromo *rbcL* mRNA (dashed line/diamonds), red-fluorescing cells (triangles), and diatom counts (squares).

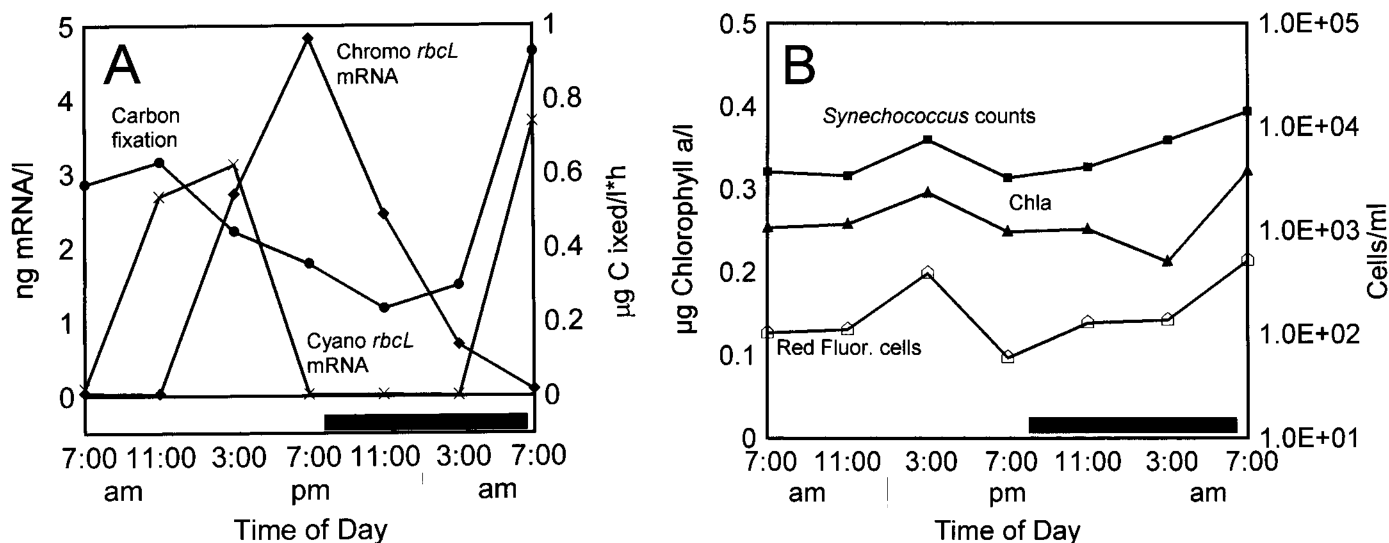

Fig. 6. Decktop diel incubator study at Sta. 5. Panel A, activity measures: carbon fixation (circles), cyano *rbcL* mRNA (dashed line), and chromo *rbcL* mRNA (diamonds). Panel B, biomass measures: PE-containing picocyanobacteria *(Synechococcus* counts, squares), red-fluorescing cells (pentagons), and Chl *a* (triangles).

transcriptional regulation. Additionally, these data suggest the presence of a temporal separation in expression of the cyano and chromo *rbcL* genes.

To further confirm or negate the observation of a temporal separation in expression of these two evolutionary RUBISCO clades, a Lagrangian study was performed in conjunction with the U.S. Department of Energy's Ocean Margin Program in the same approximate area as Sta. 5 (Fig. 1). Panel A (Fig. 7) is from the surface drifter, while Panel B is from the subsurface drifter. Sampling could only occur every 10–12 h because of experimental design constraints. As seen in the diel decktop incubator study, cyano and chromo *rbcL* mRNA levels were out of phase with one another, with cyano *rbcL* mRNA maximal in the morning and chromo mRNA maximal in the evening. These studies corroborate the temporal separation in transcription of these two clades of RUBISCO large subunit genes.

Multiple regression analysis was performed using the entire data set, the whole (nonsize fractionated) data set, the tank diel and profile data, and the profile data alone. For each data set, three regressions were performed, one each using the carbon fixation, cyano *rbcL,* and chromo *rbcL* data as the dependent variable (Table 2). When primary production was the dependent variable, *Synechococcus* cell counts were used as a significant independent variable in three of four analyses performed. Variation in Chl *a* accounted for a

