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Consistent fractionation of ^{13}C in nature and in the laboratory: Growth-rate effects in some haptophyte algae

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Abstract. The carbon isotopic fractionation accompanying formation of biomass by alkenone-producing algae in natural marine environments varies systematically with the concentration of dissolved phosphate. Specifically, if the fractionation is expressed by $\varepsilon_P \approx \delta_e - \delta_p$, where δ_e and δ_p are the $\delta^{13}C$ values for dissolved CO_2 and for algal biomass (determined by isotopic analysis of C_{37} alkadienones), respectively, and if C_e is the concentration of dissolved CO_2, μmol kg^{-1}, then $b = 38 + 160*[PO_4]$, where $[PO_4]$ is the concentration of dissolved phosphate, μM, and $b = (25 - \varepsilon_P)C_e$. The correlation found between b and $[PO_4]$ is due to effects linking nutrient levels to growth rates and cellular carbon budgets for alkenone-containing algae, most likely by trace-metal limitations on algal growth. The relationship reported here is characteristic of 39 samples ($r^2 = 0.95$) from the Santa Monica Basin (six different times during the annual cycle), the equatorial Pacific (boreal spring and fall cruises as well as during an iron-enrichment experiment), and the Peru upwelling zone. Points representative of samples from the Sargasso Sea ($[PO_4] \leq 0.1$ μM) fall above the $b = f[PO_4]$ line. Analysis of correlations expected between μ (growth rate), ε_P, and C_e shows that, for our entire data set, most variations in ε_P result from variations in μ rather than C_e. Accordingly, before concentrations of dissolved CO_2 can be estimated from isotopic fractionations, some means of accounting for variations in growth rate must be found, perhaps by drawing on relationships between $[PO_4]$ and Cd/Ca ratios in shells of planktonic foraminifera.

Introduction

Interest in measurements of the carbon isotopic composition of marine organic matter stems from the observation that fractionation of ^{13}C during photosynthesis often reflects environmental conditions at the time the organic matter was formed. In particular, the degree of fractionation is positively correlated with the concentration of dissolved carbon dioxide in seawater, C_e [*Arthur et al.*, 1985; *Popp et al.*, 1989; *Rau et al.*, 1989; *Freeman and Hayes*, 1992]. More recently, theoretical, field, and laboratory results have established that photosynthetic fractionation can be negatively correlated with microalgal growth rates [*Fry and Wainwright*, 1991; *Francois et al.*, 1993; *Goericke et al.*, 1994; *Laws et al.*, 1995]. The former consideration has suggested that measurement of the $\delta^{13}C$ of organic matter preserved in sediments may provide an indication of ancient P_{CO_2} levels [*Jasper and Hayes*, 1990; *Freeman and Hayes*, 1992; *Hayes*, 1993; *Jasper et al.*, 1994]. The latter consideration indicated that measurement of the $\delta^{13}C$ of living phytoplankton cells could be used to estimate their growth rates without the use of incubation techniques [*Laws et al.*, 1995]. In this paper we expand on this previous research and explore the relationship between carbon isotope fractionation, phytoplankton growth rate, and C_e in the alkenone-containing haptophyte algae, *Emiliania huxleyi*, and the closely related *Gephyrocapsa oceanica*.

E. huxleyi (Lohman) Hay and Mohler is a haptophyte (sensu prymnesiophyte) which occurs throughout the world's oceans, from polar regions of high productivity to the oligotrophic subtropical gyres [*Westbroek et al.*, 1993]. It is the dominant coccolithophorid in marine waters cooler than 20°C and warmer than 25°C but coexists with a diversity of related species in waters of intermediate temperatures. In addition to its importance to the carbon cycle, *E. huxleyi* produces biomarkers in the form of long-chain (C_{37}, C_{38}, and C_{39}) alkenones (see *Brassell* [1993]). The closely related *G. oceanica* also produces C_{37-39} alkenones and

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may be an important source of those compounds in some waters [*Volkman et al.*, 1995; *Conte et al.*, 1994, 1995]. Alkenones are well preserved in marine sediments, and their molecular distributions have been used to infer paleo-sea surface temperatures [*Brassell*, 1993]. While the noncalcifying haptophytes, *Crysotila* and *Isochrysis*, can also produce C_{37-39} alkenones, they are not considered a likely source of alkenones in open-oceanic waters since their distributions are restricted to coastal waters [*Marlowe et al.*, 1990]. Because of *E. huxleyi*'s cosmopolitan distribution, its importance to the carbon cycle, and its contributions to the sedimentary record [*Westbroek et al.*, 1994], it is important to understand the factors which control isotopic fractionation in this algal species.

