Limnol. Oceanogr., 47(4), 2002, 1192–1197

Sources of inorganic carbon for photosynthesis in a strain of *Phaeodactylum tricornutum*

Abstract—Diatoms are an important functional group of marine phytoplankton because of their role in the fixation of atmospheric carbon dioxide (CO_2) and transfer of organic carbon to deep waters. Carbon-concentrating-mechanisms, such as active CO_2 and bicarbonate (HCO_3^-) uptake and carbonic anhydrase activity, are believed to be essential to marine photosynthesis, because the main carbon-fixing enzyme, ribulose-1,5-bisphosphate carboxylase-oxygenase, is less than half saturated at normal seawater CO_2 concentrations. On the basis of short-term inorganic ^{14}C uptake experiments, Tortell et al. (1997; Nature 390: 243–244) recently argued that marine diatoms are capable of HCO_3^- uptake. However, as discussed herein, the extent of HCO_3^- uptake cannot be assessed on the basis of these experiments. Using short-term $^{14}CO_2$-disequilibrium experiments, we show that a clone of the marine diatom *Phaeodactylum tricornutum* takes up little or no HCO_3^- even under conditions of severe CO_2 limitation. Predicting the response of the oceans to increased CO_2 concentrations will require, among other things, a careful assessment of the extent to which marine algae take up HCO_3^- or CO_2. Because the plasmalemma of microalgae is gas permeable, all phytoplankton exchange CO_2 with the growth medium. Experimental results that are merely consistent with HCO_3^- uptake are insufficient to prove that HCO_3^- uptake is occurring. Our results are in accord with predictions based on stable carbon isotopic fractionation data. Combining isotopic disequilibrium experiments with continuous growth cultures and stable isotope fractionation experiments is a powerful tool for understanding the response of oceanic primary producers to anthropogenic CO_2 emissions as well as for interpreting paleoceanographic carbon isotope data.

Because of the effect of atmospheric CO_2 on global climate, there is increasing scientific interest in the ocean and its biota as potential sinks for anthropogenic CO_2 (Falkowski et al. 2000). An increase in atmospheric CO_2 could be buffered by a stimulation of marine photosynthesis. However, this negative feedback mechanism relies on the assumption that marine photoautotrophs are CO_2 limited and that an increase in dissolved CO_2 concentrations will intensify algal productivity (Riebesell et al. 1993).

Inorganic carbon has rarely been considered a limiting factor to marine phytoplankton growth because of its high concentration in seawater. However, <1% of the dissolved inorganic carbon (DIC) in seawater exists as CO_2 (Millero 1995), the substrate for ribulose-1,5-bisphosphate carboxylase/oxygease (Rubisco). The activity of Rubisco is less than half-saturated at normal seawater CO_2 concentrations (Badger et al. 1998). To circumvent the catalytic inefficiency of Rubisco, aquatic photoautotrophs have evolved ways to actively increase the CO_2 concentration in the vicinity of Rubisco through processes called carbon-concentrating mechanisms (CCMs). Active transport of CO_2 and/or HCO_3^- and active conversion of HCO_3^- to CO_2 by carbonic anhydrase (CA) are putative CCMs (Badger et al. 1998).

The process by which phytoplankton acquire DIC is still the subject of debate (Laws et al. 1997; Keller and Morel 1999) and remains a methodological challenge because of the difficulty in distinguishing between HCO_3^- and CO_2 uptake. Herein, we present an approach that combines the isotopic disequilibrium technique (Espie and Colman 1986) with continuous culture methodology (Laws and Bannister 1980). Combining these methods allowed us to perform short-term isotope disequilibrium experiments directly on axenic cultures without concentrating the cells (cell damage and the release of intracellular CA could occur during the concentration of cells by centrifugation) and to estimate directly the percentage of DIC uptake accounted for by CO_2 under a variety of environmental conditions. In conjunction with the isotopic disequilibrium experiments, stable carbon isotope analyses were used to determine how changes in inorganic carbon supply and demand influenced carbon isotopic fractionation (ε_p) (Laws et al. 1995). The short-term disequilibrium results can therefore better constrain carbon isotope fractionation models used to estimate ancient CO_2 concentrations (Jasper and Hayes 1990; Bidigare et al. 1999). We chose to study *Phaeodactylum tricornutum* because, although not ecologically significant, this species has been the subject of numerous inorganic carbon uptake studies (Rees 1984; Patel and Merrett 1986; Burns and Beardall 1987; Dixon and Merrett 1988; Colman and Rotatore 1995; Rotatore et al. 1995; Iglesias-Rodriguez and Merrett 1997; Burkhardt et al. 2001).