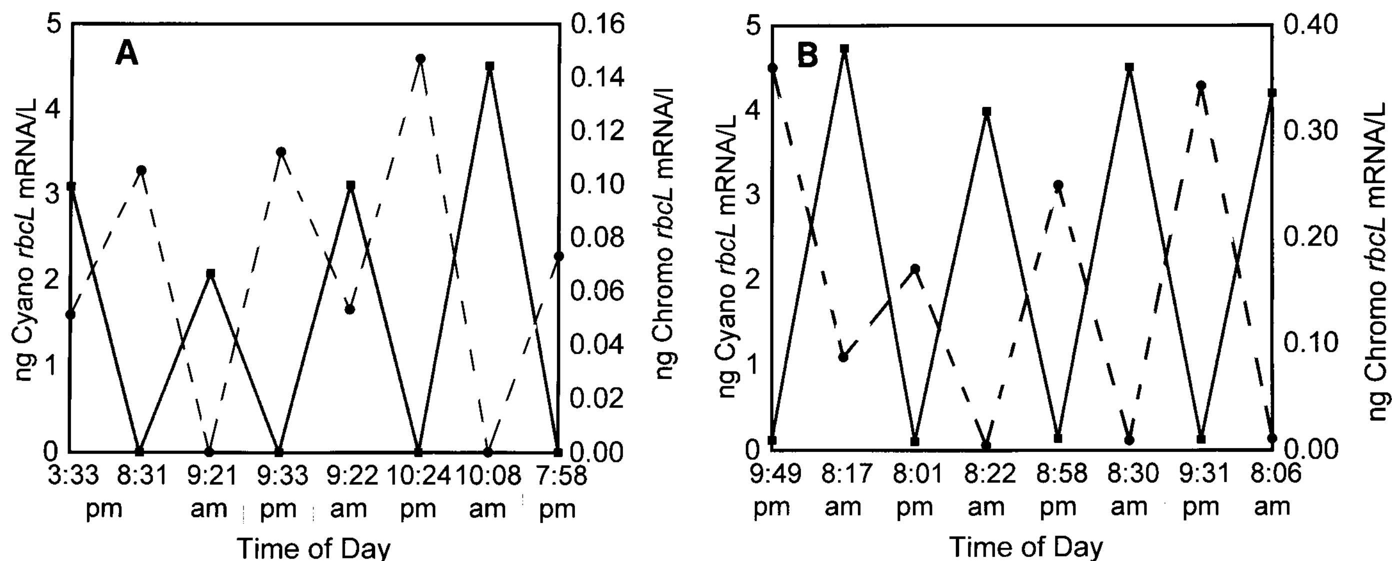

Fig. 7. Cyano (squares) and chromo (circles) *rbcL* mRNA of the surface drifter-tracked water (Panel A) or the subsurface drifter-tracked water (Panel B).

Table 2. Determination of significant independent varibles in multiple regression analyses.

Data analyzed	Dependent variable	Significant independent variables	*F* value*	*P* value	r^2
All data†	C-fix‡	Chl *a*,§ Chrom,‖ Syn¶	870	<0.0001	0.988
	Cyano#	Chrom, C-fix, Syn	44.4	<0.0001	0.812
	Chromo	Cyano	72.6	<0.0001	0.687
Unsize-fractionated samples**	C-fix	Chl *a*	2,249	<0.0001	0.989
	Cyano	C-fix, Chromo Red,†† RuB‡‡ Syn	117.1	<0.0001	0.968
	Chromo	Cyano	72.4	<0.0001	0.759
Profile and diel§§	C-fix	Chl *a*, Syn	24.2	<0.0001	0.739
	Cyano	C-fix	8.44	0.0095	0.31
	Chromo	Rub	7.58	0.0131	0.29
Profile only‖‖	C-fix	Rub, Syn	16.11	0.002	0.59
	Cyano	NS¶¶	NS	NS	NS
	Chromo	Chl *a*	12.83	0.0043	0.53

* Analysis of variance *F* value for the overall significance of the regression.
† All data are for all stations, including size-fractionated data, exclusive of the Lagrangian study from the R/V *Seward Johnson* cruise.
‡ C-fix is photosynthetic carbon fixation.
§ Chl *a* is chlorophyll *a.*
‖ Chrom is chromo *rbcL* mRNA.
¶ Syn is *Synechococcus* cell counts.
# Cyano is cyano *rbcL* mRNA.
** Unsize-fractionated samples are the data for whole-water samples only (no <5- or <1-μm data included).
†† Red is red-fluorescing cells.
‡‡ RuB is RUBISCO enzyme activity.
§§ Profile and diel signifies data from Sta. 6, 7 vertical profiles, and diel is the decktop incubator data.
‖‖ Profile only is data for Sta. 6, 7.
¶¶ NS, the regression was not significant.

