

Sources of inorganic carbon for phytoplankton in the eastern Subtropical and Equatorial Pacific Ocean

*Philippe D. Tortell*¹

Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey 08544

François M. M. Morel

Department of Geosciences, Princeton University, Princeton, New Jersey 08544

Abstract

We present the results of a field study examining inorganic carbon utilization by phytoplankton assemblages in the eastern Subtropical and Equatorial Pacific Ocean. Data from isotope disequilibrium experiments demonstrate that HCO_3^- was the principal form of inorganic carbon taken up by all of the in situ phytoplankton populations we sampled. In a cyanobacteria-dominated assemblage, HCO_3^- uptake occurred chiefly through a direct transmembrane transport mechanism. Diatom-dominated assemblages expressed extracellular carbonic anhydrase and transported CO_2 derived from the catalyzed dehydration of HCO_3^- . Direct HCO_3^- transport by the diatoms may have also occurred. In a 3-d incubation experiment, we observed the CO_2 -dependent regulation of inorganic C uptake in diatom-dominated phytoplankton assemblages. Phytoplankton assemblages grown at 150 ppm CO_2 possessed external carbonic anhydrase activity and took up HCO_3^- following its dehydration to CO_2 . In contrast, the assemblages cultured with 750 ppm CO_2 appeared to lack external carbonic anhydrase activity and rely solely on CO_2 as an exogenous source of carbon for photosynthesis. The CO_2 effect on inorganic C utilization occurred in the absence of a detectable difference in phytoplankton growth rates between the 150 and 750 ppm CO_2 treatments. Our field data provide compelling evidence that HCO_3^- utilization is prevalent in natural marine phytoplankton communities and is regulated by ambient CO_2 concentrations. We discuss the ecological and biogeochemical implications of these results.

The physiological mechanisms of inorganic carbon acquisition in marine phytoplankton remain poorly characterized. Current debate centers on the extent to which cells utilize active transport versus diffusion-based mechanisms of C uptake and depend on CO_2 versus HCO_3^- as sources of inorganic C for photosynthesis. In seawater, the vast majority of dissolved inorganic C (DIC) exists as HCO_3^- (Morel and Hering 1993), with <1% as free CO_2 —the substrate for C fixation by ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO, Cooper et al. 1969). Laboratory data suggest that diffusive CO_2 supply can potentially limit the growth of some large marine phytoplankton (Riebesell et al. 1993). Consequently, species that access the oceanic HCO_3^- pool may gain a competitive advantage over those that rely solely on CO_2 for growth and may be less sensitive to variations in atmospheric CO_2 . From a biogeochemical perspective, the inorganic C species taken up by cells can significantly affect

their C isotope composition owing to a large difference in the equilibrium $\delta^{13}\text{C}$ values of HCO_3^- and CO_2 (Mook et al. 1974). Uncertainty regarding C acquisition in phytoplankton thus limits our ability to predict the effects of CO_2 on primary production in the oceans and to interpret variations in the $\delta^{13}\text{C}$ signature of marine organic matter.

Inorganic carbon acquisition by phytoplankton has been most extensively studied in laboratory cultures of cyanobacteria and green algae. These organisms have been shown to take up HCO_3^- as a photosynthetic C source (*see* Kaplan and Reinhold [1999] for an extensive review). We use the term uptake to signify the integrated mechanism by which inorganic carbon is transferred from the external environment to the intracellular environment (e.g., the cytoplasm in eukaryotes). By this definition, HCO_3^- uptake can involve the direct transport of HCO_3^- into the cell (i.e., translocation across the outer membrane), and/or extracellular dehydration to CO_2 , catalyzed by the enzyme carbonic anhydrase (CA), followed by active or passive CO_2 transport (indirect HCO_3^- uptake). The uptake of HCO_3^- by cyanobacteria appears to occur through a direct transport system exclusively, whereas both direct and indirect mechanisms of HCO_3^- uptake have been documented in green algae (Raven 1997). Laboratory studies with several important marine phytoplankton taxa—diatoms and prymnesiophytes—suggest that these organisms also take up HCO_3^- (Burns and Beardall 1987; Nimer et al. 1997), but the extent to which natural marine phytoplankton assemblages utilize HCO_3^- as a C source for photosynthesis remains controversial.

In two recent articles, we presented field evidence for the use of CO_2 -regulated carbon concentrating mechanisms in

¹ To whom correspondence should be addressed. Present address: Department of Earth and Ocean Sciences, and Department of Botany, University of British Columbia, Vancouver, British Columbia V6T 1Z4 Canada (ptortell@eos.ubc.ca).

Acknowledgments

We thank Ken Bruland and Dave Hutchins for allowing us to participate in their research cruise and the scientists and crew of the R/V *Melville* (cruise COOK 001) for scientific and logistical support. Charlie Trick and Giacomo DiTullio kindly provided flow cytometry and taxonomic pigment data, which were used in determining the taxonomic composition of the phytoplankton communities we sampled. Geoff Smith provided assistance with sample collection. This work was funded by the Center for Environmental Bioinorganic Chemistry (CEBIC).

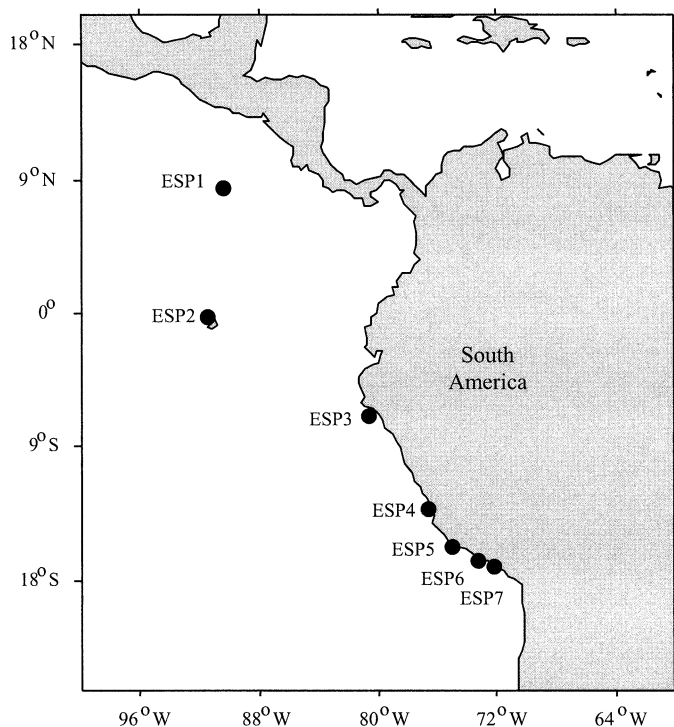


Fig. 1. Sampling locations for isotope disequilibrium experiments (ESP1, ESP3, ESP4, ESP5, ESP6), CO_2 -controlled incubations (ESP2), and ^{14}C productivity measurements (ESP4, ESP7).

coastal marine diatom populations (Tortell et al. 1997, 2000). The phytoplankton possessed a high affinity for inorganic C and were able to accumulate internal C pools significantly greater than those expected based on diffusive transport alone. Our results suggested that HCO_3^- was taken up by the phytoplankton assemblages but did not provide explicit information on the molecular nature of the C transport system or quantify the relative importance of CO_2 and HCO_3^- as exogenous inorganic C sources for photosynthesis.

The isotope disequilibrium technique was developed to study the uptake of individual inorganic C species by phytoplankton based on the slow kinetics of HCO_3^- dehydration in seawater (Johnson 1982). By measuring the rate of photosynthetic ^{14}C incorporation during a transient disequilibrium between $^{14}\text{CO}_2$ and $\text{H}^{14}\text{CO}_3^-$ in solution (Espie and Colman 1986), quantitative estimates of the HCO_3^- and CO_2

contributions to total C uptake can be obtained. Recent laboratory studies have utilized the isotope disequilibrium technique to investigate inorganic C uptake by marine diatoms and prymnesiophytes (Korb et al. 1997; Elzenga et al. 2000), but to our knowledge, this method has never been applied to natural phytoplankton populations in the oceans. In this article, we present isotope disequilibrium data demonstrating that HCO_3^- is an important source of inorganic C for phytoplankton in both coastal and open ocean environments. Our results provide evidence for direct HCO_3^- transport in a cyanobacterial assemblage and HCO_3^- uptake involving external CA coupled to a CO_2 transport system in diatom-dominated populations. We also present new data on the CO_2 -dependent regulation of C uptake by equatorial Pacific diatoms.