In this paper we show that (1) a quantifiable relationship exists between growth rate, C_e, and isotopic fractionation in laboratory chemostat experiments with *E. huxleyi* and (2) that isotopic fractionations in nature, as determined by isotopic analyses of the C_{37} alkadienone, can be related to concentrations of dissolved CO_2 and soluble reactive phosphate ($[PO_4]$). Our results suggest that growth rates of ancient alkenone-containing haptophytes may be constrained, allowing improved estimation of ancient P_{CO_2} levels, based on relationships between $[PO_4]$ and Cd/Ca ratios in shells of planktonic foraminifera.

Samples and Procedures

Chemostat. Naked and coccolith-bearing clones of the haptophyte *Emiliania huxleyi* were grown in a nitrate-limited chemostat culture at specific growth rates (μ) of 0.20 to 0.60 d^{-1} on continuous light and at constant temperature (18°C) in systems identical to that described by *Laws et al.* [1995]. The noncalcifying clone BT6 (clone CCMP373) was obtained from the Center for Culture of Marine Phytoplankton (West Boothbay Harbor, Maine), and the calcifying clone PLY B92/11 was obtained from J.C. Green (Plymouth Marine Laboratory). We measured daily cell density, chlorophyll fluorescence, concentration of total dissolved inorganic carbon (DIC), total alkalinity (to calculate C_e), and the δ_{DIC} in the growth chamber (all values of δ refer to $\delta^{13}C$ relative to the Pee Dee belemnite (PDB) standard). Samples of algal organic matter were taken for carbon isotopic analysis only after values of δ_{DIC} were within $\pm 0.1‰$ for 3 consecutive days. The day-to-day reproducibility of the growth chamber dilution rate was ± 0.01 d^{-1}. Collection, processing of algae and water samples, and all calculations are identical to those of *Laws et al.* [1995].

Natural populations. Characteristics of six sets of samples of suspended particulate matter are summarized in Table 1. Brief summaries of the methods of collection and of subsequent processing follow. The objective in each case has been the isolation and carbon-isotopic analysis of the C_{37} alkadienone. Carbon-isotopic fractionation (ε_p) values for natural populations were calculated using equation (1) of *Freeman and Hayes* [1992] which requires knowledge of the isotopic difference ($\Delta\delta$) between total biomass and the C_{37} alkadienone (see discussion given by *Jasper et al.* [1994]). The $\Delta\delta$ value we adopt here is 4‰. By comparison, F. Kenig et al. (manuscript in preparation, 1997) recently determined $\Delta\delta$ values for *E. huxleyi* clones BT6 and B92/11 and found a mean value of $4.1 \pm 0.1‰$ based on analyses of the C_{37} alkadienone from 10 culture experiments. Concentrations of carbonate species for all natural samples were determined from concentrations of DIC, phosphate, and silicate as well as total alkalinity following *Millero* [1995]. The dissociation constants for carbonic and boric acids used in this calculation were from *Dickson* [1990a, b] and *Roy et al.* [1993]. Apparent constants were corrected for the effects of pressure [*Millero*, 1979]. Isotopic composition of C_e (δ_e) for all natural samples was determined from the relative abundances of bicarbonate, carbonate, and C_e and the temperature-fractionation relationships of *Deines et al.* [1974] and *Mook et al.* [1974]. All compound-specific isotopic results reported in this study were collected using techniques described by *Hayes et al.* [1990], *Merritt and Hayes* [1994], and *Merritt et al.* [1995].