significant proportion of the variability in primary production in three of four analyses and for variation in the chromo *rbcL* mRNA in two of four analyses. Such results argue strongly for the importance of picocyanobacteria in carbon fixation in these waters. The entire data set and that for unfractionated samples, which are heavily weighted by coastal data, showed a strong influence of chromo *rbcL* mRNA, indicating the importance of chromophytes in carbon fixation in coastal waters. When cyano *rbcL* mRNA was the dependent variable, the variation in this parameter could be explained by primary production and *Synechococcus* counts in the entire data set and the unfractionated data sets, indicating the interrelationship between carbon fixation and cyano *rbcL* expression. When chromo *rbcL* mRNA was the dependent variable, cyano *rbcL* mRNA was the only significant independent variable in the entire and nonsize-fractionated data set. We think this is the result of nearshore to offshore autocorrelation, because extremely high values for both of these parameters were found at Sta. 1, 5B. In the profiles (Sta. 6, 7), chromo *rbcL* variation was effectively explained by variation in Chl *a,* as indicated in Fig. 5. Thus, the abundance of chromophytes in the SCM can account for this relationship.

Discussion

Size distribution of RUBISCO gene expression in coastal waters—Environments under the influence of plumes from Pamlico Sound (Sta. 1) and Chesapeake Bay (Sta. 3, 5) showed both active picoplankton (~30% < 1 μm) and micro/nannoplankton (40% > 5 μm) fractions in terms of carbon fixation and *rbcL* gene expression. The <1-μm fraction was characterized by cyanobacterial mRNA transcription and an abundance of PE-containing picocyanobacteria. The larger sized chromophytic *rbcL* mRNA values found here were presumably caused by diatoms, which are abundant in these waters. A similar size distribution of autotrophs was found in the Thau Lagoon in the Mediterranean (Vaquer et al. 1996) and in coastal Gulf of Mexico waters under the influence of the Tampa Bay plume (Pichard et al. 1997*a*). In the latter environment, 43% of the carbon fixation was associated with particles <1 μm in size, with 25 and 31% associated with particles >5 and 1–5 μm, respectively. Sta. 2, not in the direct plume of any estuary and perhaps influenced by Gulf Stream water (salinity = 35.8 ppt) showed a particle size distribution characteristic of autotrophic picoplankton, including picoeucaryotes, with 70% of the primary production, 44% of the cyano *rbcL* mRNA, and 93% of the chromophytic mRNA associated with particles <1 μm in size. A similar picoplankton-dominated distribution for cyano *rbcL* transcription has been found in the offshore oligotrophic waters of the SE Gulf of Mexico, where 84–100% was associated with particles <1 μm in size. However, even in these waters, the chromo *rbcL* mRNA was primarily associated with >1- or >5-μm–sized particles (Pichard et al. 1997*a*), corresponding to ca. 36% of the carbon fixation. We

do not know the nature of the chromophytes in the <1-μm fraction of Sta. 2. Chromophytic picoeucaryotes known to occur in the Atlantic include prymnesiophytes and pelagophytes (Andersen et al. 1993, 1996). Rappe et al. (1995) also noted the presence of chromophytelike algae in the subtropical Atlantic based on identification of plastid ribosomal ribonucleic acid (rRNA) genes.

The abundance of larger particles in the subsurface sample at Sta. 5B was either the result of resuspension of benthic diatoms and chlorophytes or a bloom of larger phytoplankton brought about by intrusion of cold, high-nutrient water. A steep thermocline was found at 10 m at this station. It is well known that under nutrient replete conditions or in the presence of elevated nitrate N, larger chromophytic organisms are favored over picoplankton (Malone 1980; Chisholm et al. 1992; Riegman et al. 1993; Clarke and Leakey 1996). The source of this cold, high-nutrient water is unknown, but the autotrophic size distribution and salinity (33 ppt) are indicative of estuarine water (Pichard et al. 1997*a*). For example, inside the mouth of Tampa Bay, we have previously found 88% of the cyano *rbcL* mRNA, 80% of the carbon fixation, and 60% of the Chl *a* in particles >5 μm in size (Pichard et al. 1997*a*), similar to that observed for Sta. 5B.