Materials and methods

Sample collection—Field experiments were conducted on board the R/V *Melville* during August and September 2000 (Cruise MV-Cook 001). We sampled both offshore and coastal upwelling regimes between 8°N and 18°S (Fig. 1) that varied significantly in their chemical and hydrographic properties, as well as in their primary productivity and phytoplankton species composition (Table 1). At most stations, nutrient concentrations were determined with a shipboard autoanalyzer, and total chlorophyll *a* (Chl *a*) was determined by fluorometric analysis (Parsons et al. 1984). Primary productivity was measured as described below. Dissolved CO_2 concentrations and temperature-corrected CO_2 partial pressures were obtained from potentiometric pH measurements and salinity-derived total alkalinity (Millero 1996), using the algorithm of Lewis and Wallace (1998). The taxonomic composition of phytoplankton communities was derived from high-performance liquid chromatography (HPLC) pigment analyses and flow cytometry (G. DiTullio and C. Trick pers. comm.).

To collect in situ phytoplankton assemblages for experiments, seawater (3–40 liters) was obtained from the ship's uncontaminated sampling line and filtered gently (<10 mm Hg) onto 0.6- or 5.0- μm polycarbonate membranes. Phytoplankton were resuspended in a seawater buffer containing 20 mM BICINE adjusted to pH 8.5 (± 0.05), and isotope disequilibrium experiments were begun immediately.

In addition to in situ sampling, we conducted a CO_2 ma-

Table 1. Chemical and biological properties of sampling stations.

Station ID	Nitrate (μM)	Silicate (μM)	Phosphate (μM)	CO_2 (μM)	CO_2 (ppm)	Chl <i>a</i> ($\mu\text{g L}^{-1}$)	Primary productivity ($\mu\text{g C L}^{-1} \text{h}^{-1}$)	Dominant phytoplankton taxa
ESP1	3.6	8.8	0.44	10.2	365	0.7	7.8 \pm 0.3	<i>Synechococcus</i> spp.
ESP2	5.7	5.8	0.54	3.5	150	7.0 \pm 1.7	12.4 \pm 1.6	Diatoms
	5.7	4.0	0.56	27	750	4.7 \pm 0.5	10.0 \pm 1.2	Diatoms
ESP3	16.4	6.47	1.13	16.7	481	8.5	—	Diatoms
ESP4	20.3	2.1	20.1	23.5	677	14.0	32.5 \pm 1.1	Diatoms
ESP5	17.9	2.1	15.5	—	—	1.1	—	Diatoms
ESP6	—	—	—	—	—	—	—	Cyanobacteria, nanoflagellates, diatoms
ESP7	1.8	1.24	3.1	15.0	414	—	8.2 \pm 0.3	nd

nipulation experiment with freshly upwelled, nutrient-rich seawater collected adjacent to the Galapagos Islands (Sta. ESP2). We used triplicate incubation bottles bubbled with CO₂ levels of 150 and 750 ppm, following the protocol of Tortell et al. (2000). After approximately 3 d of incubation, pooled samples from treatment replicates were collected for experiments. Samples were harvested onto 5.0- μ m filters and resuspended in the pH 8.5 seawater buffer for use in isotope disequilibrium experiments.

Isotope disequilibrium experiments—To examine inorganic C uptake by cells during steady state photosynthesis, we used the isotope disequilibrium technique as described by Elzenga et al. (2000) with several minor modifications. Concentrated cell suspensions (2 ml at \sim 60–900 μ g L⁻¹ Chl *a*) in seawater buffer were acclimated at 15°C (\pm 0.2) for 5–10 min in a rapidly stirred, thermostated O₂ electrode chamber (Hansatech). Cell suspensions were continuously mixed with a Teflon-coated magnetic stir bar and illuminated by a cool-white fluorescent bulb (\sim 400 μ E m⁻² s⁻¹). Radiolabeled C uptake was initiated by injecting 20 μ Ci ¹⁴C solution into the electrode chamber. The ¹⁴C spike solution was prepared immediately prior to experiments by diluting 20 μ Ci primary isotope stock (NEN-Dupont, 1 μ Ci μ l⁻¹; \sim 50 mCi mmol⁻¹) into 80 μ l of pH 7.0 (\pm 0.05) 50 mM HEPES buffer. To follow the time course of cellular ¹⁴C incorporation into organic matter, 200- μ l samples were withdrawn from the electrode chamber at short intervals between 5 and 90 s and rapidly dispensed into scintillation vials containing 1 ml 50% HCl (\sim 7 M). The acid solution immediately stopped photosynthesis and converted all inorganic carbon to CO₂. Samples were left to degas in a fume hood overnight and then vigorously purged with air for approximately 1 h to remove all unfixed (i.e., inorganic) carbon. Following this treatment, 5 ml scintillation cocktail (Fisher Scintisafe 50%) was added to vials, and the ¹⁴C activity in samples was measured by standard liquid scintillation procedures. Blank samples, consisting of ¹⁴C added to cell-free buffer, were also degassed and counted to correct for small amounts of residual inorganic C retained in samples. All experiments were run in duplicate.

Isotope disequilibrium theory—The isotope disequilibrium technique is used to follow C uptake by cells during a transient disequilibrium among ¹⁴C species in solution. In the ¹⁴C spike solution (pH 7.0, low ionic strength), \sim 20% of the total radiolabel exists as ¹⁴CO₂, whereas in the seawater buffer at pH 8.5, CO₂ accounts for only 0.3% of the total dissolved inorganic C pool (Morel and Hering 1993). As a result, the specific activity of CO₂ in the seawater buffer decreases significantly during the experiment as excess ¹⁴CO₂ added in the initial spike equilibrates with the bulk DIC. In contrast, the specific activity of the HCO₃⁻/CO₃²⁻ pool (hereafter referred to as the HCO₃⁻ pool) changes relatively little over time because the ¹⁴C label in these C species is close to its equilibrium value at the beginning of the experiment. Although the specific activity of CO₂ and HCO₃⁻ change throughout the experiment, the tracer ¹⁴C additions do not significantly affect the bulk DIC concentration

or the equilibrium ratios of individual C species in the seawater buffer.

The time-dependent changes in the specific activity of CO₂ and HCO₃⁻ follow a first-order exponential equation (Espie and Colman 1986).

$$SA_{CO_2,t} = SA_{DIC} + \Delta SA_{CO_2} e^{-\alpha_1 t} \quad (1)$$

$$SA_{HCO_3^-,t} = SA_{DIC} + \Delta SA_{HCO_3^-} e^{-\alpha_2 t} \quad (2)$$

SA_{DIC} is the equilibrium specific activity of the bulk DIC and thus of all individual inorganic C species, ΔSA is the change in the specific activity of CO₂ and HCO₃⁻ from initial to equilibrium values, and α_1 and α_2 are pH-, temperature-, and salinity-dependent rate constants calculated as described by Espie and Colman (1986) with temperature and salinity corrections obtained from Johnson (1982). In our experimental system, the respective values for α_1 and α_2 are 0.027 and 0.033 in the bulk solution, whereas ΔSA_{CO_2} and $\Delta SA_{HCO_3^-}$ are 166 Ci mol⁻¹ and -0.45 Ci mol⁻¹, respectively.

During steady state photosynthesis, the rate of ¹⁴C incorporation by cells, $d(\text{dpm})/dt$, is determined by the uptake rates of HCO₃⁻ and CO₂, which are assumed to be constant throughout the experiment, and by the time-dependent specific activities of these inorganic C species at the cell surface (Espie and Colman 1986). For cells transporting only CO₂, rates of ¹⁴C incorporation will be high initially, decreasing over time as the CO₂ pool approaches isotopic equilibrium (Fig. 2a). The resulting integrated time course of ¹⁴C incorporation by CO₂ users should therefore exhibit significant downward curvature (Fig. 2b). By comparison, cells that transport only HCO₃⁻ should maintain nearly constant rates of ¹⁴C fixation—and consequently exhibit a linear time course of ¹⁴C incorporation—because of the small change in the specific activity of HCO₃⁻ during the experiment (Fig. 2a,b).

A linear time course of ¹⁴C assimilation is also expected for cells that express periplasmic CA since this enzyme rapidly dissipates isotopic disequilibrium in the cell boundary layer by catalyzing the interconversion of HCO₃⁻ and CO₂. In order to distinguish between direct HCO₃⁻ transport and indirect HCO₃⁻ uptake coupled to CO₂ transport, it is therefore necessary to eliminate any potential extracellular CA activity. As in previous studies (e.g., Elzenga et al. 2000), we used a 10-min pretreatment with 20 μ M acetazolamide (AZ, a membrane-impermeable CA inhibitor) to block periplasmic CA activity in the phytoplankton assemblages. In the AZ-treated samples, the time course of ¹⁴C assimilation during isotope disequilibrium should reflect the C species transported by cells (assuming that periplasmic CA is completely inhibited).