Culture conditions—Axenic cultures of the marine diatom *P. tricornutum* Bohlin (Clone UTEX 642, Culture Collection of Algae MCDB, School of Biological Sciences, The University of Texas at Austin) were grown on modified (100 or 200 μM nitrate) f/2 medium by use of 0.2-μm sterile filtered surface seawater collected from the Hawaii Ocean Time-series, Station ALOHA (Karl and Lukas 1996). The cultures were maintained in nitrate-limited chemostats at constant temperature (22°C and 16°C), salinity (34.8‰), and irradiance (21.6 mol quanta m^{-2} s^{-1}). Light was provided by a bank of daylight fluorescent bulbs. The dissolved CO_2 concentration was controlled by mixing CO_2-free air with air containing 2% CO_2 by use of mass flow controllers. Cell concentrations in the chemostats were $\sim 10^6$ cells ml^{-1} but varied depending on the growth rate.

Chemical and stable isotope analyses—Cultures were considered in steady state when the day-to-day variability in the DIC isotopic signature was within ±0.1‰. Sampling for particulate organic carbon (POC) isotopic analysis and for isotopic disequilibrium experiments was not begun until the

culture had completed at least four doublings at a given growth rate.

DIC and $\delta^{13}C_{DIC}$ were determined as described elsewhere (Kroopnick 1985; Laws et al. 1995). The distribution of carbonate species was determined from temperature, salinity, total alkalinity, DIC, and phosphate and silicate concentrations (Roy et al. 1993; Millero 1995). Total alkalinity was determined by computer-controlled Gran titration. The precision and accuracy of alkalinity and DIC measurements were <8 μeq kg^{-1} and 10 μM, respectively. The analytical uncertainty for the carbon isotopic analyses was <0.1‰.

Samples (25 ml) for isotopic analysis of POC were filtered on precombusted Whatman GF/F glass-fiber filters and were kept frozen until analysis. Samples were vacuum dried and oxidized (with cupric oxide at 700°C overnight) in precombusted vicor tubes. The CO_2 released from the oxidation of the POC was cryogenically distilled. The amount of CO_2 was manometrically measured to determine the POC concentration. The CO_2 isotopic signature was then measured on a MAT 252 mass spectrometer (Santrock et al. 1985).

Short-term ^{14}C disequilibrium experiments—^{14}C assays were performed on 50-ml samples taken from the chemostat at steady state. Experiments were performed at growth temperature in temperature-controlled jacketed glass beakers with magnetic stirrers. Floating semitransparent plastic covers were used to decrease CO_2 exchange with the atmosphere. The experiments were carried out in front of the same light bank used in the continuous culture studies, and the irradiance was identical, 21.6 mol quanta m^{-2} s^{-1}.

Sodium bicarbonate ^{14}C with a specific activity of 50–62 Ci mol^{-1} (Amersham Pharmacia Biotech; code number CFA3) was diluted to a final concentration of 2 μCi ml^{-1} in deionized water that had been previously aerated with nitrogen gas (grade 5; BOC gases) overnight and boiled for 1 h to remove inorganic carbon. To create the ^{14}C isotopic disequilibrium, 0.5 ml (1 μCi) of the final solution was added to the 50-ml sample in the form of $^{14}CO_2$. The $^{14}CO_2$ was prepared immediately before the short-term ^{14}C experiments by acidifying $NaH^{14}CO_3$ to a pH of ~3.2 with a 0.1% HCl solution. The ^{14}C injection increased the dissolved CO_2 concentration by <5% (0.32–0.40 μM increase) in most cases and in no case by >15%. The injection decreased the pH by <0.1.

Samples (2 ml) were taken at timed intervals, with the first sample taken at 10 s. The samples were directly transferred to scintillation vials that contained 0.5 ml of 10% HCl to terminate ^{14}C incorporation and left overnight in a fume hood to degas inorganic ^{14}C (carbon that had not been fixed). Overnight degassing was experimentally shown to be sufficient to eliminate the unfixed inorganic radiocarbon. Twelve milliliters of the liquid scintillation cocktail Aquasol-2 (Packard Bioscience) were added to each sample, and the radioactive signal, which represents acid-resistant organic matter, was then measured in a Packard Tri-Carb 4640 scintillation counter.

Analytical model for the isotopic disequilibrium experiments—Carbonate (CO_3^{2-}) and HCO_3^- were considered as one pool, the HCO_3^- pool, because CO_3^{2-}/HCO_3^- interconversion is nearly instantaneous (Johnson 1982). Carbonic acid (H_2CO_3) is negligible at seawater pH (<1% of CO_2 concentration). Hence, in this model, DIC is the sum of HCO_3^- and CO_2. The concentrations of carbonate species (CO_2, HCO_3^-, CO_3^{2-}, and H_2CO_3) were determined from total alkalinity and total CO_2.

