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INORGANIC CARBON UTILIZATION BY ROSS SEA PHYTOPLANKTON ACROSS NATURAL AND EXPERIMENTAL CO_2 GRADIENTS[1]

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We present results from a field study of inorganic carbon (C) acquisition by Ross Sea phytoplankton during *Phaeocystis*-dominated early season blooms. Isotope disequilibrium experiments revealed that HCO_3^- was the primary inorganic C source for photosynthesis in all phytoplankton assemblages. From these experiments, we also derived relative enhancement factors for HCO_3^-/CO_2 interconversion as a measure of extracellular carbonic anhydrase activity (eCA). The enhancement factors ranged from 1.0 (no apparent eCA activity) to 6.4, with an overall mean of 2.9. Additional eCA measurements, made using membrane inlet mass spectrometry (MIMS), yielded activities ranging from 2.4 to 6.9 U · [μg chl *a*]$^{-1}$ (mean 4.1). Measurements of short-term C-fixation parameters revealed saturation kinetics with respect to external inorganic carbon, with a mean half-saturation constant for inorganic carbon uptake ($K_{1/2}$) of ~380 μM. Comparison of our early springtime results with published data from late-season Ross Sea assemblages showed that neither HCO_3^- utilization nor eCA activity was significantly correlated to ambient CO_2 levels or phytoplankton taxonomic composition. We did, however, observe a strong negative relationship between surface water pCO_2 and short-term ^{14}C-fixation rates for the early season survey. Direct incubation experiments showed no statistically significant effects of pCO_2 (10 to 80 Pa) on relative HCO_3^- utilization or eCA activity. Our results provide insight into the seasonal regulation of C uptake by Ross Sea phytoplankton across a range of pCO_2 and phytoplankton taxonomic composition.

Key index words: **carbonic anhydrase; diatoms; HCO_3^- uptake; inorganic carbon; *Phaeocystis*; Ross Sea**

***Abbreviations:* ANOVA, analysis of variance; Bicine, 20 mM N,N-Bis(2-hydroxyethyl) glycerine; C, carbon; CA, carbonic anhydrase; CCM, carbon concentrating mechanism; DBS, dextran-bound sulphonamide; df, degrees of freedom; DIC, dissolved inorganic carbon; DMS, dimethylsulfide; DMSP, dimethylsulfoniopropionate; DPM, disintegrations per second; eCA, extracellular carbonic anhydrase; $f HCO_3^-$, fraction of total fixed C derived from HCO_3^-; F_v/F_m, photosynthetic efficiency (maximum quantum yield of photosynthesis); JGOFS, Joint Global Ocean Flux Study; $K_{1/2}$, apparent half-saturation constant for photosynthesis; MIMS, membrane inlet mass spectrometry; NBP, *Nathaniel B. Palmer*; S_b, uncatalyzed rate of HCO_3^-/CO_2 interconversion; S_c, catalyzed rate of HCO_3^-/CO_2 interconversion; U, units of carbonic anhydrase activity; $V_{max'}$, maximum rate of C fixation measured in 10 min substrate-kinetic experiments; α, relative catalytic enhancement of HCO_3^-/CO_2 interconversion**

The Ross Sea accounts for nearly a third of total carbon fixation in the Southern Ocean and acts as a strong net annual sink for atmospheric CO_2 (Arrigo

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et al. 1998, Arrigo and Van Dijken 2007). Primary productivity in this region is dominated by large annual blooms of diatoms and the colonial haptophyte *Phaeocystis antarctica* G. Karst. whose timing and magnitude is coupled to the seasonal retreat of sea ice within the Ross Sea polynya (Smith et al. 2000b). Typically, primary productivity in the polynya increases sharply through November, reaching peak values in December/January (>2 g $C \cdot m^{-2} \cdot d^{-1}$), and thereafter declining rapidly through February (Smith et al. 1996, Arrigo and van Dijken 2003). Early season blooms are characteristically dominated by *Phaeocystis*, while diatom blooms are typically associated with the later growth season, particularly in the NW polynya (e.g., Terra Nova Bay) where a strong meltwater signal is observed (DiTullio and Smith 1996).

While both diatoms and *Phaeocystis* are responsible for high rates of C fixation (Smith et al. 1996) and vertical particle flux (Dunbar et al. 1998) in the Ross Sea, these two phytoplankton taxa have distinct impacts on regional biogeochemical cycles (Arrigo et al. 2000, Sweeney et al. 2000b, Smith and Asper 2001). Diatoms have a unique requirement for silicic acid as part of their opal frustule (Brzezinski 1985) and significantly lower nitrogen:phosphorus uptake ratios than *Phaeocystis* (Arrigo et al. 1999). *Phaeocystis*, in turn, is a strong producer of dimethylsulfoniopropionate (DMSP; Keller et al. 1989), the precursor compound to the climate-active gas dimethylsulfide (DMS). Intensive field programs including the Joint Global Flux Study (JGOFS; Smith et al. 2000a) have been aimed at elucidating the environmental parameters controlling diatom and *Phaeocystis* blooms in the Ross Sea. Based on these field programs and subsequent modeling studies, it has been suggested that mixed-layer depth and Fe supply may be key determinants of the ecological success of these phytoplankton groups (Sedwick and DiTullio 1997, Arrigo et al. 1999, 2003).

Beyond Fe supply and mixed-layer stratification, large seasonal changes have also been observed in Ross Sea pCO_2, with values ranging from ~45.6 to 10.1 Pa (450 to 100 μatm) over the course of the growing season (Bates et al. 1998, Sweeney et al. 2000a). (A value of ~38.5 Pa is expected for seawater in equilibrium with the atmosphere). Although this seasonal pCO_2 variability is among the largest recorded for any oceanic region (Takahashi et al. 2002), its effects on the physiological ecology of Ross Sea diatoms and *Phaeocystis* have thus far received very little attention.

Early laboratory work suggested that CO_2 availability could limit the growth of some Antarctic diatoms (Riebesell et al. 1993). However, more recent studies have documented the presence of carbon concentrating mechanisms (CCMs) in a variety of cultured marine phytoplankton species (see Giordano et al. 2005 for a recent review) and natural assemblages (Tortell et al. 2000, 2006, Cassar et al. 2004, Martin and Tortell 2006). The CCM acts to alleviate potential C limitation through the active transport of CO_2 and/or HCO_3^- (the dominant form of inorganic C in seawater) and the expression of carbonic anhydrase (CA), which catalyzes HCO_3^-/CO_2 interconversion. In our own recent work (Tortell et al. 2008a,b), we have documented widespread HCO_3^- use and CA activity in Ross Sea phytoplankton assemblages and demonstrated CO_2-dependent changes in phytoplankton growth rates and species composition in this region. Our published work from the Ross Sea has focused on diatom-dominated late-season phytoplankton assemblages, and we have thus far only presented cursory information on C uptake during early season blooms (Tortell et al. 2008b).