Samples 1-4 were collected during cruise TT008 of the R/V *Thomas G. Thompson* using a Flotec Tempest submersible pump in series with an Eastern MD50 magnetic-drive deck pump and a QM-A quartz microfiber filter and a 53 μm Nitex screen. They were provided by M. Bacon of the Woods Hole Oceanographic Institution. The material analyzed here represents 1/6 of each QM-A filter and includes particles in the size range 1–53 μm. Samples 5-7 were collected during cruise TT007 of the *Thompson* and were provided by A. Mix and J. Wilson of Oregon State University. They represent 3/20 splits of the >63 μm fraction from tows of the Multiple Opening and Closing Net and Environmental Sensing System (MOCNESS). Samples were processed and analyzed at Indiana University using procedures described by *Jasper et al.* [1994]. Briefly, the particulate organic matter (POM) was extracted ultrasonically once with isopropanol and twice with CH_2Cl_2/MeOH (1/1). The extracts were combined, and an alkenone-containing fraction was isolated by chromatography on silica gel. That fraction was saponified to remove alkenoates, the purity of the alkenones was checked by gas chromatography (GC; Ultra-1, 50 m), and isotope-ratio-monitoring gas chromatography-mass spectrometry (irmGCMS; Rtx-1, 50 m) was used to determine values of δ for the alkenones.

Samples 8-16 were collected during cruise TT011 of the *Thompson* in collaboration with J.K.B. Bishop (University of Victoria) using a Multiple Unit Large-Volume in situ Filtration System (MULVFS) [*Bishop et al.*, 1985]. During filtration, POM was fractionated into >53 μm (Nitex screen) and 1-53 μm (quartz-fiber filter) size classes and immediately frozen; only the 1-53 μm material has been analyzed. These samples were processed at the Skidaway Institute of Oceanography using the procedures described by *Freeman and Wakeham* [1992]. Briefly, the filters were Soxhlet extracted with CH_2Cl_2/MeOH (2/1), the lipids were saponified, and the non-saponifiable lipids (NSL) were recovered by extraction of the alkaline phase with hexane. Following derivatization of polar components (BSTFA), alkenones in the NSL fraction were quantified by GC (DB-1, 25 m) and checked for purity by GCMS (INCOS 50, DB-5, 25 m), then analyzed isotopically by irmGCMS at Penn State University.

Synoptic hydrographic and geochemical data for samples 1-16 were obtained from CTD/water-bottle casts taken immediately before or after sampling. Concentrations of DIC (ΣCO_2) and alkalinities are from D. Archer and C. Goyet, nutrient analyses are from C. Garside and P. Wheeler, and values of δ_{DIC} are from analyses by J. Zhang and P. Quay. The nutrient data and carbonate system parameters are available from the U.S. Joint Global Ocean Flux Study (JGOFS) Data Management Office via the World Wide Web (http://www1.whoi.edu/jgofs.html).

Samples 17-22 were collected during a series of cruises by the R/V *Seawatch* (Marine Science Center, University of Southern