The initial rate of ^{14}C accumulation in the organic matter pool after addition of a $^{14}CO_2$ spike reflects only CO_2 uptake, because >99% of the ^{14}C is added in the form of CO_2. Hence,

$$\text{Initial rate} = R \times SA^0_{CO_2} = R \times SA_{DIC}\frac{DIC}{CO_2} \quad (1)$$

where R is the rate of CO_2 uptake, $SA^0_{CO_2}$ is the initial specific activity of the CO_2, SA_{DIC} is the specific activity of the DIC, and CO_2 and DIC are the concentrations of carbon dioxide and dissolved inorganic carbon, respectively.

Once the ^{14}C spike has equilibrated with the seawater, the specific activities of all forms of inorganic carbon are identical, and the final rate of ^{14}C uptake is given by the equation

$$\text{Final rate} = U \cdot SA_{DIC} \quad (2)$$

where U is the uptake rate of all forms of DIC. Hence, the ratio of the initial to final ^{14}C uptake rate is

$$\left(\frac{\text{Initial rate}}{\text{Final rate}}\right) = \frac{R \times DIC}{U \times CO_2} = f\frac{DIC}{CO_2} \quad (3)$$

where f is the fraction of DIC uptake accounted for by CO_2. Hence

$$f = \left(\frac{\text{Initial rate}}{\text{Final rate}}\right)\left(\frac{CO_2}{DIC}\right) \quad (4)$$

The initial rate was estimated from the activity of ^{14}C in organic carbon after 10 s after correcting for the temperature dependent kinetic conversion of $^{14}CO_2$ to $^{14}HCO_3^-$ by use of equations from Johnson (1982). Because the initial rate is roughly two orders of magnitude larger than the final rate, care must be taken to wait at least 9–10 half-lives (i.e., ~300 s; see below) before beginning to collect data for the determination of the final slope.

Results—Figure 1 shows the pattern of ^{14}C uptake during short-term isotopic disequilibrium experiments with *P. tricornutum.* The uncatalyzed half-isotopic equilibration time between 16°C and 22°C at a pH of 8 is on the order of 30 s (Espie and Colman 1986). As expected in the case of CO_2 uptake, the initial rate of ^{14}C uptake was much greater than the rate after isotope equilibration. Differences between curves are due to differences in growth rate, algal biomass, and the specific activity of the ^{14}C in solution. Hence, one cannot determine the relative proportion of CO_2 to HCO_3^- uptake simply by looking at the temporal increase in ^{14}C activity in the organic phase. The ratio of the initial slope to final slope and the DIC and CO_2 concentrations must be known. The values of f calculated when Eq. 4 was used indicate that HCO_3^- uptake in *P. tricornutum* clone UTEX 642 is small (Fig. 2a, Table 1). CO_2 uptake is at least 84% of the total inorganic carbon uptake, even under conditions

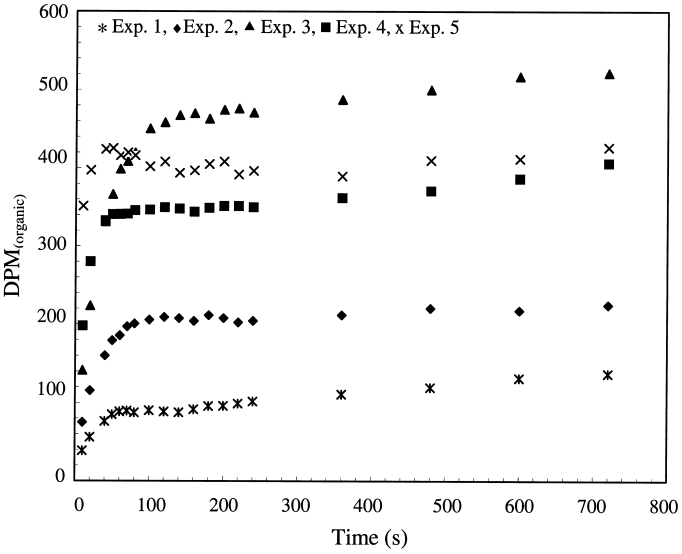

Fig. 1. Examples of the results of short-term ^{14}C experiments with *P. tricornutum* (clone UTEX 642). Each curve represents the average of 2–4 experiments. $DPM_{(organic)}$ is the activity in the acid-resistant organic matter. The variations between curves are due to differences in specific activity, algal growth, and density.

of severe CO_2 limitation (i.e., high algal growth rate and low CO_2 concentration). Because of finite mixing and sampling times and the time required for $^{14}CO_2$ to reach the site of carbon fixation within the cell, the initial rate of uptake in Eq. 4 tends to be underestimated. Hence, the figure of 84% must be regarded as a lower bound on the percentage of inorganic carbon uptake accounted for by CO_2. HCO_3^- transport could be nonexistent in this clone of *P. tricornutum*, and, if present, is minor and most likely constitutive.