In this article, we present detailed results from our field study of inorganic C acquisition by *Phaeocystis*-dominated assemblages in the early spring blooms of the Ross Sea polynya. We report measurements of photosynthetic HCO_3^- and CO_2 use, eCA, and C-fixation kinetics of phytoplankton assemblages in situ and in CO_2 manipulation experiments. We compare our results with C-uptake data from late-season blooms to examine the physiological differences in C utilization between Ross Sea diatoms and *Phaeocystis* across the annual growth cycle.

MATERIALS AND METHODS

Field sampling and hydrographic analysis. Field sampling and experiments were conducted during cruise NBP 06-08 onboard the *Nathaniel B. Palmer* between November 8 and December 8, 2006. Surface water samples (~1–5 m) were collected at a variety of sampling stations within the Ross Sea polynya and at four additional sites on a northbound transit across the polar and subantarctic frontal regions of the Southern Ocean (Fig. 1). Sampling and analytical procedures were identical to those described by Tortell et al. (2008a). Phytoplankton biomass was assessed using chl *a* concentrations, and taxonomic composition of phytoplankton assemblages was determined via HPLC analysis of accessory photosynthetic

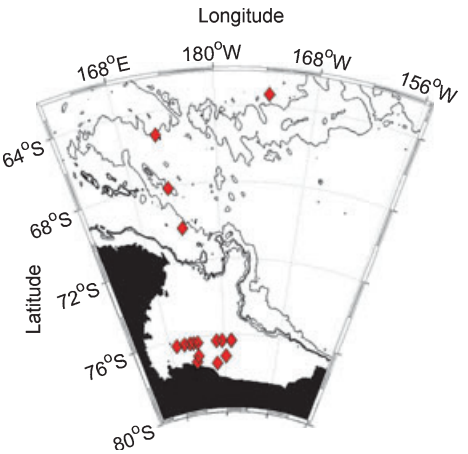

FIG. 1. Location of sampling stations in the Ross Sea, November–December 2006.

pigments. Macronutrient levels (nitrate, phosphate, and silicate) and pCO_2 were determined as described by Tortell et al. (2008a).

Seawater samples for physiological experiments were collected using 12 L Niskin bottles (General Oceanics, Miami, FL, USA) attached to a sampling rosette with conductivity, temperature, and depth sensors (CTD; Seabird Electronics, Bellevue, WA, USA), and in a few cases, using 10 L buckets deployed from the side of the ship. Phytoplankton were concentrated from the seawater samples by gravity filtering the contents of 20 L carboys onto 47 mm, 2.0 μm pore-size polycarbonate membranes (Poretics, Livermore, CA, USA) in a 4°C chamber. When flow rates through the filters were reduced significantly, phytoplankton were resuspended into 0.2 μm filtered Ross Sea water buffered with 20 mM N,N-Bis(2-hydroxyethyl) glycerine (Bicine; Sigma Inc., St. Louis, MO, USA) for subsequent physiological experiments (see below). Phytoplankton biomass in the concentrated cell suspensions was assessed by overnight extraction of 100–1,000 μL samples in 8 mL of 90% acetone followed by standard fluorometric analysis of total chl *a* concentrations (Parsons et al. 1984). Chl *a* concentrations in the cell suspensions ranged from ~0.1 to 1.5 $mg \cdot L^{-1}$.

CO_2-incubation experiments. To examine the effects of CO_2 availability on phytoplankton physiology and growth, we conducted two separate CO_2-controlled-incubation experiments following the protocols presented in Tortell et al. (2008a). Briefly, surface seawater (~5 m) was collected using a trace-metal-clean in situ pumping system and dispensed into nine trace-metal-cleaned 4 L polycarbonate bottles. An additional ~80 L of 0.2 μm filtered seawater was collected into acid-cleaned 20 L carboys for periodic dilution of samples as described below. The filtered and unfiltered seawater collected for experiments was enriched with 2 nM iron to alleviate potential limitation of this micronutrient. Phytoplankton in the incubation bottles were cultured under three CO_2 levels (10.1, 36.5, and 81 Pa, equivalent to 100, 360, and 800 ppm CO_2) delivered from commercially mixed air standards (Scott Specialty Gases, San Bernardino, CA, USA). The CO_2 mixtures were bubbled directly into the seawater samples through custom-built fittings on the incubation bottle caps. For one experiment, incubation bottles were placed in a custom-built, deck-board Plexiglas incubator maintained at ambient sea surface temperature (−1.1°C to −1.9°C) with a flow-through seawater system. In this experiment, light intensity was reduced to ~30% of ambient surface values using neutral density screening. For the other incubation experiment, bottles were placed in a temperature-controlled (1 ± 1°C) walk-in growth chamber with a constant irradiance of ~160 μmol quanta $\cdot m^{-2} \cdot s^{-1}$ provided by blue fluorescent bulbs (Philips Electronics Corporation, Andover, MA, USA, TLD 36W/18; 400–520 nm emission peak).

Phytoplankton growth was monitored by following chl *a* accumulation and nutrient consumption in daily samples from incubation bottles. When major nutrients had been drawn down to ~10% of starting values, ~90% of the remaining sample was removed for experiments and replaced with 0.2 μm filtered seawater, thus replenishing nutrient concentrations and maintaining phytoplankton in exponentially growing, semicontinuous cultures. Phytoplankton samples removed for experiments were concentrated by gravity filtration onto 2.0 μm polycarbonate membranes and processed as described above for station samples.

Physiological measurements. We utilized several methods to assess phytoplankton C-uptake physiology. The relative importance of HCO_3^- and CO_2 as photosynthetic carbon sources, and relative eCA activities were determined using the isotope disequilibrium method with concentrated phytoplankton samples in pH 8.5 buffered seawater (20 mM Bicine). We followed the exact protocol described by Tortell et al. (2008a), using an experimental temperature of 2.5°C. We describe below the essential features of the isotope disequilibrium experiments and refer the reader to several additional publications for extensive background information on the theory and experimental details of the method (Espie and Colman 1986, Elzenga et al. 2000, Martin and Tortell 2006, Rost et al. 2007).