Data analysis—Quantitative interpretation of isotope disequilibrium data is subject to several caveats. The equations derived by Espie and Colman (1986) to estimate the relative contribution of HCO₃⁻ and CO₂ to cellular C uptake assume that (1) chemical conditions within the cell boundary layer are identical to those in the bulk solution, (2) cells either lack periplasmic CA activity or this activity is completely and specifically blocked with inhibitors, and (3) the diffusion

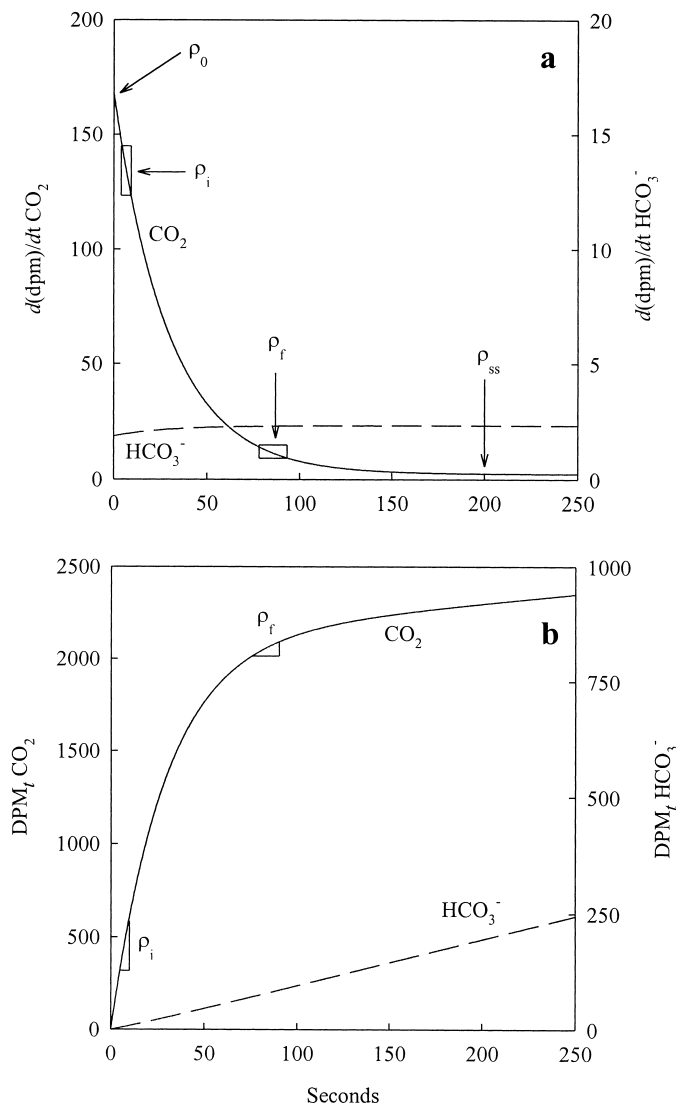


Fig. 2. Theoretical time course of instantaneous CO_2 and HCO_3^- (a) uptake rates and (b) integrated assimilation during isotope disequilibrium experiments. The differential curves in panel (a) represent the product of a constant total uptake rate and the time-dependent specific activity of CO_2 and HCO_3^- (Eqs. 1, 2). The meaning of ρ_0 , ρ_i , ρ_f , and ρ_{ss} is described in *Materials and methods*. Hollow rectangles in panel (a) indicate the time interval over which ρ_i and ρ_f were integrated by calculating an average uptake rate as shown in panel (b).

of ^{14}C label to the cell surface is effectively instantaneous (i.e., much faster than the rate of C uptake by cells). When these assumptions are met, the initial rate of ^{14}C uptake by strict HCO_3^- and CO_2 users should differ by a factor equal to the difference in the initial specific activity of these DIC species. Examination of previous isotope disequilibrium data sets (e.g., Elzenga et al. 2000) shows that the actual difference in the initial rate of ^{14}C uptake by cells taking up HCO_3^- and CO_2 is significantly lower than the difference in the initial HCO_3^- and CO_2 specific activities. This suggests that one or more of the above assumptions may be invalid. For this reason, and also because we conducted experiments

with diverse phytoplankton assemblages rather than mono-specific cultures, we have adopted a heuristic, semiquantitative approach to the analysis of our isotope disequilibrium data.

Our analysis is based on examining the degree of curvature in the ^{14}C time course data by comparing the rates of ^{14}C assimilation during the first 10 s of experiments (ρ_i ; Fig. 2) with the uptake rates observed during the final 15 s of experiments from 75–90 s (ρ_f ; Fig. 2). These rates were determined by linear regression of ^{14}C activity versus time between the 5- and 10-s time points (ρ_i) and the 75- and 90-s time points (ρ_f) ($n = 4$ in both cases).

In theory, the ratio of the true initial and true steady state ^{14}C uptake rates (ρ_0/ρ_{ss} ; Fig. 2a) should vary between 0.86 for strict HCO_3^- users and ~ 70 for cells that take up only CO_2 . These lower and upper limits for ρ_0/ρ_{ss} correspond to the respective changes in the specific activities of HCO_3^- and CO_2 from the beginning of the experiments until the isotopes fully equilibrate (i.e., $\Delta\text{SA}_{\text{HCO}_3^-}/\text{SA}_{\text{DIC}} = 0.86$, and $\Delta\text{SA}_{\text{CO}_2}/\text{SA}_{\text{DIC}} = 70$). In practice, however, because complete isotopic equilibration requires ~ 200 s under our experimental conditions, the ^{14}C uptake rates measured between 75 and 90 s (ρ_f) differ from the true steady state values (ρ_{ss} ; Fig. 2a). The difference between ρ_f and ρ_{ss} is small for cells that take up only HCO_3^- but significant for CO_2 users (Fig. 2a). In addition, averaging the initial ^{14}C uptake rate over the first 10 s of experiments (ρ_i) leads to a considerable underestimation of the true initial rate (ρ_0) for cells that take up CO_2 and a slight overestimation for cells that take up HCO_3^- (see Fig. 2a).

The expected values of ρ_i and ρ_f for CO_2 and HCO_3^- users can be calculated using the theoretical time course of ^{14}C uptake (Fig. 2a) by integrating the uptake rates between 5 and 10 s and between 75 and 90 s, respectively. This empirical analysis yields ρ_i/ρ_f values of ~ 1 for strict HCO_3^- users and ~ 10 for strict CO_2 users. These values can be used as a semiquantitative indication of the importance of HCO_3^- and CO_2 to total cellular uptake, with ρ_i/ρ_f estimates close to 1 (i.e., linear time course) indicating that HCO_3^- is the predominant source of inorganic C for cells and values close to 10 (significant curvature in the time course) indicating that CO_2 dominates C uptake. Although our semiquantitative approach does not yield precise estimates of the relative contributions of HCO_3^- and CO_2 to total C uptake, it does provide a robust semiquantitative interpretation of the data.

Productivity studies—We examined the effects of CA inhibitors on phytoplankton productivity at two locations, Stas. ESP4 and ESP7 (see Fig. 1 and Table 1 for station information). Surface seawater was collected from an in situ pumping system and dispensed into acid-cleaned 250-ml polycarbonate bottles. Triplicate (Sta. ESP7) and quadruplicate (Sta. ESP4) samples were incubated for 6 h in a flow-through seawater tank, with either 20 or 50 μCi ^{14}C , and harvested by gentle filtration onto GF/C filters. After overnight acidification with 1 ml of 1% H_3PO_4 , radioactivity in samples was determined by liquid scintillation counting. At Sta. ESP4, only AZ was used in experiments, whereas both AZ and its membrane-permeable analog ethoxymolamide

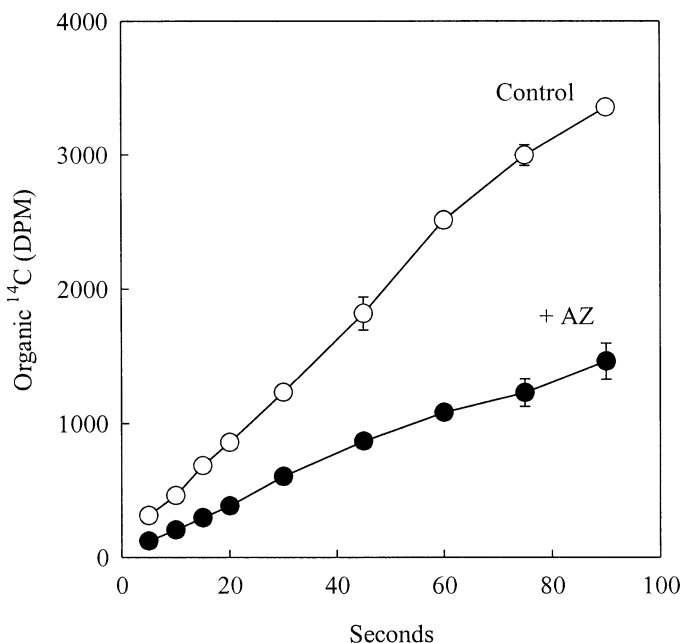


Fig. 3. Time course of ^{14}C assimilation during isotope disequilibrium experiments with the ESP1 phytoplankton assemblage. The community was dominated by cyanobacteria, and phytoplankton were collected for experiments using $0.6\text{-}\mu\text{m}$ filters.