No experiments with carbonic anhydrase inhibitors were performed. Because extracellular CA would decrease the time required to reach isotopic equilibrium and would therefore lower the estimate of the initial slope, isotopic disequilibrium experiments with CA inhibitors would not significantly affect the $\%CO_2$ uptake, which is already close to 100%. *P. tricornutum* clone UTEX 642 has in fact been shown not to produce external CA (John-McKay and Colman 1997).

The ratio of microalgal carbon specific growth rate to CO_2 concentration (μ/CO_2) is a useful proxy for the CO_2 demand/supply ratio. Here it was used as a surrogate for the extent of CO_2 limitation to which the microalga was exposed. A >75-fold increase in μ/CO_2 (0.008–0.619 kg μmol^{-1} d^{-1}) did not significantly change the percentage of uptake accounted for by CO_2 (84% vs. 88% CO_2 uptake, respectively; Fig. 2a). In other words, induction of HCO_3^- transport in response to CO_2 limitation was not observed. As opposed to what has recently been proposed with respect to natural populations (Tortell et al. 1997), not only is HCO_3^- transport across the plasmalemma in *P. tricornutum* small (if any), but it is not inducible over the range of μ/CO_2 reported in field studies (Tortell et al. 2000).

Discussion—Theoretical models predict that if passive diffusion of CO_2 accounts for all DIC uptake, the relationship between ε_p and μ/CO_2 should be linear, and indeed this is the case for *P. tricornutum* when $\mu/CO_2 \leq 0.15$ kg μmol^{-1} d^{-1} (Fig. 2b). However, the relationship becomes distinctly nonlinear at higher μ/CO_2 (Fig. 2b), as has been reported in several laboratory and field studies on various microalgal species (Tortell et al. 2000). Because HCO_3^- uptake appears to be minimal and is not inducible in this clone of *P. tri-*

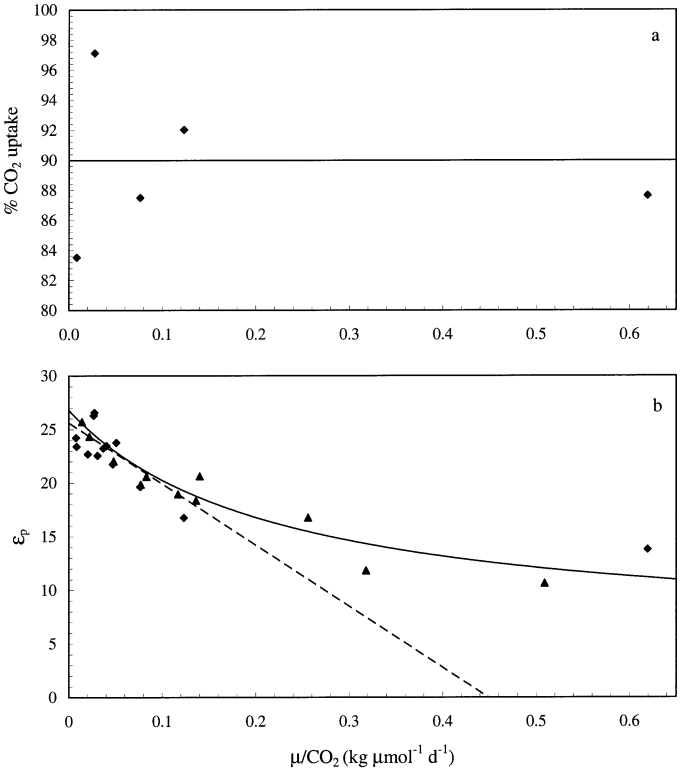

Fig. 2. (a) Percentage of CO_2 uptake, as determined by the isotopic disequilibrium experiments, vs. μ/CO_2 for *P. tricornutum* (clone UTEX 642). Each point is the average of 2–4 experiments. The horizontal line is the average $\%CO_2$ uptake (90%). SEs vary from 2.6% ($\mu/CO_2 = 0.619$) to 16.7% ($\mu/CO_2 = 0.027$). (b) Carbon isotope fractionation (ε_p) vs. μ/CO_2 for *P. tricornutum* (diamonds, this study). The SE of the ε_p is $\pm$0.05‰, and the SE of the μ/CO_2 measurements is about $\pm$5% of the mean value at the given growth rate. Triangle symbols are *P. tricornutum* (clone CCMP1327) data from Laws et al. (1997). Diamond symbols are from this study. The dashed line is the relationship between ε_p and μ/CO_2 predicted by the passive diffusion model of Laws et al. 1995. The continuous line is a nonlinear fit to the data (see Laws et al. 1997).

Table 1. Summary of the experimental results. $\%CO_2$ uptake = 100 *f*.