The isotope disequilibrium technique was designed to follow ^{14}C fixation by phytoplankton during a transient disequilibrium between $^{14}CO_2$ and $H^{14}CO_3^-$ in solution. Isotopic disequilibrium is initiated by injecting a $^{14}CO_2$-rich spike (pH 7.0) to cell suspensions at pH 8.5. Following the initial ^{14}C spike, the specific activity of CO_2 decreases exponentially as isotopic equilibrium is reestablished. Phytoplankton utilizing CO_2 as an external photosynthetic C source thus show significant curvature in the time course of ^{14}C accumulation, with high initial rates of isotope uptake decreasing to lower steady-state values. Note that the high initial rates of ^{14}C fixation in CO_2 users do not reflect higher rates of total C uptake, but rather the high specific activity (i.e., dpm ^{14}C per mol C) of the CO_2 pool in solution. By comparison, the HCO_3^-/CO_3^{2-} pool remains relatively uniformly labeled (i.e., near-constant specific activity) during the experiment, and the use of HCO_3^- as a photosynthetic C source results in a nearly linear ^{14}C time course. Linear time-course kinetics can also result from eCA expression, which dissipates isotopic disequilibrium in solution. Experiments were thus conducted in the presence and absence of eCA-specific inhibitors (see below).

Two physiological parameters can be derived from the analysis of the ^{14}C time-course data: $fHCO_3^-$ and α. These parameters correspond, respectively, to the fraction of photosynthetically fixed C derived from HCO_3^- and the eCA-catalyzed rate of HCO_3^-/CO_2 conversion in the cell boundary layer. Both $fHCO_3^-$ and α were calculated from each experiment by fitting ^{14}C time-course data to the equations presented in Martin and Tortell (2006) using the nonlinear least-squares regression algorithm in Sigma Plot (v.10.0, Systat Software Inc., Chicago, IL, USA).

Additional direct measurements of eCA activity were made by MIMS (Badger and Price 1989) using the protocols and instrumentation described by Tortell et al. (2008a). The MIMS eCA assays were based on measuring the rate of ^{18}O loss from doubly labeled CO_2 ($^{13}C^{18}O_2$) in pH 8.0 buffered seawater (20 mM Bicine) at a temperature of 2.5°C, and the eCA activity, U, was calculated following Badger and Price (1989) as follows:

$$U = S_c/S_b - 1 \quad (1)$$

where the S_c is the catalyzed rate measured with phytoplankton samples and the S_b is the uncatalyzed rate measured with the blank buffer (i.e., pH 8.0 buffered seawater with no added cells). The final enzyme activity values were normalized to the chl *a* content measured fluorometrically in 100–200 μL aliquots of concentrated cell suspensions (Parsons et al. 1984).

We used a ^{14}C-based method to estimate the substrate-dependent kinetics of inorganic C fixation (i.e., apparent half-saturation constants, $K_{1/2}$, and maximum C-fixation rates, V_{max}). These experiments were conducted by measuring short-term C fixation over a range of external C concentrations in a seawater buffer (20 mM Bicine, pH 8.0). Prior to experiments, inorganic C was removed from the assay buffer by purging ~20 mL aliquots with CO_2-free air for at least 3 h. One milliliter aliquots of phytoplankton concentrated in C-free buffer were dispensed into polypropylene microcentrifuge tubes and placed in a custom-made, temperature-controlled

glass chamber (2.5°C). The incubation tubes were illuminated from the side with 200 μmol quanta · m^{-2} · s^{-1} irradiance provided by a full sun spectrum halogen bulb (Life-GLO, T5-HO, 6,700 k, Rolf C. Hagen Inc., Montreal, Quebec, Canada). To initiate experiments, various amounts of $H^{14}CO_3^-$ (GE Healthcare Biosciences, Baie d'Urfé, Quebec, Canada) and $H^{12}CO_3^-$ were added to each tube sequentially, with a delay of 60 s between successive additions. The $^{14}C/^{12}C$ additions were adjusted to yield a final concentration of total inorganic carbon ranging from ~50 to 4,000 μM, with a final specific activity of 2.7 mCi · $mmol^{-1}$ (100 MBq · $mmol^{-1}$). After 10 min of incubation, 600 μL samples were rapidly transferred into 600 μL of 6N HCl in 7 mL scintillation vials and vortexed to terminate reactions. Sample vials were then placed on a shaker table to degas evolved $^{14}CO_2$ for at least 12 h, and the ^{14}C activity of residual labeled organic compound samples was measured after adding 5 mL of Scintisafe scintillation cocktail (Fisher Scientific, Ottawa, ON, Canada) using a Beckman LS6500 liquid scintillation counter (Beckman-Coulter, Mississauga, ON, Canada) with automatic quench correction. Background activity levels in cell-free blanks were subtracted from all experimental samples.

Kinetic parameters $V_{max'}$ and $K_{1/2}$ were derived from the ^{14}C data using nonlinear, least-squares regression of the hyperbolic Michaelis–Menten equation:

$$V = V_{max'} \times S/(S + K_{1/2}) \quad (2)$$

where V and V_{max} are the rate of C fixation at any given external C concentration, S, and the maximum fixation rate, respectively. [Note that the maximum C-fixation rates obtained from this analysis ($V_{max'}$) are not directly comparable to steady-state C-uptake rates measured in traditional (12–24 h) ^{14}C-incubation experiments. The 10 min rates reflect the total cellular capacity for C fixation, while longer-term rates include a significant contribution of respiration and other C-loss processes.] Phytoplankton at all stations showed the expected saturation-type behavior of C fixation, and we were able to obtain reasonable data fits to the hyperbolic equation, with mean relative standard errors of 9% and 32% for $V_{max'}$ and $K_{1/2}$, respectively. Carbon-fixation rates were normalized to cell biomass as measured by fluorometric chl *a* determinations in 100–200 μL aliquots of concentrated cell suspensions.

For most of the physiological assays, replicate assays were performed in the presence and absence of dextran-bound sulphonamide (DBS; Ramidus, Lund, Sweden). This chemical is a potent, membrane-impermeable CA inhibitor (Sültemeyer et al. 1990) that is believed to selectively inhibit eCA activity. The inhibitor was added to cell suspensions at a final concentration of 100 μM prior the measurements.