(EZ) were used at Sta. ESP7. In all cases, inhibitors were used at a final concentration of $20\ \mu\text{M}$.

Results

In situ samples—At the time of our sampling, primary production at Sta. ESP1 (located in the Costa Rica upwelling dome) was dominated by a nearly monospecific bloom of cyanobacteria (*Synechococcus* spp.), as judged by flow cytometry and microscopic examinations of samples (C. Trick, D. Kirchman pers. comm.). Phytoplankton assimilation of ^{14}C during isotope disequilibrium experiments was essentially linear over time, both in the presence and absence of AZ (Fig. 3). Qualitatively, the linearity of the time course data provides compelling evidence that HCO_3^- was an important photosynthetic C source for the assemblage. Indeed, the ratio of the initial and final ^{14}C uptake slopes (ρ_i/ρ_f) for both control and AZ-treated samples was close to 1 (Table 2), indicating that HCO_3^- uptake accounted for essentially all of the total C demands of the assemblage. The linearity of the ^{14}C time course data for AZ-treated samples suggests that HCO_3^- uptake occurred through a direct transport mechanism rather than catalyzed extracellular dehydration. The presence of AZ did, however, substantially decrease the rate of ^{14}C assimilation by the assemblage (Fig. 3), suggesting a possible role of CA in the HCO_3^- transport process (see Discussion).

Flow cytometry and taxonomic pigment analyses indicated that samples collected from Stas. ESP3, ESP4, and ESP5 were dominated by diatoms (C. Trick, G. DiTullio pers. comm.; Table 1). In all three of these assemblages (which were sampled using $5\text{-}\mu\text{m}$ filters), the ^{14}C assimilation time course differed significantly between control and AZ-treated

Table 2. Data analysis of isotope disequilibrium experiments (see Materials and methods for an explanation of ρ_i/ρ_f).

Station		ρ_i/ρ_f (\pm SE)
ESP1	Control	1.2 ± 0.6
	+AZ	1.1 ± 0.6
ESP3	Control	2.9 ± 0.8
	+AZ	10.0 ± 0.2
ESP4	Control	5.1 ± 0.2
	+AZ	10.9 ± 0.7
ESP5	Control	3.9 ± 0.7
	+AZ	10.9 ± 1.8
ESP6	Control	2.9 ± 0.4
	+AZ	3.7 ± 0.1

samples, with greater curvature in the presence of the inhibitor (Fig. 4). Estimates of ρ_i/ρ_f ranged from ~ 3 to 5 for control samples and from 10 to 11 in AZ-treated samples (Table 2). These values indicate that both HCO_3^- and CO_2 were taken up by the assemblages and that a large fraction of the HCO_3^- uptake occurred through CA-catalyzed dehydration coupled to CO_2 transport. To compare the time course data from the three diatom-dominated assemblages, we corrected for differences in total C uptake rates by normalizing all of the ^{14}C activities to the values obtained in the ESP3 experiment (Fig. 4a). Following this normalization, ^{14}C assimilation time courses were quantitatively similar for all AZ-treated samples (Fig. 5a), but significant differences existed among control samples in the amount of curvature present in the ^{14}C time courses (Fig. 5b). The differences in curvature can be interpreted as variability among the assemblages in the relative importance of HCO_3^- uptake and external CA expression. Taken together, the results from Stas. ESP3, ESP4, and ESP5 indicate that HCO_3^- was an important source of inorganic C for diatom-dominated assemblages. Although our data demonstrate that HCO_3^- uptake occurred through catalyzed dehydration coupled to CO_2 transport, they do not rule out direct HCO_3^- transport by the diatom assemblages.

The phytoplankton community at Sta. ESP6 was characterized by a mixed population of large diatoms, nanoflagellates, and cyanobacteria (phycocyanin- and phycoerythrin-containing) present in roughly equal biomass proportions (Table 1). For both control and AZ-treated samples (collected on a $0.6\text{-}\mu\text{m}$ filter), the time course of ^{14}C assimilation showed a level of curvature intermediate between that expected for assemblages dominated by HCO_3^- users and CO_2 users, with ρ_i/ρ_f values of ~ 3 (Fig. 6; Table 2). These values indicate that the C uptake pathway in this assemblage involved the transport of both HCO_3^- and CO_2 , with HCO_3^- as the dominant source of inorganic C for photosynthesis.

Effects of AZ and EZ on phytoplankton productivity—In a 6-h productivity experiment conducted at Sta. ESP4, $20\text{-}\mu\text{M}$ AZ additions had no discernible effect on ^{14}C uptake by the phytoplankton assemblage (Fig. 7; *t*-test, $P = 0.3$). In the second sampling location (ESP7), for which no corresponding isotope disequilibrium experiments were run, a 40% reduction in productivity was observed in AZ-treated bottles (Fig. 8; *t*-test, $P < 0.001$). When this assemblage

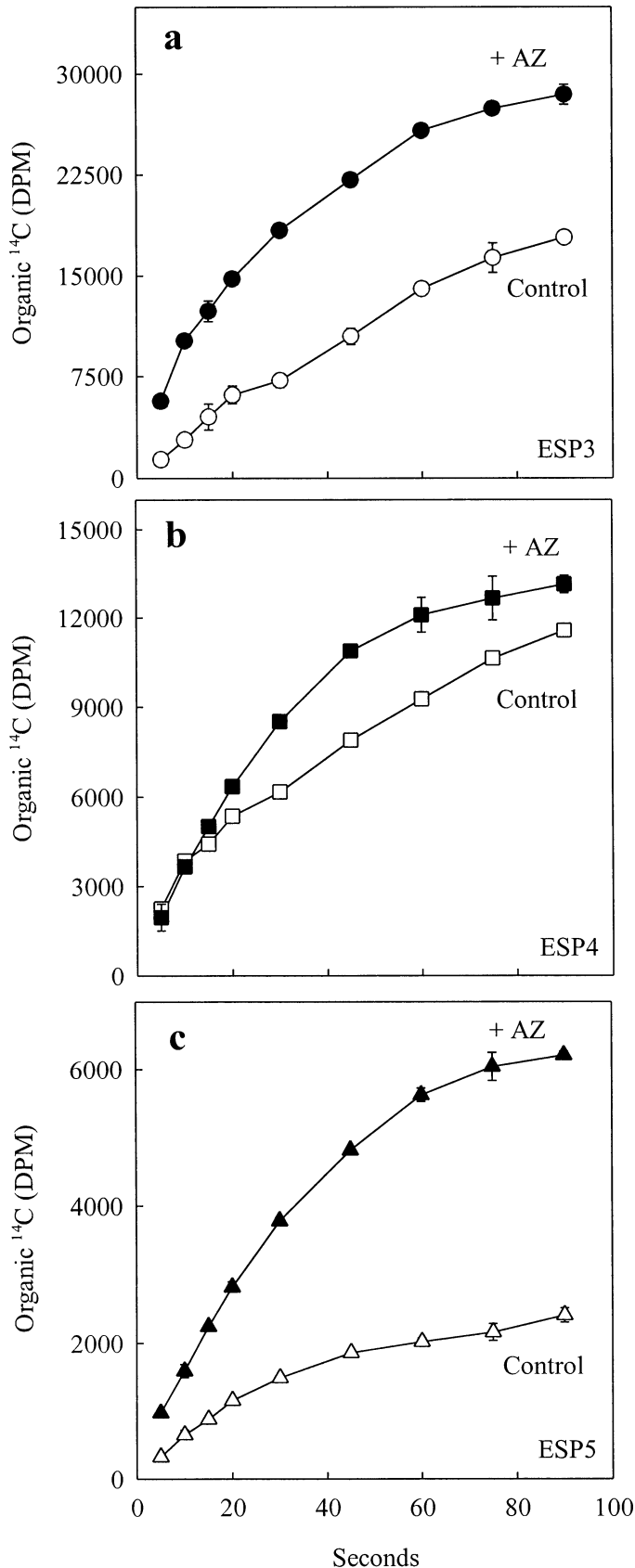


Fig. 4. Time course of ^{14}C assimilation during isotope disequilibrium experiments with the diatom-dominated phytoplankton assemblages at Stas. (a) ESP3, (b) ESP4, and (c) ESP5. All experiments were conducted with phytoplankton collected on 5.0- μm filters.