Experiment	Temperature (°C)	μ/CO_2	DIC (μmol kg^{-1})	CO_2 (μmol kg^{-1})	ε_p (‰)	$\%CO_2$ uptake
1	22	0.008	2329	31.3	23.42	84
2	16	0.027	1987	8.9	26.56	97
3	16	0.076	2012	12.2	19.65	88
4	22	0.123	1951	8.5	16.74	92
5	22	0.619	1654	2.3	13.80	88

cornutum, the nonlinearity of the relationship between ε_p and μ/CO_2 must reflect active uptake of CO_2, as has been suggested by Laws et al. (1997).

It has recently been argued on the basis of short-term inorganic ^{14}C uptake experiments that HCO_3^- is an important source of inorganic carbon for diatoms (Tortell et al. 1997). This scientific correspondence, entitled, ''Active uptake of bicarbonate by diatoms,'' has been frequently cited as evidence of bicarbonate uptake (Nimer et al. 1999; Lane and Morel 2000; Taraldsvik and Myklestad 2000; Burkhardt et al. 2001; Rau et al. 2001). In the experiments presented by Tortell et al. (1997), inorganic ^{14}C equilibrated in a seawater solution (P. D. Tortell, pers. comm.) was added to natural samples of seawater dominated by large diatoms. The fact that ^{14}C uptake was observed within 10 s was erroneously interpreted by the authors (p. 243) as evidence of ''active uptake of HCO_3^- in the field,'' and they argued, ''Carbonic anhydrase is therefore required to catalyze intracellular dehydration of actively imported HCO_3^-.'' Their conclusion was based on the fact that >99% of the ^{14}C activity was present in the form of HCO_3^-. However, in tracer kinetics, the uptake of a labeled substrate is determined by the substrate's specific activity (e.g., activity per mol; see Eqs. 1 and 2), not its activity (Lambrecht and Rescigno 1983; Espie and Colman 1986). Because the inorganic ^{14}C was allowed to equilibrate in a seawater solution prior to addition to the seawater samples, the specific activities of all forms of inorganic carbon were identical throughout the experiment. Hence, contrary to the authors' conclusions, the fact that uptake was observed during the first 10 s proved nothing regarding the form of inorganic carbon being taken up by the microalgae.

In contrast, if the inorganic ^{14}C spike is not initially in isotopic equilibrium with the inorganic carbon in the medium, the change in the rate of ^{14}C uptake as the ^{14}C equilibrates with the inorganic carbon in the medium may provide insights about the form of inorganic carbon crossing the plasmalemma (Espie and Colman 1986). The change in uptake rate will be most apparent if the spike contains ^{14}C primarily in the form of CO_2 (Elzenga et al. 2000). Under these conditions, the initial specific activity of the CO_2 in the seawater will be ~100 times greater than the equilibrium-specific activity. The initial uptake rate of ^{14}C will therefore be ~100 times greater than the final uptake rate if the microalgae are taking up CO_2 exclusively. Comparison of the initial and final ^{14}C uptake rates therefore allows a quantitative assessment of the percentage of DIC uptake accounted for by CO_2 (Eq. 4, Fig. 1). To the extent that HCO_3^- was being taken up, a $H^{14}CO_3^-$ injection would produce only a small change in ^{14}C uptake kinetics over time, because the change in specific activity of $H^{14}CO_3^-$ would be small (i.e., most inorganic carbon in seawater is in the form of HCO_3^-).

Our results by no means preclude the possibility that some photosynthetic eukaryotes use HCO_3^- as an inorganic carbon source. In some cases, HCO_3^- conversion to CO_2 is catalyzed by an external carbonic anhydrase, and CO_2 is the form of inorganic carbon that crosses the plasmalemma (Elzenga et al. 2000). Some eukaryotic microalgae may in fact actively transport HCO_3^- across the plasmalemma (Elzenga et al. 2000), but reports that microalgae take up bicarbonate to the exclusion of CO_2 must be viewed with caution (Elzenga et al. 2000), because there is no microalga whose plasmalemma is known to be impermeable to CO_2. Indeed, the fact that carbon isotopic fractionation is always observed implies that some of the internal inorganic carbon leaks out of the cell and therefore that the plasmalemma is permeable to CO_2.

Conflicting reports on carbon uptake mechanisms in *P. tricornutum* seem to be attributable to the use of different clones and growth conditions in culture studies. John-McKay and Colman (1997) found that *P. tricornutum* clone UTEX 642 lacks external CA activity. Our results are consistent with their work. Other strains of *P. tricornutum* show different levels of external CA activity (John-McKay and Colman 1997). In contrast to the work of Rees (1984), Dixon and Merrett (1988), Colman and Rotatore (1995), and Rotatore et al. (1995), we could not find evidence of direct bicarbonate transport across the plasmalemma. Burkhardt et al. (2001) recently showed that a different strain of *P. tricornutum*, also with low external CA activity, demonstrated a preference for CO_2 uptake, although HCO_3^- uptake was observed. They also found that *Thalassiosira weissflogii*, another marine diatom, preferentially takes up HCO_3^- and concurrently has a high external CA activity. This result seems counterintuitive, because these two physiological processes (HCO_3^- transport and HCO_3^- conversion to CO_2) compete for the same substrate. Because of the apparently large intra- and interspecific variations in inorganic carbon uptake mechanisms among microalgae, extrapolation of our results from a single clone to natural populations or even to other clones of *P. tricornutum* is unjustified.