RESULTS AND DISCUSSION

General hydrographic and biological properties of sampling sites. Biological and chemical characteristics for each in situ station are presented in Table 1. Our study was conducted during the initial phase of the phytoplankton growth season in the Ross Sea polynya. At the beginning of our survey, much of the Ross Sea was still ice covered with only a small region of open water. We initially observed large oxygen undersaturation and pCO_2 values well above atmospheric equilibrium (>40.5 Pa), indicative of a net heterotrophic system (C. Gueguen and P. Tortell, unpublished data). During our ~3 week Ross Sea survey, the rapid onset of the spring phytoplankton bloom was evident from a significant increase in chl *a* (from 0.6 to 3.2 μg · L^{-1}) and drawdown of CO_2 to values as low as 25.3 Pa (Table 1). As is typical for this region, macronutrient concentrations were uniformly high (>20 μM for nitrate and >70 μM for silicic acid), while sea surface temperature ranged from −1.9°C to −1.1°C and salinity ranged from 32.54 to 34.09. *P. antarctica* dominated phytoplankton assemblages in most of the Ross Sea stations we sampled. There were, however, two stations dominated by diatoms (stations 41 and 44) and two stations (14 and 32) with high abundances of both diatoms and *Phaeocystis*. Station 45 was located on the outer edge of the continental margin separating the Ross Sea from the open Southern Ocean. This station had elevated chl *a* associated with significant Si drawdown and a mixed diatom/*Phaeocystis* phytoplankton assemblage. Other Southern Ocean stations farther north (46–48) had lower chl *a* levels and diatom-dominated phytoplankton assemblages.

Table 1. Locations and biological/chemical characteristics of sampling stations.

Station	Latitude deg. S	Longitude deg. E	Chl *a* (μg · L^{-1})	NO_3 (μmol · L^{-1})	Dominant taxa	pCO_2 (Pa)	SiO_2 (μmol · L^{-1})
14	77.0017	174.0469	0.56	28.80	Mixed	40.9	81.96
20	76.3011	173.2996	1.15	28.20	*Phaeocystis*	37.7	88.16
21	76.3001	169.598	0.71	26.00	*Phaeocystis*	32.9	85.92
23	76.2999	172.5142	0.92	26.30	*Phaeocystis*	35.8	80.13
28	76.2999	178.3414	1.46	27.90	*Phaeocystis*	33.7	81.6
32	77.1005	180.4048	0.91	27.30	Mixed	36.5	84.39
35	77.4499	178.2994	1.21	25.40	*Phaeocystis*	31.7	86.16
38	77.2997	173.5983	2.77	24.60	*Phaeocystis*	31.0	82.02
41	76.2996	174.1719	1.71	25.30	Diatoms	33.5	76.50
42	76.2977	171.2525	3.21	26.00	*Phaeocystis*	27.4	83.53
43	76.3005	181.6593	2.73	24.20	*Phaeocystis*	25.3	84.41
44	76.3003	179.5929	2.94	24.10	Diatoms	29.3	77.71
45	70.2794	174.2993	2.63	29.30	Mixed	31.6	59.33
46	68.1106	173.0429	0.40	29.70	Diatoms	31.3	70.96
47	65.233	172.494	1.23	–	Diatoms	32.6	–
48	63.395	173.538	1.02	–	Diatoms	35.8	–

HCO_3^- utilization in natural phytoplankton assemblages. Figure 2 presents the results of isotope disequilibrium experiments for stations 41, 42, and 45 where phytoplankton assemblages were diatom dominated, *Phaeocystis* dominated, and taxonomically mixed, respectively. For all experiments (both control and DBS-treated replicates), the time-course data could not be adequately fitted with a CO_2-only model (dashed line Fig. 2), indicating that HCO_3^- was a significant photosynthetic carbon source for the phytoplankton assemblages. Table 2 shows the quantitative values for $fHCO_3^-$ derived from the analysis of ^{14}C time-course data. Unfortunately, isotope disequilibrium results are not available for the first seven stations of our survey due to a problem with the seawater buffer used for experiments. Values of $fHCO_3^-$ ranged from 78% to 91% in control experiments, with a mean value of 86%, indicating that HCO_3^- was the major source of inorganic C for photosynthesis. There was no statistically significant difference between $fHCO_3^-$ measured for stations in the Ross Sea polynya (stations 38–44) and those sampled along our northbound transit through the Southern Ocean (stations 45–48) (*t*-test, $P = 0.45$, df = 7).

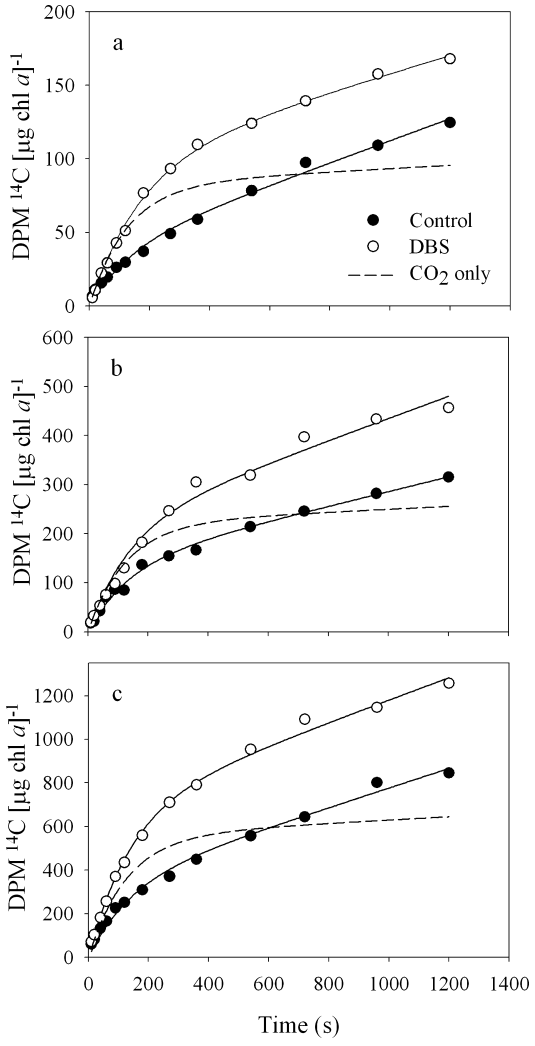

FIG. 2. Typical results of isotope disequilibrium experiments with Ross Sea phytoplankton assemblages at station 41 (a), 42 (b), and 45 (c). DBS, dextran-bound sulphonamide; DPM, disintegrations per minute.

The addition of DBS led to increased curvature in ^{14}C time-course data, providing qualitative evidence for eCA activity in the phytoplankton assemblages. The apparent increase in initial ^{14}C-fixation rates in DBS-treated cells is attributable to an increased residence time of high specific activity CO_2 in solution, rather than an increase in total C-uptake rates (Martin and Tortell 2006). Quantitative analysis of the ^{14}C time-course data showed that DBS-treated cells showed lower apparent HCO_3^- utilization, with $fHCO_3^-$ ranging from 65% to 83% and averaging 74%, as compared to 86% for control experiments. This result may have several explanations. In experiments run without DBS additions, model fits treat the HCO_3^-/CO_2 interconversion rate, α, as a free parameter (see Martin and Tortell 2006), whereas a fixed constant is used for DBS-treated cells under the assumption that the inhibitor effectively eliminates eCA activity. This difference in the empirical curve-fitting procedure can lead to some uncertainty in $fHCO_3^-$ (see Rost et al. 2007). A second possibility is that the time delay in processing DBS-treated samples (control experiments were run first, followed by DBS-treated replicates) could have induced slight physiological stress on cells, leading to the observed decrease in $fHCO_3^-$. In recent studies, we have measured photosynthetic efficiency (F_v/F_m) of concentrated Antarctic phytoplankton samples (P. Tortell, C. Payne, and A. Alderkamp, unpublished data) and observed a 5%–25% reduction over time, indicating that cells experience some physiological stress, which may increase the longer cells remain in the concentrated suspension.