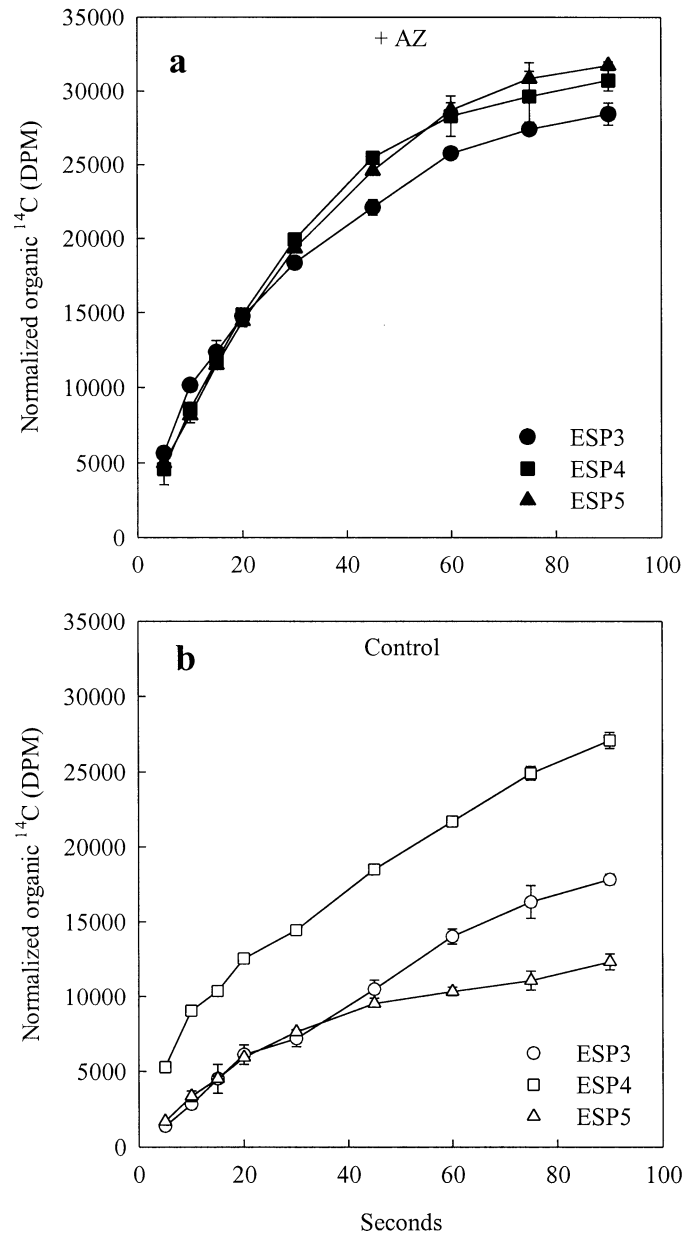


Fig. 5. Normalized ^{14}C uptake in the ESP3, ESP4, and ESP5 diatom-dominated phytoplankton assemblages in (a) AZ-treated and (b) control samples. The absolute value of ^{14}C activity in all assemblages was normalized to the +AZ treatment of the ESP3 assemblage.

was treated with 20 μM EZ, ^{14}C uptake rates were further reduced by 75% relative to the control rate (Fig. 7; *t*-test, $P < 0.001$).

CO₂ incubations—We conducted an incubation experiment to examine the effects of CO_2 manipulations on inorganic C uptake by equatorial Pacific phytoplankton (ESP2). Based on taxonomic pigment analysis (G. R. DiTullio pers. comm.), the phytoplankton community at the beginning of the experiment was highly diverse, containing roughly equal biomass of diatoms, haptophytes, cryptophytes, prasinophy-

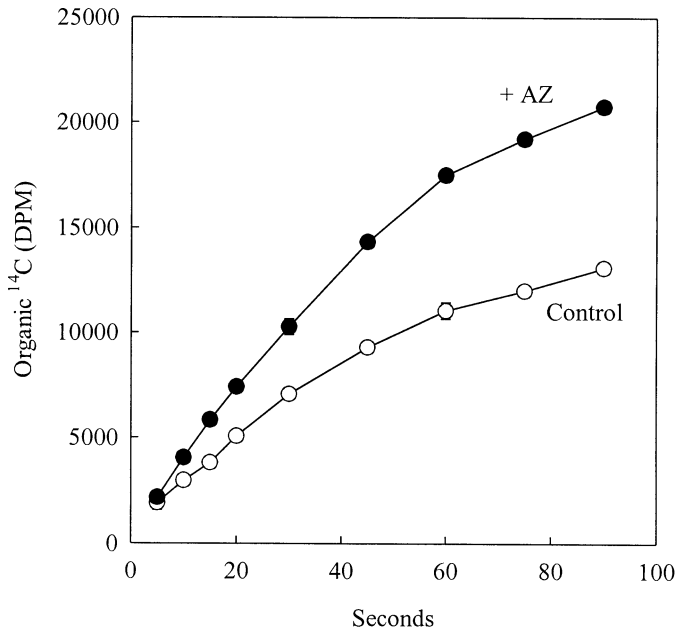


Fig. 6. Time course of ¹⁴C assimilation during isotope disequilibrium experiments with a mixed phytoplankton assemblage (ESP6) containing a significant biomass of cyanobacteria, nanoflagellates, and diatoms. Samples were collected with 0.6- μ m filters.

tes, and dinoflagellates ($\sim 0.5 \mu\text{g}$ total Chl *a* L^{-1}). After approximately 3 d incubation, both the 150- and 750-ppm CO_2 treatment bottles were dominated by diatoms ($\sim 90\%$ of biomass; total Chl *a* $\approx 6 \mu\text{g}$ L^{-1}), most notably large *Nitzschia* species. No significant differences in phytoplankton species composition could be detected between the CO_2 treatments by either flow cytometry or pigment analysis (C. Trick, G. DiTullio pers. comm.). Primary productivity and phyto-

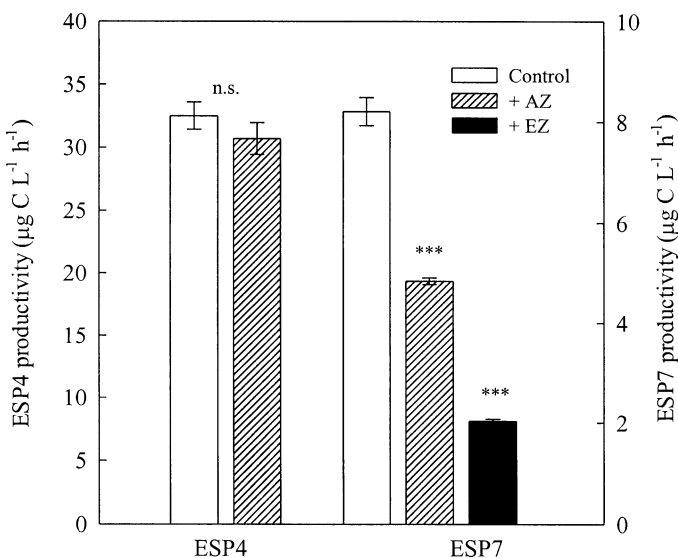


Fig. 7. Effects of acetazolamide (AZ) and ethoxylzolamide (EZ) on carbon fixation in seawater samples from Stas. ESP4 and ESP7. Stars indicate a statistically significant difference (*t*-test, $P < 0.001$), and n.s. denotes no significant difference ($P > 0.1$).

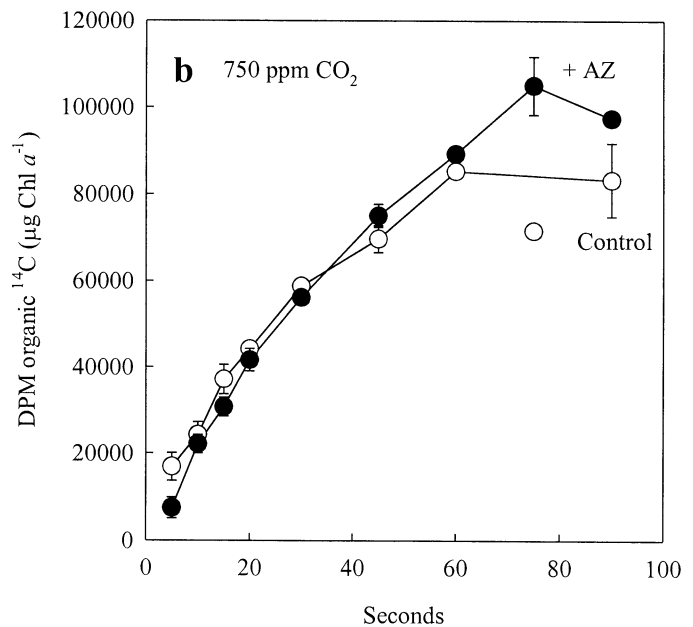
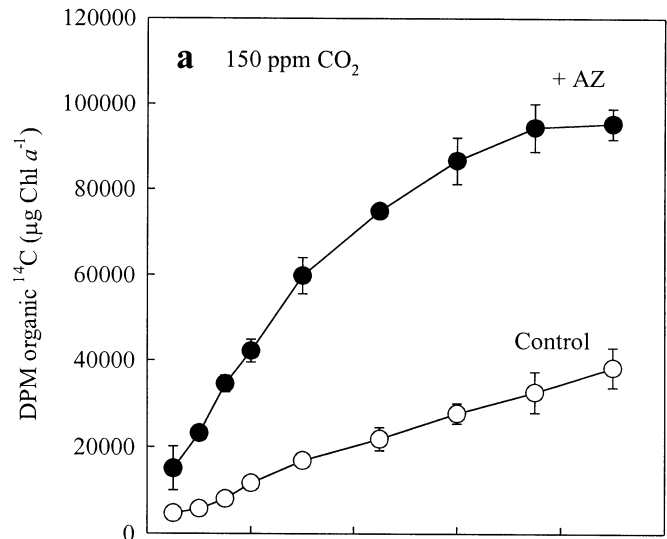