The methodological approach we present in this note can be used to better understand carbon uptake in marine photoautotrophs. At issue is whether the form of inorganic carbon that crosses the plasmalemma is HCO_3^- or CO_2. There is no question that some active transport is required in almost all cases. The fact that we did not observe an increase in HCO_3^- uptake in response to CO_2 limitation does not preclude the presence of an inducible active CO_2 uptake mechanism. The short-term disequilibrium experiments only tell which form of inorganic carbon crosses the plasmalemma, not whether this transport is active or passive. Hence, it is probable that as μ/CO_2 became large, the cells were actively taking up CO_2. In fact, Rotatore et al. (1995) found evidence of active CO_2 transport in this particular strain of *P. tricornutum* (UTEX 642).

To predict the response of the biological pump and oceanic carbon sequestration to increases in dissolved CO_2 concentrations, it will be important to perform definitive experiments to determine what forms of inorganic carbon are transported in various phytoplankton species. Isotopic disequilibrium experiments carried out on continuous growth cultures in combination with stable isotope fractionation experiments provide a powerful mechanism for addressing this question.

Nicolas Cassar and Edward A. Laws

Department of Oceanography
School of Ocean and Earth Science and Technology
University of Hawaii
Honolulu, Hawaii 96822

Brian N. Popp

Department of Geology and Geophysics
School of Ocean and Earth Science and Technology
University of Hawaii
Honolulu, Hawaii 96822

Robert R. Bidigare

Department of Oceanography
School of Ocean and Earth Science and Technology
University of Hawaii
Honolulu, Hawaii 96822

References

BADGER, M. R., T. J. ANDREWS, S. M. WHITNEY, M. LUDWIG, D. C. YELLOWLEES, W. LEGGAT, AND G. D. PRICE. 1998. The diversity and coevolution of Rubisco, plastids, pyrenoids, and chloroplast-based CO_2-concentrating mechanisms in algae. Can. J. Bot. **76:** 1052–1071.

BIDIGARE, R. R., AND OTHERS. 1999. Iron-stimulated changes in ^{13}C fractionation and export by equatorial Pacific phytoplankton: Toward a paleogrowth rate proxy. Paleoceanography **14:** 589–595.

BURKHARDT, S., G. AMOROSO, U. RIEBESELL, AND D. SÜLTEMEYER. 2001. CO_2 and HCO_3^- uptake in marine diatoms acclimated to different CO_2 concentrations. Limnol. Oceanogr. **46:** 1378–1391.

BURNS, B. D., AND J. BEARDALL. 1987. Utilization of inorganic carbon by marine microalgae. J. Exp. Mar. Biol. Ecol. **107:** 75–86.

COLMAN, B., AND C. ROTATORE. 1995. Photosynthetic inorganic carbon uptake and accumulation in two marine diatoms. Plant Cell Environment **18:** 919–924.

DIXON, G. K., AND M. J. MERRETT. 1988. Bicarbonate utilization by the marine diatom *Phaeodactylum tricornutum* 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 isotopic 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 isotopic disequilibrium technique. Plant Physiol. **80:** 863–869.

FALKOWSKI, P. G., AND OTHERS. 2000. The global carbon cycle: A test of our knowledge of Earth as a system. Science **290:** 291–296.

IGLESIAS-RODRIGUEZ, M. D., AND M. J. MERRETT. 1997. Dissolved inorganic carbon utilization and the development of extracellular carbonic anhydrase by the marine diatom *Phaeodactylum tricornutum.* New Phytol. **135:** 163–168.

Acknowledgments—We thank Dr. Ulf Riebesell and an anonymous reviewer for their very constructive comments on this manuscript. We thank Terri Rust and David Hashimoto for help with isotopic analyses and laboratory cultures. We are also grateful to the HOT program and its personnel for their assistance in collecting the seawater for media. This research was partially supported by National Science Foundation grants OCE-9633091 (to B.N.P., R.R.B., E.A.L., and Stuart G. Wakeham), OCE-9521332 (to B.N.P. and E.A.L.) and OCE-9725966 (to E.A.L.) and by a NSERC scholarship to N. C. This is SOEST contribution number 5932.

JASPER, J. P., AND J. M. HAYES. 1990. A carbon isotope record of CO_2 levels during the late Quaternary. Nature **374:** 462–464.

JOHN-MCKAY, M. E., AND B. COLMAN. 1997. Variation in the occurrence of external carbonic anhydrase among strains of the marine diatom *Phaeodactylum tricornutum* (bacillariophyceae). J. Phycol. **33:** 988–990.