Notwithstanding the above caveats, our results demonstrate that the HCO_3^- was the dominant photosynthetic C source for early spring Ross Sea/Southern Ocean assemblages. HCO_3^- utilization in DBS-treated cells cannot be attributed to eCA-catalyzed HCO_3^- dehydration coupled to CO_2 uptake and is thus interpreted as evidence for HCO_3^- transport across the cell membrane (Espie and Colman 1986). Our isotope disequilibrium results are consistent with recent studies in a number of ocean regions (Cassar et al. 2004, Martin and Tortell 2006, Tortell et al. 2006, 2008a) and further strengthen the case for the widespread use of HCO_3^- as a dominant inorganic C source for marine photosynthesis.

eCA in natural phytoplankton assemblages. The relative enhancement of HCO_3^-/CO_2 interconversion (α) calculated from the isotope disequilibrium ^{14}C time-course data ranged from 1.0- to 6.4-fold, with an overall mean enhancement factor of 2.9 ± 1.9

TABLE 2. Calculated carbon-uptake parameters for in situ station samples.

Station	$f\mathrm{HCO_3^-}$ +DBS	$f\mathrm{HCO_3^-}$ control	Catalytic enhancement	eCA ($\mathrm{U \cdot [\mu g\ chl\ } a\mathrm{]^{-1}}$)	$V_{max'}$ ($\mathrm{mg\ C \cdot [mg\ chl\ } a\mathrm{]^{-1} \cdot h^{-1}}$)	$K_{1/2}$ ($\mu\mathrm{mol \cdot L^{-1}}$ DIC)
14[a]	–	–	–	2.7	0.064 ± 0.006	379 ± 134
20	–	–	–	4.2	0.135 ± 0.016	332 ± 139
21	–	–	–	2.4	0.140 ± 0.017	228 ± 109
23	–	–	–	3.9	0.146 ± 0.020	360 ± 174
28	–	–	–	3.6	0.137 ± 0.019	306 ± 151
32	–	–	–	4.1	0.076 ± 0.003	247 ± 35
35	–	–	–	3.3	0.180 ± 0.019	354 ± 135
38	0.72 ± 0.02	0.89 ± 0.01	1.2 ± 0.002	3.2	0.289 ± 0.020	693 ± 128
41	0.70 ± 0.02	0.78 ± 0.08	1.3 ± 0.003	3.8	0.188 ± 0.017	243 ± 80
42	0.70 ± 0.02	0.88 ± 0.02	1.6 ± 0.002	4.0	–	–
43	0.67 ± 0.02	0.83 ± 0.03	1.0 ± 0.002	4.2	–	–
44	0.74 ± 0.02	0.85 ± 0.04	3.7 ± 0.026	4.8	0.216 ± 0.016	439 ± 95
45	0.65 ± 0.03	0.79 ± 0.03	2.2 ± 0.005	6.4	–	–
46	0.83 ± 0.01	0.88 ± 0.04	3.7 ± 0.034	–	–	–
47	0.82 ± 0.02	0.91 ± 0.02	4.9 ± 0.040	6.9	–	–
48	0.82 ± 0.01	0.91 ± 0.05	6.4 ± 0.171	–	–	–
Mean	0.74 ± 0.07	0.86 ± 0.05	2.9 ± 1.9	4.1 ± 1.25	0.162 ± 0.016	383 ± 118

DBS, dextran-bound sulphonamide; DIC, dissolved inorganic carbon; eCA, extracellular carbonic anhydrase.
[a]No isotope disequilibrium data are available for stations 14–35 due to a problem with the ^{14}C buffer used for these experiments.

(Table 2). Additional eCA estimates derived from direct MIMS-based measurements are also presented in Table 2. The range of these MIMS-derived eCA activity estimates ($\mathrm{U \cdot [\mu g\ chl\ } a\mathrm{]^{-1}}$) was 2.4–6.9, with an average of 4.1 ± 1.3. Although the range of values derived from the two eCA activity estimates (MIMS and ^{14}C) is similar, this agreement is fortuitous since the measurements are not quantitatively comparable (see Rost et al. 2007). Nonetheless, qualitative comparisons of ^{14}C-based and MIMS-based eCA estimates indicate some discrepancies between the methods. In particular, station 43, which showed no apparent eCA activity based on ^{14}C assays (catalytic enhancement factor of 1.0), did show activity in MIMS assays. Rost et al. (2007) have discussed the limitations associated with ^{14}C-derived eCA measurements, urging caution in the interpretation of such results, particularly in phytoplankton that possess high relative $\mathrm{HCO_3^-}$ transport (as observed here). We thus believe that MIMS-derived eCA measurements are likely more robust than those derived from ^{14}C experiments. We have, nonetheless, included ^{14}C-based eCA estimates for the purposes of comparison with our previous work in the Ross Sea (see below) where only limited MIMS measurements are available.

Both ^{14}C- and MIMS-based estimates suggested relatively little eCA activity in the early season Ross Sea phytoplankton assemblages we sampled. The values we observed are somewhat low compared to previously reported measurements for laboratory cultures of many temperate species. For example, Rost et al. (2003) reported activities (in units equivalent to those used here) of $\sim$10 $\mathrm{U \cdot (\mu g\ chl\ } a\mathrm{)^{-1}}$ for laboratory cultures of *Phaeocystis globosa* (isolates from the N. Sea) and values up to 30 $\mathrm{U \cdot (\mu g\ chl\ } a\mathrm{)^{-1}}$ for *Skeletonema costatum*. By comparison, much lower eCA activities were measured by Mitchell and Beardall (1996) in a laboratory study of the Antarctic diatom *Nitzschia frigida*, with a mean value $<<1$ $\mathrm{U \cdot (\mu g\ chl\ } a\mathrm{)^{-1}}$. This laboratory result, combined with our field observations, suggests that eCA may play a relatively minor role in C uptake by Antarctic phytoplankton. This result is consistent with the high proportion of direct $\mathrm{HCO_3^-}$ transport by the phytoplankton assemblages.