Table 1. Natural Populations and Pertinent Environmental Conditions

Sample	Field Designation	Date	d, m	T, °C	ΣCO_2, μmol kg^{-1}	Alk, μeq kg^{-1}	Salinity	δ_{DIC}, ‰	PO_4, μM	$Si(OH)_4$, μM	NO_3+NO_2, μM
					Equatorial Pacific at 140°W (TT007/TT008)						
1	9N-0414, 9°N	April 14, 1992	2	27.10	1936	2261	34.56	1.58	0.20	3.59	0.00
2	5N-0412, 5°N	April 12, 1992	2	28.41	1933	2257	34.46	1.59	0.36	4.24	0.46
3	EQ-0326, 0°	March 26, 1992	2	28.41	1986	2293	35.03	1.46	0.59	2.12	3.02
4	2S-0321, 2°S	March 22, 1992	2	28.85	1982	2298	35.06	1.57	0.50	2.42	3.11
5	MCN 15/7, 0°	Feb. 23, 1992	17	28.38	1987	2319	35.04	1.46	0.55	2.51	3.13
6	MCN 20/7, 2°S	Feb. 29, 1992	15	28.62	1977	2318	35.06	1.57	0.46	2.43	2.64
7	MCN 21/8, 5°S	March 1,1992	6	28.66	1979	2330	35.28	1.63	0.44	2.02	2.53
					Equatorial Pacific at 140°W (TT011)						
8	Station 6, 2°N	Aug. 26, 1992	35	24.47	2038	2314	34.91	1.20	0.40	1.95	5.99
9	Station 8, 0°	Aug. 30, 1992	15	24.71	2032	2315	35.11	1.20	0.56	2.33	6.68
10	Station 8, 0°	Aug. 30, 1992	40	24.61	2036	2317	35.12	1.20	0.53	2.57	6.71
11	Station 10, 2°S	Sept. 4, 1992	15	25.30	2024	2326	35.09	1.25	0.45	1.95	6.24
12	Station 10, 2°S	Sept. 4, 1992	40	25.12	2023	2321	35.07	1.25	0.50	2.04	6.30
13	Station 12, 5°S	Sept. 9, 1992	16	25.93	2011	2313	35.14	1.35	0.53	1.42	4.92
14	Station 12, 5°S	Sept. 9, 1992	40	25.88	2011	2315	35.14	1.35	0.57	1.39	4.92
15	Station 15, 12°S	Sept. 14, 1992	25	26.49	2005	2377	36.00	1.65	0.32	0.21	0.60
16	Station 15, 12°S	Sept. 14, 1992	50	26.48	2003	2378	36.00	1.65	0.35	0.50	0.47
					Santa Monica Basin, 38 km Offshore Southern California						
17	1-40	March 14, 1991	40	14.34	2111	2338	33.45	1.14	0.39	4.1	2.6
18	2-30	May 22, 1991	30	13.05	2131	2332	33.45	1.65	0.75	NA[a]	3.7
19	3-25	Aug. 6, 1991	25	15.20	2091	2343	33.49	1.86	0.36	2.7	0.3
20	4-40	Oct. 30, 1991	40	13.51	2126	2342	33.27	1.31	0.58	5.3	2.8
21	5-40	Feb. 5, 1992	40	14.78	2104	2348	33.29	1.85	0.47	2.4	0.8
22	6-40	April 12, 1992	40	17.65	2117	2338	33.38	1.67	0.83	5.9	6.3
					Peru Upwelling Zone[b]						
23	014, 13.5°S, 25 km	Oct. 15, 1992	10	15.5	2183	2309	34.93	-0.42	2.4	14.5	20.0
24	025, 13.5°S, 32 km	Oct. 16, 1992	10	15.5	2030	2339	34.90	0.49	1.0	2.8	1.2
25	041, 13.5°S, 80 km	Oct. 18, 1992	10	17.0	2097	2324	35.03	0.49	1.2	1.3	8.8
26	049, 13.5°S, 93 km	Oct. 19, 1992	10	16.7	2093	2318	35.00	0.60	1.1	0.9	3.7
27	075, 13.5°S, 80 km	Oct. 24, 1992	10	17.8	2072	2356	35.02	0.77	1.2	0.1	5.5
28	109, 12°S, 84 km	Oct. 30, 1992	10	18.0	2118	2330	35.05	0.81	1.4	3.0	12.4
29	122, 12°S, 75 km	Nov. 1, 1992	10	18.1	2103	2319	35.01	0.20	1.3	3.5	10.8
30	133, 12°S, 65 km	Nov. 3, 1992	10	17.1	2066	2334	35.01	0.06	1.2	3.0	10.2
					Bermuda Atlantic Time-series Study Site, 31°50'N 64°10'W						
31	38	Nov. 12, 1991	5	23.4	2026	2291	36.58	1.53	0.0	0.58	0.0
32	39	Dec. 10, 1991	5	22.7	2036	2290	36.78	1.50	0.0	0.71	0.0
33	41	Feb. 12, 1992	5	19.3	2060	2286	36.68	1.41	0.0	0.82	0.5
34	42	March 10, 1992	5	19.8	2054	2285	36.66	1.40	0.1	0.68	0.0
35	43	April 21, 1992	5	20.1	2066	2285	36.77	1.40	0.0	0.64	0.0
36	45	June 17, 1992	5	23.3	2041	2283	36.46	1.45	0.0	0.57	0.0
37	46	July 14, 1992	5	26.6	2033	2286	36.37	1.49	0.0	0.73	0.0
38	47	Aug. 18, 1992	5	28.1	2024	2286	36.44	1.51	0.1	0.69	0.0
39	48	Sept. 15, 1992	5	26.8	2012	2288	36.28	1.56	0.0	0.72	0.0

Table 1. (continued)