Fig. 8. Isotope disequilibrium results obtained with phytoplankton assemblages preconditioned for ~ 3 d with (a) 150 ppm CO_2 or (b) 750 ppm CO_2 .

plankton growth rates were also unaffected by the CO_2 manipulations. Productivity, as measured in 6-h ¹⁴C uptake experiments, averaged $11 (\pm 1.4 \text{ SE}) \text{ mg C L}^{-1} \text{ h}^{-1}$. Normalizing these C uptake rates to particulate organic carbon (POC) concentrations (assuming $45 \text{ g POC g}^{-1} \text{ Chl } a$; Montagnes et al. 1994) yields a corresponding growth rate of 1.2 d^{-1} for the phytoplankton assemblage. This value is consistent with previous growth rate estimates for trace metal-replete equatorial Pacific diatoms (e.g., Chavez et al. 1991).

Despite the lack of CO_2 -dependent differences in primary production and phytoplankton growth rates, we observed a distinct CO_2 effect on short-term cellular C uptake as mea-

sured in isotope disequilibrium experiments. Phytoplankton grown at 150 ppm CO_2 exhibited a time course of ^{14}C uptake that was approximately linear in the control samples and significantly curved in the presence of AZ (Fig. 8a). This result indicates that HCO_3^- was the dominant C source for the low- CO_2 assemblage and that a significant fraction of HCO_3^- uptake occurred through catalyzed extracellular dehydration coupled to CO_2 transport. In contrast, phytoplankton preconditioned at high CO_2 (750 ppm) did not appear to express external CA and were chiefly dependent upon CO_2 for uptake, as judged by the high degree of curvature in the ^{14}C assimilation time courses and the quantitative similarity between control and AZ-treated samples (Fig. 8b). Overall, our incubation results demonstrate the CO_2 -dependent regulation of external CA expression and HCO_3^- uptake. In the absence of significant taxonomic differences between the low- and high- CO_2 treatments, these changes in C uptake can be attributed to cell-specific physiological effects.

Discussion

The goal of this field study was to investigate the sources of inorganic C utilized by phytoplankton in the eastern Subtropical and Equatorial Pacific Ocean and to examine the effects of CO_2 manipulations on cellular C uptake pathways. Although previous studies of C acquisition in natural phytoplankton communities have reported measurements of CA activity, cellular carbon concentration factors, and photosynthetic kinetics (Berman-Frank et al. 1994; Berman-Frank and Erez 1996; Tortell et al. 1997, 2000), the relative importance of HCO_3^- and CO_2 as C sources for photosynthesis has thus far not been explicitly examined. Our main qualitative observation, based on isotope disequilibrium experiments, is that HCO_3^- uptake—either through direct transport or catalyzed extracellular dehydration coupled to CO_2 transport—is widespread among marine phytoplankton and is physiologically regulated in a manner that enables natural assemblages to maintain rapid growth rates over a large range of ambient CO_2 concentrations.

Mechanisms of HCO_3^- utilization—All of the phytoplankton assemblages we examined utilized HCO_3^- as an exogenous inorganic C source, but significant variability existed among populations in the apparent mechanisms of HCO_3^- uptake (Fig. 9). Our data suggest that C uptake in a cyanobacteria-dominated community occurred largely through direct HCO_3^- transport (Fig. 3), whereas diatom-dominated assemblages expressed external CA (to various extents) and transported CO_2 derived from HCO_3^- dehydration, in addition to potentially transporting HCO_3^- directly (Figs. 4, 8). In a diverse phytoplankton assemblage containing diatoms, cyanobacteria, and nanoflagellates, the observed kinetics of ^{14}C incorporation (Fig. 6) suggested that C uptake occurred through a mixture of direct HCO_3^- transport (presumably by cyanobacteria and possibly by nanoflagellates) and indirect HCO_3^- uptake (presumably by diatoms). Our field results are generally consistent with laboratory data on the mechanisms of C acquisition in monospecific phytoplankton cultures.

Carbon uptake studies with marine and freshwater cyanobacteria have provided strong evidence for the active up-

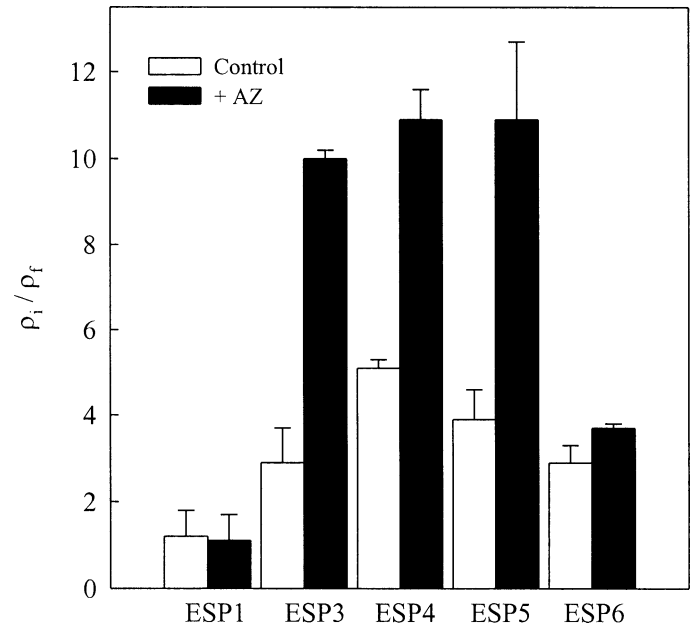


Fig. 9. Comparison of isotope disequilibrium results for in situ assemblages.

take of both CO_2 and HCO_3^- by these organisms (see reviews in Espie et al. 1991; Miller et al. 1991). Because all cyanobacteria examined thus far appear to lack periplasmic CA activity, the uptake of HCO_3^- by the cells has been attributed to a direct transport mechanism. Although external CA activity has not been detected in cyanobacteria, it has been suggested that the HCO_3^- transporter may, itself, contain a transmembrane CA-like subunit (Price and Badger 1989). Evidence for this comes from experiments in which CA inhibitors decreased C uptake rates in laboratory cultures without affecting intracellular CA activity. In our experiment with the *Synechococcus*-dominated assemblage at Sta. ESP1, we observed a similar decrease in HCO_3^- transport rates in the presence of AZ (Fig. 4). Irrespective of the underlying molecular mechanisms, the isotope disequilibrium results from this station provide the first field evidence of direct HCO_3^- transport in a cyanobacteria-dominated marine phytoplankton community.

Laboratory studies suggest that HCO_3^- utilization is also widespread among diatoms, although the mechanisms of uptake appear to differ significantly from those in cyanobacteria. Several authors have presented circumstantial evidence for HCO_3^- transport in diatoms (Colman and Rotatore 1988, 1995; Dixon and Merrett 1988). More direct evidence exists for CA-catalyzed HCO_3^- dehydration at the cell surface coupled to a CO_2 transport system (Rotatore and Colman 1992; Rotatore et al. 1995), as we observed in the diatom-dominated assemblages at Stas. ESP3, ESP4, and ESP5 (Fig. 5). In such a C uptake system, external CA activity acts to maintain maximum CO_2 concentrations at the cell surface. When external CA is inhibited through AZ treatment, laboratory phytoplankton cultures have been shown to deplete nearly all free CO_2 from solution, indicating that the rate of CO_2 uptake by cells can greatly exceed the rate of uncatalyzed HCO_3^- dehydration in the medium (Sültemeyer et al. 1989;

Rotatore et al. 1995). It follows from this that the inhibition of external CA activity should significantly reduce CO₂ supply rates to the cells, potentially resulting in CO₂ limitation of photosynthesis. Indeed, Elzenga et al. (2000) observed a decrease of ~25% in steady state ¹⁴C fixation rates in AZ-treated cultures of *Phaeocystis globosa*. We have recently observed a similar AZ effect in the centric diatom *Thalassiosira weissflogii* (unpubl. data). Although these inhibitor studies should be interpreted with caution (given potential nonspecific inhibitor effects), they suggest that external CA activity plays a significant role in supplying CO₂ to cells for photosynthesis.