Carbon-uptake kinetics. Typical results from carbon-uptake kinetic experiments are shown in Figure 3 for a *Phaeocystis*-dominated assemblage (station 20), mixed assemblage (station 32), and diatom-dominated assemblage (station 44). For all assemblages, we observed saturation kinetics of C-fixation indicative of transport-mediated C uptake. Quantitative estimates for apparent half-saturation constants ($K_{1/2}$) and maximum short-term fixation rates ($V_{max'}$) derived from these assays are shown in Table 2. $V_{max'}$ ranged from 0.06 to $\sim$0.3 $\mathrm{mg\ C \cdot (mg\ chl\ } a\mathrm{)^{-1} \cdot h^{-1}}$, while $K_{1/2}$ ranged from 230 to 700 $\mu\mathrm{mol \cdot L^{-1}}$ DIC with an overall mean of 383 $\mu\mathrm{mol \cdot L^{-1}}$.

The mean $K_{1/2}$ value we measured across our sampling stations (<400 $\mu\mathrm{mol \cdot L^{-1}}$ DIC) is slightly higher than previous measurements made for temperate marine phytoplankton assemblages (Tortell et al. 1997, 2000), yet still below the DIC concentration of typical marine waters (>2,000 $\mu\mathrm{mol \cdot L^{-1}}$). In contrast, half-saturation constants of C fixation by RUBISCO in vitro are significantly higher than oceanic CO_2 concentrations. The discrepancy between in vivo and in vitro C-fixation kinetics provides primary evidence for the operation of a CCM (Badger

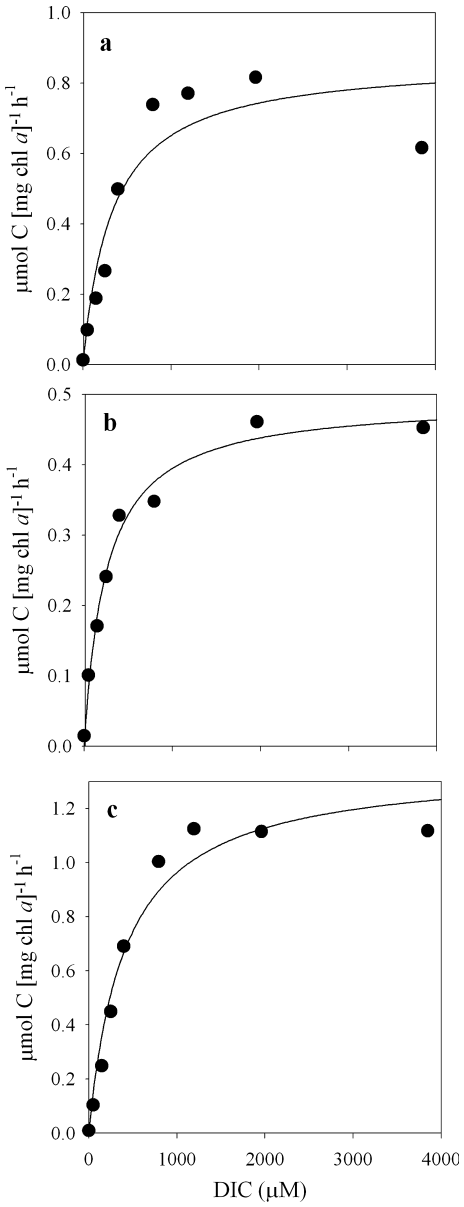

FIG. 3. Typical results of in situ C-uptake kinetic experiments. Data were obtained with the Ross Sea phytoplankton assemblages at station 20 (a), 32 (b), and 44 (c), respectively. DIC, dissolved inorganic carbon.

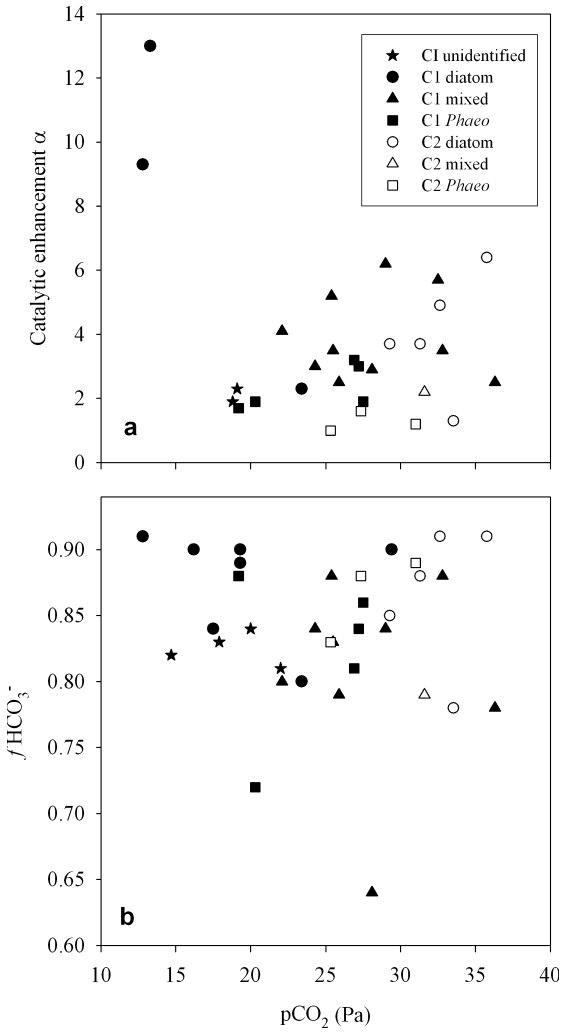

FIG. 4. Relationship between pCO_2 and eCA activity (a), $fHCO_3^-$ (b) for two Ross Sea surveys. In the key, C1 and C2 refer to CORSACS 1 and CORSACS 2, which are the late-season and early season Ross Sea cruises, respectively. eCA, extracellular carbonic anhydrase.

et al. 1998). Despite the operation of a CCM, our kinetic measurements suggest that C fixation by Ross Sea phytoplankton may be slightly undersaturated at ambient DIC concentrations. For example, cells with a $K_{1/2}$ DIC of 400 µM would achieve ~85% of their maximum photosynthetic rate at a DIC concentration of ~2,200 µM. This result suggests the possibility of a CO_2 effect on C-fixation rates (see below).