JOHNSON, K. S. 1982. Carbon dioxide hydration and dehydration kinetics in seawater. Limnol. Oceanogr. **27:** 849–855.

KARL, D. M. AND R. LUKAS. 1996. The Hawaii Ocean Time–series (HOT) program: Background, rationale and field implementation. Deep-Sea Res. II **43:** 129–156.

KELLER, K., AND F. M. M. MOREL. 1999. A model of carbon isotopic fractionation and active carbon uptake in phytoplankton. Mar. Ecol. Prog. Ser. **182:** 295–298.

KROOPNICK, P. 1985. The distribution of ^{13}C in ΣCO_2 in the world oceans. Deep-Sea Res. **32:** 57–84.

LAMBRECHT, R. M., AND A. RESCIGNO. 1983. Tracer kinetics and physiologic modeling: theory to practice. Society of Nuclear Medicine. Springer-Verlag.

LANE, T. W., AND F. M. M. MOREL. 2000. Regulation of carbonic anhydrase expression by zinc, cobalt, and carbon dioxide in the marine diatom *Thalassiosira weissflogii*. Plant Physiol. **123:** 345–352.

LAWS, E. A., AND T. T. BANNISTER. 1980. Nutrient- and light-limited growth of *Thalassiosira fluviatilis* in continuous culture, with implications for phytoplankton growth in the ocean. Limnol. Oceanogr. **25:** 457–473.

———, R. R. BIDIGARE, AND B. N. POPP. 1997. Effect of growth rate and CO_2 concentration on carbon isotopic fractionation by the marine diatom *Phaeodactylum tricornutum*. Limnol. Oceanogr. **42:** 1552–1560.

———, B. N. POPP, R. R. BIDIGARE, M. C. KENNICUTT, AND S. A. MACKO. 1995. Dependence of phytoplankton carbon isotopic composition on growth rate and $[CO_2]_{aq}$: Theoretical considerations and experimental results. Geochim. Cosmochim. Acta **59:** 1131–1138.

MILLERO, F. J. 1995. Thermodynamics of the carbon dioxide system in seawater. Geochim. Cosmochim. Acta. **59:** 661–677.

NIMER, N. A., C. BROWNLEE, AND M. J. MERRETT. 1999. Extracellular carbonic anhydrase facilitates carbon dioxide availability for photosynthesis in the marine dinoflagellate *Prorocentrum micans.* Plant Physiol. **120:** 105–111.

PATEL, B. N., AND M. J. MERRETT. 1986. Inorganic-carbon uptake by the marine diatom *Phaeodactylum tricornutum.* Planta **169:** 222–227.

RAU, G. H., F. P. CHAVEZ, AND G. E. FRIEDERICH. 2001. Plankton $^{13}C/^{12}C$ variations in Monterey Bay, California: Evidence of non-diffusive inorganic carbon uptake by phytoplankton in an upwelling environment. Deep-Sea Res. I **48:** 79–94.

REES, T. A. V. 1984. Sodium dependent photosynthetic oxygen evolution in a marine diatom. J. Exp. Bot. **35:** 332–337.

RIEBESELL, U., D. A. WOLF-GLADROW, AND V. SMETACEK. 1993. Carbon dioxide limitation of marine phytoplankton growth rates. Nature **361:** 249–251.

ROTATORE, C., B. COLMAN, AND M. KUZMA. 1995. The active uptake of carbon dioxide by the marine diatoms *Phaeodactylum tricornutum* and *Cyclotella* sp. Plant Cell Environ. **18:** 913–918.

ROY, R. N., AND OTHERS. 1993. The dissociation constants of carbonic acid in seawater in salinities 5 to 45 ‰ and temperatures 0 to 45°C. Mar. Chem. **44:** 249–267.

SANTROCK, J., S. A. STUDLEY, AND J. H. HAYES. 1985. Isotopic analyses based on the mass spectrum of carbon dioxide. Anal. Chem. **57:** 1444–1448.

TARALDSVIK, M., AND S. MYKLESTAD. 2000. The effect of pH on growth rate, biochemical composition and extracellular carbohydrate production of the marine diatom *Skeletonema costatum.* Eur. J. Phycol. **35:** 189–194.

TORTELL, P. D., G. H. RAU, AND F. M. M. MOREL. 2000. Inorganic carbon acquisition in coastal Pacific phytoplankton communities. Limnol. Oceanogr. **45:** 1485–1500.

———, J. R. REINFELDER, AND F. M. M. MOREL. 1997. Active uptake of bicarbonate by diatoms. Nature **390:** 243–244.