Vertical distribution of RUBISCO gene expression—Both profiles at Sta. 6, 7 (data not shown) indicated a spatial separation of the cyanobacterial/green clade of *rbcL* evolution (Form IB) from the chromophytic (Form ID *rbcL*) phytoplankton, with the former in the upper portion of the water column and the chromophytes located at the SCM. The peak in cyano *rbcL* mRNA (50 m) coincided with the peak in maximal PE-containing cyanobacteria (*Synechococcus* counts), while the peak in chromo *rbcL* mRNA coincided with an abundance of red-fluorescent cell counts (presumably picoeucaryotes) and diatom counts. A similar vertical distribution of cyano and chromo *rbcL* mRNA was observed in the oligotrophic regions of the SE Gulf of Mexico (Pichard et al. 1997*a*). In that study, both flow cytometry and pigment analyses were performed. *Synechococcus* cells were most abundant in the surface waters (3–25 m), with *Prochlorococcus* abundant (2 to 3 $\times$ 10^5 cells ml^{-1}) at 50 m and picoeucaryotes at the SCM (68–80 m). Pigment analysis of the SCM indicated the presence of fucoxanthin, 19′-butanoyloxyfucoxanthin, and 19′-hexanoyloxyfucoxanthin, indicative of diatoms and other heterokont chromophytes, pelagophytes, and prymnesiophytes, respectively. Zeaxanthin, indicative of prochlorophytes and *Synechococcus,* peaked in waters above the SCM. Others have found evidence for picoeucaryote dominance of the SCM. The proportion of pelagophytes was found to increase with depth at Bermuda and Hawaii and to dominate the SCM (>100 m; Andersen et al. 1996). Letelier et al. (1993) found prymnesiophytes and chrysophytes to make up 35% of the autotrophic biomass at the SCM at station ALOHA, Hawaii. Furuya (1990) found the SCM to be composed primarily of picoeucaryotes in the West Pacific Ocean. Others have found prochlorophytes abundant at the SCM (Shimada et al. 1993).

Diel regulation of rbcL *gene expression in coastal waters*—Our decktop tank incubator study showed clear evidence of diel regulation of *rbcL* transcript abundance, with greater concentrations of *rbcL* transcripts in the light than in the dark. This could have been caused by changes in the rate of message production (transcriptional regulation) or modulation of transcript degradation (posttranscriptional regulation). A previous study conducted in the Gulf of Mexico indicated diel regulation of cyano *rbcL* transcript abundance in decktop incubator studies of natural populations, Lagrangian studies of a water mass, and a laboratory study with a *Prochlorococcus* strain in culture (Pichard et al. 1996). We did not anticipate the temporal shift observed between cyano and chromo *rbcL* expression, whereby cyano transcription occurred in the morning, followed by chromo *rbcL* expression in the afternoon. Diel regulation of *rbcL* transcription has been studied in cyanobacteria (Chow and Tabita 1994; Li and Tabita 1994) and in the chromophyte *Heterosigma carterae* (Reynolds et al. 1993). In both of these groups of organisms, transcription occurred in the light, then began to decrease toward the end of the light cycle, reaching a minimum during the dark cycle. An ''anticipatory'' increase in transcription occurs near the end of the dark phase, prior to receiving any cue by light (Reynolds et al. 1993; Watson and Tabita 1996). Why these two types of transcription are offset in natural phytoplankton populations is unknown.

Diel fluctuations in photosynthesis and diurnal rhythms in phytoplankton production have been known for years (Doty and Oguri 1957; Verduin 1957; Sournia 1974). Cuhel et al. (1984) observed synthesis of protein in algae at night, and Collos et al. (1989) found nocturnal increases in Chl *a* and particulate N, with decreases in these parameters during the day. Hama and Handa (1992*a*) found production of glucose in the day by phytoplankton populations and consumption at night. These investigators also found amino acid synthesis to occur in the morning (0900–1200 h), with fatty acid synthesis occurring in the afternoon. This was attributed to nitrogen consumption in the morning, resulting in N-limited fatty acid synthesis in the afternoon (Hama and Handa 1992*b*). Prezlin et al. (1987) found no clear diel pattern in RUBISCO activity for water samples enclosed in 200-liter vats for 38 h but observed an overall increase in activity over the incubation period. We found evidence of diurnal levels in RUBISCO specific activity, which were probably due in part to transcriptional and other forms of regulation (Hartman and Harpel 1994; Tabita 1995).

The reason for the temporal separation between cyano and chromophytic clade *rbcL* expression may be that the main mode of regulation in picocyanobacteria is at the transcriptional level, whereas the eucaryotic chromophytes are under other regulatory mechanisms. The synthesis of RUBISCO in chromophytes could be more linked to cell division (or other cellular processes), which might occur in the early evening or at night. Synthesis of RUBISCO may occur late in the day and the enzyme stored in an inactive form, then activated in the morning in the chromophytes. Potential mechanisms involved in enzyme activation or inactivation, respectively, include RUBISCO activase (RA), which cleaves RuBP from the uncarbamylated RUBISCO (Hartman and Harpel 1994), or the binding of 2-carboxyarabinitol 1-phosphate (CA1P) to the active (carbamylate) form of the en-

zyme to inactivate it. Both CA1P removal and RUBISCO activase are dependent on light.