C acquisition across natural and experimental CO_2 gradients. Our results indicate that HCO_3^- utilization and eCA activity are relatively insensitive to natural and experimental CO_2 gradients during early season blooms. Over a natural pCO_2 range from ~29 to 41 Pa, we observed no statistically significant trends in $fHCO_3^-$ or eCA (measured by either ^{14}C assays or MIMS measurements) ($r^2 < .35$, $P > 0.05$). We have obtained similar results during late-season blooms (Tortell et al. 2008a), and the combined data from our early and late-season surveys show no CO_2-dependent trend in either $fHCO_3^-$ or eCA (Fig. 4) across the full seasonal range in Ross Sea pCO_2 (12 to 41 Pa). This result is strengthened by data obtained from our CO_2-controlled incubations in which $fHCO_3^-$ and eCA (measured by either MIMS or ^{14}C experiments) showed no statistically significant difference across an ~8-fold pCO_2 range (Table 3). This observation is consistent with previous incubation results obtained with late-season, diatom-dominated

TABLE 3. Carbon-uptake parameters for incubation samples.

Treatment	[a]$f\mathrm{HCO_3^-}$	$K_{1/2}$ (μmol · L^{-1} DIC)	eCA (U · [μg chl *a*]$^{-1}$)
Incubation 1			
10.1 Pa	0.74 ± 0.04	357 ± 109	28.6 ± 11.9
36.5 Pa	0.67 ± 0.07	468 ± 127	24.1 ± 7.6
81 Pa	0.77 ± 0.04	597 ± 99	19.7 ± 1.6
Incubation 2			
10.1 Pa	0.89 ± 0.04	618 ± 154	27.1 ± 6.8
36.5 Pa	0.90 ± 0.01	256 ± 99	19.2 ± 1.6
81 Pa	0.98 ± 0.04	895 ± 303	19.5 ± 4.5

DBS, dextran-bound sulphonamide.
[a]Results are presented from DBS-treated experiments due to high parameter estimation errors for control experiments.

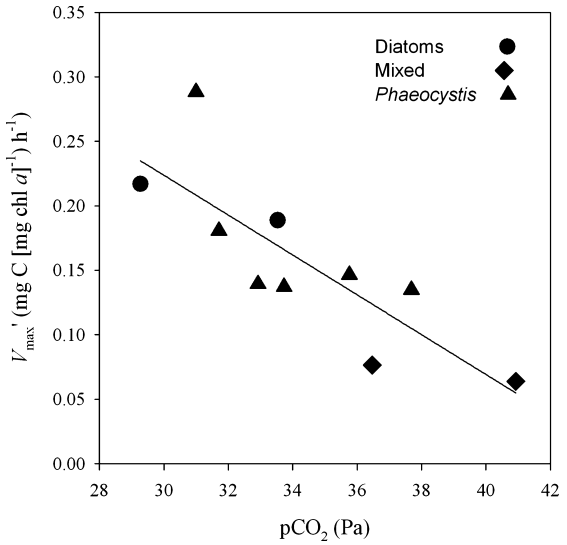

FIG. 5. Relationship between maximum short-term C-fixation rates V_{max}' and pCO_2 for sampling stations. Symbols denote the dominant taxonomic groups present for each sample.

phytoplankton assemblages in the Ross Sea (Tortell et al. 2008a).

The lack of CO_2-dependent regulation of eCA or $f\mathrm{HCO_3^-}$ appears to be a very robust observation for Ross Sea phytoplankton assemblages across a range of growth conditions. In contrast, results from other marine phytoplankton assemblages (Tortell et al. 2002) and a number of laboratory experiments (see Badger and Price 1994 for a comprehensive review) have demonstrated significant CO_2-dependent regulation of eCA activity. The explanation for this discrepancy is presently unclear, though it may be ascribed to interspecific differences in the CO_2-dependent regulation of inorganic C uptake (Rost et al. 2003, Trimborn et al. 2008).

In contrast to eCA and $f\mathrm{HCO_3^-}$, we did observe a relationship between CO_2 concentrations and short-term C fixation measured in substrate-dependent kinetic experiments. For the early season assemblages, there was a strong inverse correlation between ambient CO_2 levels and maximum short-term ^{14}C-fixation rates (V_{max}') measured in kinetic assays ($r^2 = 0.68$, df = 10, $P = 0.003$; Fig. 5). V_{max}' increased ~4.5-fold over a range of surface water pCO_2 from ~41 to 29 Pa, indicating that phytoplankton in low-CO_2 waters possessed a greater capacity for gross short-term C fixation. In contrast, we observed no correlation between CO_2 and $K_{1/2}$ across our survey stations or in our incubation experiments, suggesting that the qualitative nature of the C-uptake system was unaffected by ambient CO_2 levels.

Interpretation of the apparent CO_2-V_{max}' relationship is confounded by the potential covariation between CO_2 and several environmental parameters, and it is difficult to infer mechanistic causality from the observed correlation shown in Figure 5. Two important variables in the Ross Sea are Fe supply and mixed-layer depth. During our survey, neither of these variables was significantly correlated to pCO_2 across the sampling stations ($r^2 < 0.1$ for both variables). Similarly, the apparent pCO_2-V_{max}' relationship cannot be explained by changes in phytoplankton productivity or species composition. Net primary productivity, measured in 24 h incubation experiments (W. O. Smith, unpublished data), showed no significant relationship with ambient CO_2 concentrations across our sampling stations ($r^2 < 0.01$, $n = 19$), while no clear taxonomic pattern was observed in the V_{max}'-CO_2 relationship (Fig. 5).

Unfortunately, V_{max}' data are not available for our incubation experiments due to a problem with chl normalization of samples. However, results from previous field studies in several oceanic regions have demonstrated a CO_2-dependent regulation of short-term C-fixation rates in controlled-incubation experiments and increased abundances of RUBISCO under low-CO_2 conditions (Tortell et al. 2000, 2006, Martin and Tortell 2006). We thus believe that the correlation observed in Figure 5 does indeed result from the CO_2-dependent regulation of gross C fixation in the Ross Sea phytoplankton assemblages we examined.

CO_2-dependent changes in short-term C-fixation rates (Fig. 5) do not necessarily imply similar effects on steady-state primary productivity. As discussed above, we observed no significant relationship between ambient pCO_2 and in situ primary productivity measured in 24 h ^{14}C-incubation experiments. Moreover, in previous work, we observed a small but statistically significant increase in phytoplankton growth rates and primary productivity with increasing CO_2 in 5 d CO_2-manipulation experiments (>5 d) (Tortell et al. 2008b). The explanation for these contrasting results may relate to the CO_2-dependent costs of C acquisition/assimilation. Under low-CO_2 conditions, many phytoplankton upregulate inorganic C transport, gross C fixation, and the expression of key CCM enzymes (e.g., RUBISCO/carbonic anhydrase). This upregulation

may impose additional resource/energy requirements that decrease the efficiency of net C fixation due, for example, to increased respiratory demands.