In our field experiments, the effect of CA inhibition on ¹⁴C uptake rates by the phytoplankton assemblages is difficult to ascertain because the time scale of our isotope disequilibrium experiments was insufficient to establish the true steady state C uptake rates of cells (see *Isotope disequilibrium theory section above*). However, our 6-h productivity experiments provide some information on the importance of CA for the growth of phytoplankton assemblages in situ. At Sta. ESP7, we observed a decrease of ~25% in ¹⁴C incorporation in response to a 20- μ M acetazolamide (AZ) treatment and an even greater response to the membrane-permeable CA inhibitor, ethoxycarbonylacetamide (EZ) (Fig. 7). The differential effects of AZ and EZ suggest that both extracellular and intracellular CA activity are required for photosynthesis as has been previously documented in laboratory cultures (Badger and Price 1994). Unlike the ESP7 assemblage, phytoplankton collected at Sta. ESP4 showed no significant change in productivity following AZ additions (Fig. 7). This result suggests that extracellular CA activity was less important for the growth of the phytoplankton at this station. Indeed, isotope disequilibrium experiments with the ESP4 assemblage showed a relatively small difference in the ¹⁴C incorporation time courses of control and AZ-treated samples (Fig. 4b), providing evidence that this assemblage possessed less external CA activity than the other diatom-dominated populations we examined (Fig. 4a,c). The insensitivity of the ESP4 assemblage to AZ in our productivity experiment indicates that the inhibitor was not toxic to cells per se. An AZ-dependent decrease in carbon fixation rates may, therefore, be a useful bioassay for high-level external CA expression in natural phytoplankton assemblages.

CO₂ effects on C acquisition—Our incubation experiment demonstrates that HCO₃⁻ uptake in equatorial Pacific diatoms is regulated specifically by ambient CO₂ concentrations (the total DIC concentration in bottles was affected only slightly by the CO₂ manipulations; see Tortell et al. 2000). Under low CO₂ conditions (150 ppm), phytoplankton assemblages expressed external CA and utilized HCO₃⁻ as a primary source of inorganic C (Fig. 8a). In contrast, assemblages grown under high CO₂ (750 ppm) did not possess detectable external CA activity and exhibited a time course of ¹⁴C assimilation indicative of CO₂ as the dominant form of C taken up (Fig. 8b).

The regulation of inorganic C acquisition by CO₂ has previously been documented in a variety of phytoplankton taxa in laboratory experiments and in diatom-dominated coastal marine phytoplankton assemblages. These studies have

shown that external CO₂ concentrations can affect the expression of CA and RubisCO (Aizawa and Miyachi 1986; Tortell et al. 2000), the size of intracellular C pools (Aizawa and Miyachi 1986), C:N:P ratios (Burkhardt et al. 1999), and, in laboratory diatom cultures, the expression of a C₄ metabolic pathway (Reinfelder et al. 2000). In a previous field study of the California Upwelling regime, we observed a significant difference in short-term ¹⁴C uptake rates between low-CO₂ and high-CO₂ preconditioned diatom-dominated phytoplankton assemblages (Tortell et al. 2000). Over the range of external DIC concentrations used in these experiments (total DIC = 100–300 μ M; CO₂ = 0.5–1.5 μ M), low-CO₂ assemblages assimilated ¹⁴C about twofold faster than high-CO₂ assemblages. In light of our results from the equatorial Pacific incubation experiments (Fig. 8), we suggest that the lower C uptake rates of high-CO₂ samples from the California Upwelling resulted from a decreased capacity for HCO₃⁻ uptake by the phytoplankton.

Although incubation experiments provide the most direct evidence for CO₂ effects on inorganic C acquisition by phytoplankton, the CO₂-dependent regulation of C uptake may also be observable over the course of phytoplankton blooms as CO₂ is significantly depleted from surface waters in situ. Indeed, a temporal increase in CA expression has been reported during blooms of *Peridinium gatunense* in Lake Kinneret, coincident with a large photosynthetic CO₂ drawdown (Berman-Frank et al. 1994, 1998). To our knowledge, no comparable data exist for marine phytoplankton communities, even though CO₂ concentrations as low as ~150 ppm have been observed during periods of intense primary production in coastal marine waters (e.g., Codispoti et al. 1982; Bates et al. 1998). Based on our incubation results, such low CO₂ concentrations would be expected to induce HCO₃⁻ uptake and external CA activity by marine diatoms.

Among the diatom-dominated assemblages we have sampled for C uptake studies (Tortell et al. 1997, 2000; present study), we have observed significant differences in cellular C concentration factors, CA expression, and HCO₃⁻ uptake. Although much of this variability may reflect differences in species composition among the phytoplankton assemblages, variations in seawater CO₂ concentrations may also be a contributing factor. In support of this, we found that the diatom assemblage collected from waters with the highest CO₂ concentrations (Sta. ESP4, Table 1) possessed the lowest apparent levels of CA expression and HCO₃⁻ uptake as judged by the small difference in the control and +AZ time course data (Fig. 5b). In future, it may be possible to systematically study the influence of CO₂ drawdown on C uptake by repeated sampling of phytoplankton populations over the course of a bloom.

Implications for phytoplankton ecology and marine biogeochemistry—Our field results bear direct relevance to the question of C limitation of marine primary production. Riebesell et al. (1993) presented evidence for CO₂ limitation of diatoms based on laboratory growth experiments and a C uptake model that assumed cells did not use HCO₃⁻ as a source of inorganic carbon. Our field data indicate that this assumption could be incorrect for many natural diatom-dominated assemblages. The uptake of HCO₃⁻ by diatoms as part

of a carbon concentrating mechanism explains why the growth rates of natural diatom assemblages are often largely insensitive to wide CO₂ variations (Tortell et al. 2000).

In general, the mechanisms of inorganic C acquisition employed by phytoplankton should influence their growth responses to CO₂ variations (Raven 1991; Raven and Johnston 1991). The taxonomic variability we observed in C uptake among the phytoplankton assemblages (compare Sta. ESP1 with Stas. ESP3, ESP4, and ESP5) may therefore have significant ecological implications. Species that possess direct HCO₃⁻ transport mechanisms (e.g., cyanobacteria) may be less CO₂-sensitive than those that rely solely on CO₂ uptake or indirect HCO₃⁻ uptake via external CA-catalyzed dehydration. It has been suggested that the dominance of cyanobacteria in high-pH (i.e., low-CO₂) freshwaters is attributable to the ability of these organisms to utilize the abundant HCO₃⁻ ion as a direct source of inorganic C for photosynthesis (Shapiro 1973). The extent to which differential C acquisition systems affect competition among marine phytoplankton remains to be determined.

Variability in C acquisition among phytoplankton is also significant in the context of stable C isotope biogeochemistry. Direct HCO₃⁻ transport in cyanobacteria, as observed at Sta. ESP1 and previously in laboratory studies (Espie et al. 1991), could contribute significantly to the low ¹³C discrimination in these organisms (Popp et al. 1998), owing to the ¹³C enrichment of HCO₃⁻ ($\delta^{13}\text{C} \approx 0\text{‰}$) relative to CO₂ ($\delta^{13}\text{C} \approx -8\text{‰}$, Mook et al. 1974). Active HCO₃⁻ transport has also been suggested as an explanation for the low C isotope fractionation observed in coastal diatom assemblages under conditions of high growth rates, low CO₂ concentrations, or both (Fry and Wainright 1991; Pancost et al. 1999; Tortell et al. 2000). The results of this field study suggest, however, that HCO₃⁻ uptake in diatom-dominated assemblages occurs to a large extent through the catalyzed dehydration of HCO₃⁻ coupled to CO₂ transport. Because carbonic anhydrase does not affect the isotopic equilibrium between DIC species (Goericke et al. 1994), cells that transport CO₂ derived from the catalyzed dehydration of HCO₃⁻ will not show an isotopic enrichment associated with HCO₃⁻ use. Rather, high $\delta^{13}\text{C}$ values in diatoms could result from low inorganic C leakage out of cells because of, for example, a C₄ metabolic pathway (Reinfeldt et al. 2000). Although the exact mechanistic controls on C isotope fractionation in phytoplankton remain poorly constrained, isotope models clearly must incorporate a variety of C acquisition systems in order to accurately describe the variability in marine organic matter $\delta^{13}\text{C}$ values.