Sample	Field Designtion	Date	d, m	T, °C	ΣCO_2, µmol kg^{-1}	Alk, µeq kg^{-1}	Salinity	δ_{DIC}, ‰	PO_4, µM	$Si(OH)_4$, µM	NO_3+NO_2, µM
					IronExII, 4°-8°S 105°-110°W						
40	03, 4.04°S, 104.79°W	May 29, 1995	3	25.06	2009	2253	35.11	1.03	0.78	5.10	10.30
41	10, 5.42°S, 106.85°W	June 3, 1995	3	25.40	2011	2265	35.11	1.17	0.80	5.10	10.30
42	05, 4.66°S, 105.36°W	May 31, 1995	3	25.41	2009	2259	35.09	1.16	0.78	5.00	10.45
43	07, 4.72°S, 106.40°W	June 1, 1995	3	25.36	2007	2261	35.10	1.19	0.77	4.95	10.00
44	12, 5.75°S, 107.09°W	June 5, 1995	3	25.21	1995	2264	35.12	1.35	0.66	2.80	7.75
45	15, 5.77°S, 107.33°W	June 6, 1995	3	25.29	1998	2275	35.14	1.33	0.66	3.20	8.00
46	17, 6.20°S, 107.90°W	June 7, 1995	3	25.21	1996	2267	35.15	1.30	0.65	2.10	8.15
47	24, 7.35°S, 109.62°W	June 12, 1995	3	25.60	1990	2273	35.18	1.34	0.60	0.70	7.20
48	26, 7.46°S, 109.90°W	June 13, 1995	3	25.70	1990	2265	35.16	1.36	0.64	1.05	8.20

[a]NA, not available.
[b]Kilometers equal to distance offshore.

California). Suspended particles were collected using submersible pumps and filters (Nitex screen with 20 µm apertures in series with glass-fiber filters that retained particles larger than 0.7 µm). Samples were processed at Skidaway Institute of Oceanography using procedures described by *Freeman and Wakeham* [1992]. Briefly, half of each 293 mm glass-fiber filter was extracted with CH_2Cl_2/MeOH (2/1), and an alkenone-containing fraction was isolated by chromatography on silica gel. A portion of that fraction was saponified to remove alkenoates, and the purity of the alkenones was checked by GCMS at Skidaway. The alkenones were analyzed isotopically by irmGCMS at Indiana University. Standard techniques were used to determine concentrations of phosphate [*Solorzano and Strickland*, 1968], nitrate and nitrite [*Strickland and Parsons*, 1972], and silicate [*Atlas et al.*, 1971]. The concentration and isotopic composition of DIC as well as alkalinity were determined in the laboratories of C.D. Keeling at the Scripps Institution of Oceanography.

Samples 23-30 were collected during cruise SJ1092 of the R/V *Seaward Johnson*. Large-volume filtration samples were obtained by pumping seawater through an on-deck filter containing a 20 µm Nitex screen and a glass-fiber filter, thus obtaining two size fractions, >20 µm and 1-20 µm. Analytical procedures duplicated those applied to samples 8-16. Alkenones were analyzed by GC and GCMS at Skidaway and by irmGCMS at Penn State [*Freeman and Wakeham*, 1992]. Concentrations of alkenones in the >20 µm fraction were too low for isotopic analysis. Hydrographic, DIC, and alkalinity data were obtained from CTD and Niskin casts. The concentration of DIC was determined by acidification of the sample with phosphoric acid and quantification of the released CO_2 on a UIC Model 5012 carbon dioxide coulometer. Total alkalinity was determined by acidimetric titration as described by *Gran* [1952] and *Hannson and Jagner* [1973] (see also *Dickson* [1981]). A. Dickson of Scripps Institute of Oceanography provided a standard and calibrated the titrating acid. The overall uncertainty is 3-5 µmol kg^{-1}. Concentrations of phosphate were determined at the time of sampling [*Strickland and Parson*; 1972] (modified for 1.0 mL sample volumes). Values of δ_{DIC} were determined by isotopic analysis of CO_2 released from samples by treatment with H_3PO_4.