The CO_2-dependent regulation of cellular C fixation has implications for phytoplankton ecology in the Ross Sea. Increased gross C-fixation rates under low-CO_2 conditions (Fig. 5) could increase cellular resource requirements (Raven and Johnston 1991). Although macronutrients are plentiful in the Ross Sea, micronutrients such as Fe can become limiting in surface waters (Coale et al. 2003), and light intensities can be subsaturating under conditions of deep vertical mixing. It is thus possible that CO_2 availability can interact with other potentially limiting factors to influence phytoplankton productivity in the Ross Sea. It remains unclear, at present, how this CO_2 stimulation may differentially affect diatoms and *Phaeocystis* (we have thus far been unable to directly test the effects of CO_2 on mixed assemblages). Additional controlled experiments are thus needed to directly test the effects of CO_2 on mixed diatom/phytoplankton assemblages, with attention given to other potentially important interacting variables, such as light and trace metals.

Taxonomic and seasonal effects on C acquisition. To examine potential taxonomic and seasonal differences in $f\mathrm{HCO_3^-}$ and eCA activity, we combined results from our springtime survey (this paper) with the results of Tortell et al. (2008a) obtained in the later growth period (December 2005 to January 2006). As shown in Figure 6a, the compiled results of our two cruises indicate that the relative utilization of HCO_3^- as a photosynthetic C source varied little across the annual growth season or between taxonomic groups. All mean $f\mathrm{HCO_3^-}$ values were >0.8, irrespective of sampling time or phytoplankton taxonomic composition, and a two-way analysis of variance (ANOVA) indicated no statistically significant effects of sampling time or taxonomic composition on HCO_3^- utilization (Table 4). In contrast, relative eCA activity (as measured by ^{14}C experiments) did show consistent differences across the seasonal cycle and between taxonomic groups (Fig. 6b, Table 4). For both early and late-season phytoplankton assemblages, we observed a clear pattern of higher eCA activity in diatom-dominated assemblages relative to *Phaeocystis*-dominated assemblages, with intermediate values in taxonomically mixed assemblages. This result is consistent with a previous study in the Bering Sea demonstrating higher eCA levels in diatom-dominated assemblages compared to flagellate-dominated assemblages (Martin and Tortell 2006). Higher eCA levels in Bering Sea diatoms were attributed to their larger cell sizes (and hence diffusive boundary layers) relative to the nanoflagellates. In the Ross Sea, however, the majority of *Phaeocystis* occurred in colonial forms with aggregate sizes (>1,000 μm) larger than any of the diatom species we observed. An alternative explanation for higher eCA activity in Ross Sea diatoms may relate to the hypothesized role of eCA in a C-recycling system, which scavenges CO_2 leaked out of cells via conversion to HCO_3^- and subsequent transport (Trimborn et al. 2008; see above). Under this

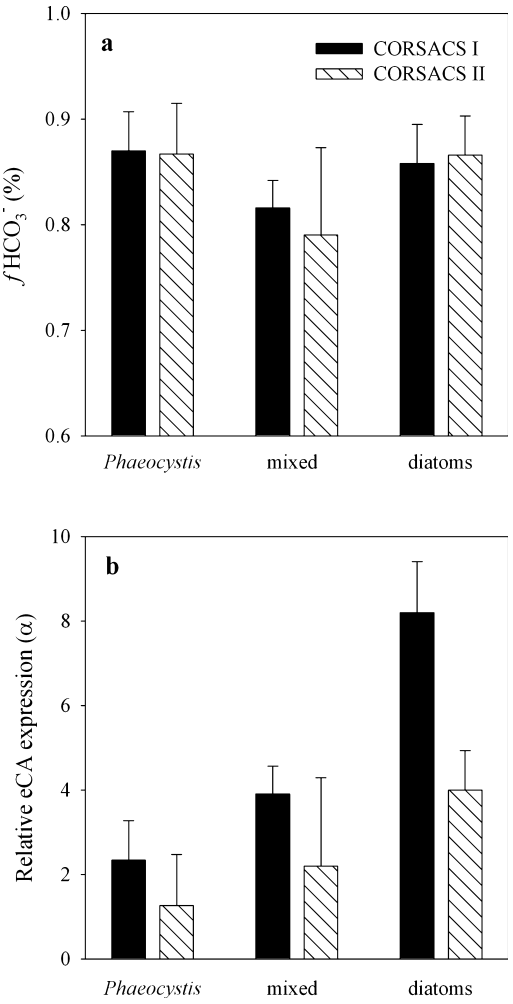

FIG. 6. Compilation of $f\mathrm{HCO_3^-}$ and eCA (^{14}C-based) measurements for two Ross Sea cruises, CORSACS I (December 2005/January 2006), CORSACS II (November 2006), across phytoplankton groups with different taxonomic compositions. eCA, extracellular carbonic anhydrase.

TABLE 4. Results of two-way analysis of variance demonstrating seasonal and taxonomic effects on relative HCO_3^- uptake and extracellular carbonic anhydrase (eCA) activity.

Dependent variable	Independent variable	Degrees of freedom	Mean square	*F*-ratio	*P*
$f\mathrm{HCO_3^-}$	Cruise	1	0.000	0.032	0.86
	Taxa	2	0.006	0.843	0.44
	Cruise × taxa	2	0.000	0.056	0.95
eCA	Cruise	1	22.5	5.16	0.034
	Taxa	2	35.8	8.21	0.003
	Cruise × taxa	2	4.89	1.12	0.344

hypothesis, higher eCA activity in diatoms would indicate a more active C-recycling pathway.

The compiled results of our two Ross Sea cruises also demonstrate a statistically significant trend toward higher eCA activity in the later season (December 2005/January 2006) populations (Table 4). This apparent seasonal difference was seen across all taxonomic groups and thus cannot be simply explained by the greater prevalence of diatoms in the late-season blooms. Previous field studies have reported increased eCA levels over the course of a dinoflagellate bloom in Lake Kineret, coincident with a decline in CO_2 availability (Berman-Frank et al. 1998). In contrast, our results suggest that CO_2 availability does not exert a strong direct influence on eCA activity in the Ross Sea. Similarly, differences in bulk primary productivity cannot explain the apparent seasonal difference in eCA expression. The ^{14}C primary-productivity measurements from >60 stations (W. O. Smith, unpublished data) revealed no systematic differences between phytoplankton C-fixation rates between our two survey cruises (*t*-test: $t = 1.9$, df = 67, $P = 0.20$). Thus, differences in relative C supply/demand do not appear to drive higher eCA activity in later-season blooms. Other environmental factors, such as mixed-layer stratification and surface irradiance levels (both of which increase in the later growth season), may have played a role in regulating eCA activity. Our measurements do not allow us to directly test this possibility.

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