References

- AIZAWA, K., AND S. MIYACHI. 1986. Carbonic anhydrase and CO₂ concentrating mechanisms in microalgae and cyanobacteria. *FEMS Microbiol. Rev.* **39**: 215–233.
- BADGERM M. R., AND G. D. PRICE. 1994. The role of carbonic anhydrase in photosynthesis. *Ann. Rev. Plant. Physiol. Mol. Biol.* **45**: 369–392.
- BATES, N. R., D. A. HANSELL, AND C. A. CARLSON. 1998. Distribution of CO₂ species, estimates of net community production, and air-sea CO₂ exchange in the Ross Sea polynya. *J. Geophys. Res.* **103**(C2): 2883–2896.
- BERMAN-FRANK, I., AND J. EREZ. 1996. Inorganic carbon pools in the bloom-forming dinoflagellate *Peridinium gatunense*. *Limnol. Oceanogr.* **41**: 1780–1789.
- , I., T. ZOHARY, J. EREZ, AND Z. DUBINSKY. 1994. CO₂ availability, carbonic anhydrase, and the annual dinoflagellate bloom in Lake Kinneret. *Limnol. Oceanogr.* **39**: 1822–1834.
- , J. EREZ, AND A. KAPLAN. 1998. Changes in inorganic carbon uptake during the progression of a dinoflagellate bloom in a lake ecosystem. *Can. J. Bot.* **76**: 1043–1051.
- BURKHARDT, S., U. RIEBESELL, AND I. ZONDERVAN. 1999. Effect of CO₂ concentration on C:N:P ratio in marine phytoplankton: A species comparison. *Limnol. Oceanogr.* **44**: 683–690.
- BURNS, B. D., AND J. BEARDALL. 1987. Utilization of inorganic carbon by marine microalgae. *J. Exp. Mar. Biol. Ecol.* **107**: 75–86.
- CHAVEZ, F. P., AND OTHERS. 1991. Growth-rates, grazing, sinking, and iron limitation of Equatorial Pacific phytoplankton. *Limnol. Oceanogr.* **36**: 1816–1833.
- CODISPOTI, L. A., G. E. FRIEDRICH, R. L. IVERSON, AND D. W. HOOD. 1982. Temporal changes in the inorganic carbon system of the southeastern Bering Sea during spring 1980. *Nature* **296**: 242–234.
- COLMAN, B., AND C. ROTATORE. 1988. Uptake and accumulation of inorganic carbon by a freshwater diatom. *J. Exp. Bot.* **39**: 1025–1032.
- , AND ———. 1995. Photosynthetic inorganic carbon uptake and accumulation in two marine diatoms. *Plant Cell. Environ.* **18**: 919–924.
- COOPER, T. D., D. FILMER, M. WISHNICK, AND M. D. LANE. 1969. The active species of “CO₂” utilized by ribulose diphosphate carboxylase. *J. Biol. Chem.* **244**: 1081–1083.
- DIXON, G. K., AND M. J. MERRETT. 1988. Bicarbonate utilization by the marine diatom *Phaeodactylum tricorutum* Bohlin. *New Phytol.* **109**: 47–51.
- ELZENGA, J. T. M., H. B. A. PRINS, AND J. STEFELS. 2000. The role of extracellular carbonic anhydrase activity in inorganic carbon utilization of *Phaeocystis globosa* (Prymnesiophyceae): A comparison with other marine algae using the isotope disequilibrium technique. *Limnol. Oceanogr.* **45**: 372–380.
- ESPIE, G. S., AND B. COLMAN. 1986. Inorganic carbon uptake during photosynthesis. I. A theoretical analysis using the isotope disequilibrium technique. *Plant Physiol.* **80**: 863–869.
- , A. G. MILLER, R. A. KANDASAMY, AND D. T. CANVIN. 1991. Active HCO₃⁻ transport in cyanobacteria. *Can. J. Bot.* **69**: 936–944.
- FRY, B., AND S. W. WAINRIGHT. 1991. Diatom sources of ¹³C-rich carbon in marine food webs. *Mar. Ecol. Prog. Ser.* **76**: 149–157.
- GOERICKE, R., J. P. MONTOYA, AND B. FRY. 1994. Physiology of isotopic fractionation in algae and cyanobacteria, p. 187–221. *In* K. Lajtha and B. Michener [eds.], *Stable isotopes in ecology and environmental science*. Blackwell.
- JOHNSON, K. S. 1982. Carbon dioxide hydration and dehydration kinetics in seawater. *Limnol. Oceanogr.* **27**: 849–855.
- KAPLAN A., AND L. REINHOLD. 1999. CO₂ concentrating mechanisms in photosynthetic microorganisms. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **50**: 539–559.
- KORB, R. E., P. J. SAVILLE, A. M. JOHNSTON, AND J. A. RAVEN. 1997. Sources of inorganic carbon for photosynthesis by three species of marine diatom. *J. Phycol.* **33**: 433–440.
- LEWIS, E., AND D. W. R. WALLACE. 1998. Program developed for CO₂ system calculations. ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee.
- MILLER, A. G., G. S. ESPIE, AND D. T. CANVIN. 1991. Active transport of CO₂ in cyanobacteria. *Can. J. Bot.* **69**: 1032–1039.
- MILLERO, F. J. 1996. *Chemical oceanography*. CRC Press.

- MONTAGNES, D. J. S., J. BURGESS, P. J. HARRISON, AND F. J. R. TAYLOR. 1994. Estimating carbon, nitrogen, protein, and chlorophyll *a* from volume in marine phytoplankton. *Limnol. Oceanogr.* **39**: 1044–1060.
- MOOK, W. G., J. C. BOMMERSON, AND W. H. STAVERMAN. 1974. Carbon isotope fractionation between dissolved bicarbonate and gaseous carbon dioxide. *Earth Planet. Sci. Lett.* **22**: 169–176.
- MOREL, F. M. M., AND J. G. HERRING. 1993. Principles and applications of aquatic chemistry. Wiley.
- NIMER, N. A., M. D. IGLESIAS-RODRIGUEZ, AND M. J. MERRETT. 1997. Bicarbonate utilization by marine phytoplankton species. *J. Phycol.* **33**: 625–631.
- PANCOST, R. D., K. H. FREEMAN, AND S. G. WAKEHAM. 1999. Controls on the carbon-isotope composition of compounds in Peru surface waters. *Org. Geochem.* **30**: 319–340.
- PARSONS, T. R., Y. MAITA, AND C. M. LALLI. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon.
- POPP, B. N., E. A. LAWS, R. R. BIDIGARE, J. E. DORE, K. L. HANSON, AND S. G. WAKEHAM. 1998. Effect of phytoplankton cell geometry on carbon isotopic fractionation. *Geochim. Cosmochim. Acta* **62**: 69–77.
- PRICE, G. D. AND M. R. BADGER. 1989. Ethoxycarbonyl inhibition of CO₂ uptake in the cyanobacterium *Synechococcus* PCC7942 without apparent inhibition of internal carbonic anhydrase activity. *Plant Physiol.* **89**: 37–43.
- RAVEN, J. A. 1991. Implications of inorganic carbon utilization: Ecology, evolution, and geochemistry. *Can. J. Bot.* **69**: 908–924.
- . 1997. Inorganic carbon acquisition by marine autotrophs. *Adv. Bot. Res.* **27**: 87–209.
- , AND A. M. JOHNSTON. 1991. Mechanisms of inorganic carbon acquisition in marine phytoplankton and their implications for the use of other resources. *Limnol. Oceanogr.* **36**: 1701–1714.
- REINFELDER, J. R., A. M. L. KRAPIEL, AND F. M. M. MOREL. 2000. Unicellular C₄ photosynthesis in a marine diatom. *Nature* **407**: 996–999.
- RIEBESSELL, U., D. A. WOLF-GLADROW, AND V. SMETACEK. 1993. Carbon dioxide limitation of marine phytoplankton growth rates. *Nature* **361**: 249–251.
- ROTATORE, C., AND B. COLMAN. 1992. Active uptake of CO₂ by the diatom *Navicula pelliculosa*. *J. Exp. Bot.* **43**: 571–576.
- , ———, AND M. KUZMA. 1995. The active uptake of carbon dioxide by the marine diatoms *Phaeodactylum tricoratum* and *Cyclotella* sp. *Plant Cell Environ.* **18**: 913–918.
- SHAPIRO, J. 1973. Blue-green algae: Why they become dominant. *Science* **179**: 382–384.
- SÜLTEMEYER, D. F., A. G. MILLER, G. S. ESPIE, H. P. FOCK, AND D. T. CANVIN. 1989. Active CO₂ transport by the green alga *Chlamydomonas reinhardtii*. *Plant Physiol.* **89**: 1213–1219.
- TORTELL, P. D., J. R. REINFELDER, AND F. M. M. MOREL. 1997. Active uptake of bicarbonate by diatoms. *Nature* **390**: 243–244.
- , G. H. RAU, AND F. M. M. MOREL. 2000. Inorganic carbon acquisition in coastal Pacific phytoplankton communities. *Limnol. Oceanogr.* **45**: 1485–1500.

Received: 3 July 2001

Accepted: 21 January 2002

Amended: 15 February 2002