The bimodal regulatory pattern of *rbcL* transcription observed was for coastal water (Sta. 5 and environs), which were under the influence of an estuarine plume in these studies. It is not known if the *rbcL* transcription of the chromophytes of the SCM are regulated out of phase or in phase with the picocyanobacteria in stratified water columns of oligotrophic environments such as Sta. 6, 7, near Bermuda.

The carbon fixation associated with chromophytes (particularly diatoms and prymnesiophytes) at the SCM has been termed new production, because these organisms are generally N-limited in the euphotic zone and use nitrate as their major source of nitrogen, which is advected into the photic zone. They are viewed as the backbone of the "biological pump," or the mechanism of transport of organic matter into the deep ocean (Cochlan et al. 1991; Claustre 1994). Picocyanobacteria such as *Synechococcus* and *Prochlorococcus* (cyanophytic *rbcL* clade) are viewed as "recycled production" because of their efficient use of recycled N (believed to be ammonium or organic N) and the fact that production is often balanced by grazing (Latasa et al. 1997). We propose that, in stratified oligotrophic water columns, cyano *rbcL* mRNA may be evidence for the abundance of recycled production, whereas chromophytic *rbcL* mRNA may be an indicative of new production. Our observations of this phenomenon are limited to the SE Gulf of Mexico and the tropical Atlantic near Bermuda. We would like to test this hypothesis in other tropical and temperate oceanic environments in conjunction with alternate methods for clade-specific production such as specific pigment labeling (DiTullio et al. 1993; Goericke and Welschmeyer 1994; Latasa et al. 1997).

Our results clearly indicate a separation of the cyanophytic and chromophytic *rbcL* expression in terms of three parameters: (1) particle size, with chromophytic *rbcL* expression associated with larger particles and cyano *rbcL* expression associated with the <1-μm–size fraction, at least for coastal waters; (2) time of day, with cyano *rbcL* expression in the morning and chromophytic *rbcL* expression in the afternoon; and (3) in space or position in the water column, with low nutrient, brightly lit upper water column for the cyano *rbcL* expression and the dimly lit, bottom of the photic zone for chromophytic *rbcL* expression. It appears as if these RUBISCO clades evolved to take advantage of these size, space, and time niches in oceanic ecosystems. Selective pressures enabling such niche occupation are not completely understood but probably involve nutrient utilization (the efficient utilization of low levels of recycled N by the cyanobacteria/prochlorophytes in the upper water column compared to nitrate utilization at the SCM by the chromophytic phytoplankton) and pigment composition/adaptation to light regimes. For example, the chromophytic eucaryotes may be better adapted to use the low levels of light found at the SCM. Additionally, the chromophytic algal RUBISCOs are known to have a greater specificity for CO_2 over O_2 (τ value) than the cyanophytic/green algal forms of the enzyme (Read and Tabita 1994). Thus, the chromophytes would be less likely to photorespire, which results in a net loss of energy and reductant (Raven 1993). Molecular efficiency (greater ratio of carboxylase to oxygenase) would be an advantage for carbon fixation late in the day in coastal surface waters, when photosynthetically produced oxygen levels may be maximal and light intensities are less. It should be noted that observations regarding the τ values for RUBISCO have been made for cultures of marine chromophytes and one *Synechococcus* species and may not be representative of natural populations. Direct testing of specificities of naturally occurring RUBISCO enzyme preparations are needed to verify or negate this hypothesis. Alternatively, differences in CO_2-concentrating mechanisms (CCMs) could counter effects of molecular efficiencies of RUBISCO. These and other interesting possibilities that might explain the vertical separation of organisms containing these two evolutionary clades of *rbcL* are fertile areas of research.

References

Andersen, R. A., R. R. Bidigare, M. D. Keller, and M. Latasa. 1996. A comparison of HPLC pigment signatures and electron microscopic observations for oligotrophic waters of the North Atlantic and Pacific Oceans. Deep-Sea Res. II **43:** 517–537.

———, G. W. Saunders, M. P. Paskind, and J. P. Sexton. 1993. Ultrastructure and 18S rRNA gene sequence for *Pelagomonas colceolata* gen. et sp. nov. and the description of a new algal class, the Pelagophyceae classis nov. J. Phycol. **29:** 701–715.

Campbell, L., L. P. Shapiro, and E. Haugen. 1994. Immunochemical characterization of eukaryotic ultraplankton from the Atlantic and Pacific Oceans. J. Plankton Res. **16:** 35–51.

———, and D. Vaulot. 1994. The importance of *Prochlorococcus* to community structure in the central North Pacific Ocean. Limnol. Oceanogr. **39:** 954–961.