Received: 23 July 2001
Amended: 13 February 2002
Accepted: 5 March 2002

Limnol. Oceanogr., 47(4), 2002, 1197–1201

Benthic photosynthesis in an acidic mining lake (pH 2.6)

Abstract—Natural neutralization of acidic mining lakes is usually limited by the availability of organic carbon. We investigated whether benthic photosynthesis could contribute to primary production in an acidic mining lake (pH 2.6). The occurrence and light dependence of benthic photosynthesis in the lake was investigated using oxygen microelectrodes. Oxygen microprofiles measured in light and darkness were significantly different, indicating photosynthetic activity. The photic zone was 300 μm thick and the highest photosynthetic activity was found at the sediment surface, which was covered by a dense layer of diatoms. These algae, predominantly *Eunotia* spp. and *Pinnularia obscura,* were found to be adapted to low light intensities. The community compensation irradiance was 6.8 μE m^{-2} s^{-1}, corresponding to an annual mean compensation depth of 1.8 m. These results imply that 13% of the lake area could have a net efflux of oxygen from the sediment. Even at an irradiance as low as 1.2 μE m^{-2} s^{-1}, photosynthetic activity was detected. The relatively low light requirements for benthic photosynthesis in this acidic environment may be due to an efficient absorption of red light, the dominant wavelength available in this ferric iron-rich lake. Our results suggest that benthic photosynthesis can play an important role in the biogeochemistry of acidic mining lakes.

In mining areas, the oxidation of pyrite and marcasite associated with coal or metal ores leads to the formation of acid mine drainage (AMD). Lakes fed by AMD, either by groundwater or surface flow, are usually extremely acidic with a pH ranging from 2 to 4. In these lakes the pH is buffered by ferric iron: $Fe^{3+} + 3H_2O \Leftrightarrow Fe(OH)_3 + 3H^+$, and the high iron content of the water leads to the typical reddish color of such lakes. An understanding of the ecosystem structure and function of these lakes is essential for the development of appropriate remediation strategies (Geller et al. 1998).

Enclosure experiments and in-situ observations indicate that the process of natural neutralization within acidic lakes depends on the amount of organic carbon available as a substrate for iron and sulfate reduction and on lake mixing and oxygen supply (Davison et al. 1995; Klapper and Schultze 1995). Therefore, primary production could influence the acidity of these lakes by producing organic carbon and liberating oxygen. Planktonic primary production in acidic mining lakes is usually low (2.7 mmol C m^{-2} d^{-1}, Gyure et al. 1987; 0.08–16.5 mmol C m^{-2} d^{-1}, Lessmann et al. 1999) because of low phytoplankton biomass and low biomass-specific production in these lakes (Nixdorf and Kapfer 1998; Lessmann et al. 1999). However, existing estimates of primary production only take into account pelagic photosynthesis. It is not known if, and to what extent, photosynthesis by benthic algae contributes to the primary production of acidic mining lakes. In a literature survey, about half of the lakes reviewed had benthic algal production equal to or higher than phytoplankton production (Vadeboncoeur et al. 2001). Mining lakes are typically shallow. In such lakes, benthic photosynthesis can make a significant contribution to carbon fixation (Sand-Jensen and Borum 1991). The present study was undertaken to determine whether, and to what extent, benthic photosynthesis takes place in an acidic mining lake.

The study was carried out in Mining Lake 111 (ML111) in the Lusatian mining district in Germany (51°29′N, 13°38′E). The lake has a surface area of 0.11 km^2, a mean depth of 4.7 m, and a maximum depth of 10.2 m (Büttner et al. 1998). The lake pH was 2.6 and the titratable acid ($K_{B8.2}$) was 15.5 mM. Concentrations of SO_4^{2-}, Fe^{3+}, and Al^{3+} were considered high at 12.5 mM, 2.5 mM, and 1.5 mM, respectively (Friese et al. 1998; Herzsprung et al. 1998). Acidity is supplied continuously by groundwater inflow. No natural neutralization of the water has been observed since the formation of the lake in 1958.

On 8 August 2000, two sediment cores were collected at a water depth of 7 m and transported at 4°C to a climate chamber. Four hours after sampling, the cores were immersed in an aquarium containing original lake water at an in situ temperature of 9°C. The water in the aquarium was continuously bubbled with air containing 5% CO_2. Concentration of CO_2 increased from 4.8 to 10.3 mg C L^{-1} over the course of the experiments. CO_2 was not assumed to limit photosynthesis and fell within the range of in situ concentrations at the depth sampled (6.1–16.6 mg C L^{-1}). The cores were preincubated for 12 h at a photosynthetically available radiation (PAR) of 1.7 μE m^{-2} s^{-1}, corresponding to typical in situ PAR measured 7 m deep on a cloudy summer day (assuming subsurface PAR 700 μE m^{-2} s^{-1}). A specially designed optical device, consisting of halogen and fluorescent lamps, colored acetate transparencies, and a layer of circulating deionized water, was used to simulate the characteristic red light spectrum (Fig. 1a). PAR was measured