Chisholm, S. W., and others. 1992. *Prochlorococcus marinus* nov. gen sp.: An oxyphototrophic marine prokaryote containing divinyl chlorophyll *a* and *b*. Arch. Microbiol. **157:** 297–300.

———, R. J. Olsen, E. R. Zettler, R. Goericke, J. B. Waterbury, and N. A. Welschmeyer. 1988. A novel free-living prochlorophyte abundant in the oceanic euphotic zone. Nature **334:** 340–343.

Chow, T. J., and F. R. Tabita. 1994. Reciprocal light-dark transcriptional control of *nif* and *rbc* expression and light-dependent posttranslational control of nitrogenase activity in *Synechococcus* sp. strain RF-1. J. Bacteriol. **176:** 6281–6285.

Clarke, A., and R. J. G. Leakey. 1996. The seasonal cycle of phytoplankton, macronutrients, and the microbial community in a nearshore Antarctic marine ecosystem. Limnol. Oceanogr. **41:** 1281–1294.

Claustre, H. 1994. The trophic status of various oceanic provinces as revealed by phytoplankton pigment signatures. Limnol. Oceanogr. **35:** 1206–1210.

Cochlan, W. P., P. J. Harrison, and K. L. Denman. 1991. Diel periodicity of nitrogen uptake by marine phytoplankton in nitrate-rich environments. Limnol. Oceanogr. **36:** 1689–1700.

Collos, Y., C. Descolas-Gros, M. Fontugne, A. Mortain-Bertrand, M. J. Chretiennot-Dinet, and M. G. Frikha. 1992. Carbon and nitrogen dynamics during growth and degradation of phytoplankton under natural surface irradiance. Mar. Biol. **112:** 491–496.

———, S. Y. Maestrini, and J. M. Robert. 1989. Nocturnal synthesis and diurnal degradation of phytoplankton biomass in surface waters. Mar. Biol. **101:** 457–462.

Colman, B. 1989. Photosynthetic carbon assimilation and the sup-

pression of photorespiration in cyanobacteria. Aquat. Bot. **34:** 211–231.
CUHEL, R. L., R. B. ORTNER, AND D. R. S. LEAN. 1984. Night synthesis of protein by algae. Limnol. Oceanogr. **29:** 731–744.
DESCOLAS-GROS, C., AND M. R. FONTUGNE. 1985. Carbon fixation in marine phytoplankton: Carboxylase activities and stable carbon-isotope ratios; physiological and paleoclimatological aspects. Mar. Biol. **87:** 1–6.
DITULLIO, G. R., D. A. HUTCHINS, AND K. W. BRULAND. 1993. Interaction of iron and major nutrients controls phytoplankton growth and species composition in the tropical North Pacific Ocean. Limnol. Oceanogr. **38:** 495–508.
DOTY, M. S., AND M. OGURI. 1957. Evidence for a photosynthetically daily periodicity. Limnol. Oceanogr. **2:** 37–40.
FALKOWSKI, P. A., AND J. A. RAVEN. 1997. Aquatic photosynthesis. Blackwell.
FURUYA, K. 1990. Subsurface chlorophyll maximum in the tropical and subtropical western Pacific Ocean: Vertical profiles of phytoplankton biomass and its relationship with chlorophyll *a* and particulate organic carbon. Mar. Biol. **107:** 529–539.
GOERICKE, R., AND N. A. WELSCHMEYER. 1993. The carotenoid labeling method: Measuring specific rates of carotenoid synthesis in natural phytoplankton communities. Mar. Ecol. Prog. Ser. **98**: 157–171.
HAMA, J., AND N. HANDA. 1992*a.* Diel photosynthetic production of cellular organic matter in natural phytoplankton populations, measured with ^{13}C and gas chromatography/mass spectrometry. I. Monosaccharides. Mar. Biol. **112:** 175–181.
———, AND ———. 1992*b.* Diel photosynthetic production of cellular organic matter in natural phytoplankton populations, measured with ^{13}C and gas chromatography/mass spectrometry. II. Fatty acids and amino acids. Mar. Biol. **112:** 183–190.
HARTMAN, F. C., AND M. R. HARPEL. 1994. Structure, function, regulation, and assembly of d-Ribulose-1,5-bisphosphate carboxylase/oxygenase. Annu. Rev. Biochem. **63:** 197–234.
HOLM-HANSEN, O., AND B. RIEMANN. 1978. Chlorophyll *a* determination: Improvements in methodology. Oikos **30:** 438–447.
ITTURIAGA, R., AND B. G. MITCHELL. 1986. Chroococcoid cyanobacteria: A significant component in the food web dynamics of the open ocean. Mar. Ecol. Prog. Ser. **28:** 291–297.
JIANG, S. C., J. M. THURMOND, S. L. PICHARD, AND J. H. PAUL. 1992. Concentration of microbial populations from aquatic environments by Vortex Flow Filtration. Mar. Ecol. Prog. Ser. **80:** 101–107.
JOHNSON, P. W., AND J. MCN. SIEBURTH. 1979. Chroococcoid cyanobacteria in the sea: A ubiquitous and diverse phototrophic biomass. Limnol. Oceanogr. **24:** 928–935.
LATASA, M., M. R. LANDRY, L. SCHLUTER, AND R. R. BIDIGARE. 1997. Pigment-specific growth and grazing rates of phytoplankton in the central equatorial Pacific. Limnol. Oceanogr. **42:** 289–298.
LETELIER, R. M., R. R. BIDIGARE, D. V. HEIBEL, M. ONDRUSEK, C. D. WINN, AND D. M. KARL. 1993. Temporal variability of phytoplankton community structure based on pigment analysis. Limnol. Oceanogr. **38:** 1420–1437.
LI, L-A., AND F. R. TABITA. 1994. Transcription control of ribulose bisphosphate carboxylase/oxygenase activase and adjacent genes in *Anabaena* species. J. Bacteriol. **176:** 6697–6706.
LI, W. K. W. 1994. Primary productivity of prochlorophytes, cyanobacteria, and eukaryotic ultraplankton: Measurements from flow cytometric sorting. Limnol. Oceanogr. **39:** 169–175.
———, D. V. SUBBA-RAO, W. G. HARRISON, J. C. SMITH, J. J. CULLEN, B. IRWIN, AND T. PLATT. 1983. Autotrophic picoplankton in the tropical ocean. Science **219:** 292–295.
MALONE, T. C. 1980. Algal size, p. 433–463. *In* I. Morris [ed.], The physiological ecology of phytoplankton. Univ. of California.
———, H. W. DUCKLOW, E. R. PEELE, AND S. E. PIKE. 1991. Picoplankton carbon flux in Chesapeake Bay. Mar. Ecol. Prog. Ser. **78:** 11–22.
MARSHALL, H., AND K. K. NESIUS. 1996. Phytoplankton composition in relation to primary production in Chesapeake Bay. Mar. Biol. **125:** 611–617.
MORSE, D., P. SALOIS, P. MARKOVIC, AND J. W. HASTINGS. 1995. A nuclear-encoded form II RuBisCO in dinoflagellates. Science (USA) **268:** 1622–1624.
PAUL, J. H., AND S. L. PICHARD. 1995. Extraction of DNA and RNA from aquatic environments, p. 153–177. *In* J. D. Trevors and J. D. Van Elsas [eds.], Nucleic acids in the environments: Methods and applications. Springer-Verlag.
———, AND ———. 1998. Phytoplankton activity through the measurement of ribulose bisphosphate carboxylase gene expression (RuBisCO), p. 207–226. *In* K. E. Cooksey [ed.], Molecular approaches to the study of the ocean. Chapman and Hall.
PICHARD, S. L., L. CAMPBELL, K. CARDER, J. B. KANG, J. PATCH, F. R. TABITA, AND J. H. PAUL. 1997*a.* Analysis of ribulose bisphosphate carboxylase gene expression in natural phytoplankton communities by group-specific gene probing. Mar. Ecol. Prog. Ser. **149:** 239–253.
———, ———, J. B. KANG, F. R. TABITA, AND J. H. PAUL. 1996. Regulation of ribulose bisphosphate carboxylase gene expression in natural phytoplankton communities. I. Diel rhythms. Mar. Ecol. Prog. Ser. **139:** 257–265.
———, ———, AND J. H. PAUL. 1997*b.* Diversity of ribulose bisphosphate carboxylase/oxygenase Form I gene (*rbcL*) in natural phytoplankton communities. Appl. Environ. Microbiol. **63:** 3600–3606.
———, M. E. FRISCHER, AND J. H. PAUL. 1993. Ribulose bisphosphate carboxylase gene expression in subtropical marine phytoplankton populations. Mar. Ecol. Prog. Ser. **101:** 55–65.
PORTIS, A. R. 1995. The regulation of RuBisCO by RuBisCO activase. J. Exp. Bot. **46:** 1285–1291.
PREZLIN, B. B., H. E. GLOVER, AND L. CAMPBELL. 1987. Effects of light intensity and nutrient availability on diel patterns of cell metabolism and growth in populations of *Synechococcus* spp. Mar. Biol. **95:** 469–480.
RAPPE, M. S., P. F. KEMP, AND S. J. GIOVANNONI. 1995. Abundant chromophyte plastid 16S ribosomal RNA genes found in a clone library from Atlantic Ocean seawater. J. Phycol. **31:** 979–988.
RAVEN, J. A. 1993. Carbon: A phycocentric view, p. 123–151. *In* G. T. Evans and M. J. R. Fasham [eds.], Towards a model of ocean biogeochemical processes. NATO ASI Series, v. I10. Springer-Verlag.
READ, B. A., AND F. R. TABITA. 1992. A hybrid ribulose bisphosphate carboxylase/oxygenase enzyme exhibiting a substantial increase in substrate specificity factor. Biochemistry **31:** 5553–5560.
———, AND ———. 1994. High substrate specificity factor ribulose bisphosphate carboxylase/oxygenase from eukaryotic marine algae and properties of recombinant cyanobacterial rubisco containing ''algal'' residue modifications. Arch. Biochem. Biophys. **312:** 210–218.
REYNOLDS, A. E., B. L. MCCONAUGHY, AND R. A. CATTOLICO. 1993. Chloroplast genes of the marine alga *Heterosigma carterae* are transcriptionally regulated during a light/dark cycle. Mol. Mar. Biol. Biotechnol. **2:** 121–128.
RIEGMAN, R., B. R. KUIPERS, A. A. M. NOORDELOOS, AND H. J. WITTE. 1993. Size-differential control of phytoplankton and the structure of plankton communities. Neth. J. Sea Res. **31:** 255–265.
ROWAN, R., S. M. WHITNEY, A. FOWLER, AND D. YELLOWLEES.

1996. Rubisco in marine symbiotic dinoflagellates: Form II enzymes in eukaryotic oxygenic phototrophs encoded by a nuclear multigene family. Plant Cell **8:** 539–553.

SHIMADA, A., T. HASEGAWA, I. UMEDA, N. KADOYA, AND T. MARUYAMA. 1993. Spatial mesoscale patterns of West Pacific picophytoplankton as analyzed by flow cytometry: Their contribution to subsurface chlorophyll maxima. Mar. Biol. **115:** 209–215.

SOURNIA, A. 1974. Circadian periodicities in natural phytoplankton populations of marine phytoplankton. Adv. Mar. Biol. **12:** 325–389.

———. 1978. Phytoplankton manual. UNESCO monographs on oceanographic methodology, v. 6. UNESCO.

STOCKNER, J. G. 1988. Phototrophic picoplankton: An overview from marine and freshwater ecosystems. Limnol. Oceanogr. **33:** 765–775.

TABITA, F. R. 1988. Molecular and cellular regulation of autotrophic carbon dioxide fixation in microorganisms. Microbiol. Rev. **52:** 155–189.

———. 1995. The biochemistry and metabolic regulation of carbon metabolism and CO_2 fixation in purple bacteria, p. 885–914. *In* R. E. Blankenship, M. T. Madigan, and C. E. Bauer [eds.], Anoxygenic photosynthetic bacteria. Kluwer.

VAQUER, A., M. TROUSSELLIER, C. COURTIES, AND B. BIBENT. 1996. Standing stock and dynamics of picophytoplankton in the Thau Lagoon (northwest Mediterranean coast). Limnol. Oceanogr. **41:** 1821–1828.

VERDUIN, J. 1957. Daytime variations in phytoplankton photosynthesis. Limnol. Oceanogr. **2:** 333–336.

VERNET, M., B. G. MITCHELL, AND O. HOLM-HANSEN. 1990. Adaptation of *Synechococcus in situ* determined by variability in intracellular phycoerythrin-543 at a coastal station off the Southern California coast, USA. Mar. Ecol. Prog. Ser. **63:** 9–16.

WATERBURY, J. B., S. W. WATSON, R. R. GUILLARD, AND L. E. BRAND. 1979. Widespread occurrence of a unicellular, marine, planktonic cyanobacterium. Nature **277:** 293–294.

WATSON, G. M. F., AND F. R. TABITA. 1996. Regulation, unique gene organization, and unusual primary structure of carbon fixation genes from a marine phycoerythrin-containing cyanobacterium. Plant Mol. Biol. **32:** 1103–1116.

———, AND ———. 1997. Microbial ribulose-1,5-bisphosphate carboxylase/oxygenase: A molecule for phylogenetic and enzymological investigations. FEMS Microbiol. Lett. **146:** 13–22.

WEBBER, D. F., AND J. C. ROFF. 1996. Influence of Kingston Harbor on the phytoplankton community of the nearshore Hellshire Coast, southeast Jamaica. Bull. Mar. Sci. **59:** 245–258.

Received: 17 December 1997
Accepted: 4 September 1998
Amended: 4 August 1998