Samples 31-39 were collected by the staff of the Bermuda Biological Station for Research using the R/V *Weatherbird II*. Suspended particles were obtained by on-deck filtration of 700-1400 L seawater pumped from 5 to 10 m depth. The glass-fiber filters (Gelman A/E, 293 mm, nominal porosity equal to 1 µm) were frozen until processing as described for samples 8-16. Alkenones were quantified by GC and checked for purity by GCMS at Skidaway, then analyzed isotopically by irmGCMS at Indiana University. Synoptic hydrographic and geochemical data, with the exception of isotopic compositions of DIC, were obtained from CTD/water-bottle casts taken immediately before or after sampling. Total phosphate and reactive silicate were analyzed using a Technicon Auto Analyzer II with detection limits of 0.03 and 0.2 µmol kg^{-1}, respectively. DIC was determined coulometrically using a Single-Operator Multi-Metabolic Analyzer (SOMMA) system similar to that described by *Johnson et al.* [1993]. Nutrient, hydrographic, and DIC data are available from the U.S. JGOFS Data Management Office (http://www.bbsr.edu/bats/batsdata.html). Total alkalinity data are from *Bates et al.* [1996a, Figure 3a]. These data are well described by the relationship TA = 66.576*Salinity - 47.155 [*Bates et al.*, 1996a] except during cruise 41 when coccolithophore calcification apparently produced a drawdown in TA [*Bates et al.*, 1996b]. The isotopic composition of DIC was determined in the laboratories of C.D. Keeling at the Scripps Institution of Oceanography albeit not on synoptic samples. Values in Table 1 are for monthly samples that were averaged over 3 years previous to sample collection.

Samples 40-48 were collected during the IronEx II in situ fertilization experiment aboard R/V *Melville*. Suspended particles were obtained with a Challenger Oceanic in situ pump deployed at a depth of 3 m. Seawater (400-1000 L) was pumped sequentially through a 20 µm nylon prefilter and a GF/F glass-fiber filter (nominal porosity equal to 0.7 µm). Both nylon and GF/F filters were frozen in liquid nitrogen until processing. Only results from the analysis of the small size fraction have been used in this study since concentrations of alkenones in the >20 µm fraction were generally too low for isotopic analysis. However, one of the samples of larger particles contained concentrations of alkenones adequate for isotopic analyses. Observed values of δ were in good agreement with results from the corresponding sample of smaller particles. Alkenones were extracted, quantified, and checked for purity at the Skidaway Institute of Oceanography as described for samples 8-16, then analyzed isotopically by irmGCMS at University of Hawaii (Ultra-1, 50 m). Samples for analysis of

total phosphate, reactive silicate, and nitrate plus nitrite were collected from CTD rosette casts deployed concurrently with the in situ pump and analyzed by S. Tanner (Moss Landing Marine Laboratories). Concentrations of DIC and alkalinity were determined at the University of Miami, Rosenstiel School of Marine and Atmospheric Sciences (RSMAS). Values of δ_{DIC} were determined at the University of Hawaii using a technique modified from *Kroopnick* [1985] (see *Laws et al.* [1995] for details).

Results and Discussion

Chemostat cultures. The results summarized in Table 2 and in Figure 1 extend those of *Laws et al.* [1995], showing that the relationship between ε_P, μ, and C_e for *E. huxleyi* takes the same form as that observed previously for the diatom *Phaeodactylum tricornutum*. Thus ε_P is related to μ/C_e by the equation shown in Figure 1. While this linear relationship is consistent with the uptake of DIC by diffusion of CO_2, the results of this study do not preclude uptake by other mechanisms. [*Laws et al.*, 1997]. For example, *Nimer et al.* [1996] conclude that the DIC requirement for a high-calcifying strain of *E. huxleyi* (clone CCMP88E) is met by bicarbonate uptake and diffusive CO_2 transport under certain growth conditions. In the present study, similar fractionation patterns were obtained for a noncalcifying and calcifying clone of *E. huxleyi*. However, the slopes of the relationship between ε_P and μ/C_e obtained for *E. huxleyi* and *P. tricornutum* [*Laws et al.*, 1995] are significantly different and can be largely accounted for by differences in their respective surface area-to-volume ratios (B.N. Popp et al., manuscript in preparation, 1997). In these chemostat experiments, growth rate and C_e were varied by factors of 3- and 28-fold, respectively (Table 2). Attempts to achieve higher growth rates for clones BT6 and B92/11 were unsuccessful and resulted in cells "washing out" of the growth chamber. Thus much of the variability observed for ε_P was caused by the wide range of C_e used in the chemostat experiments. Accordingly, the coefficient of determination for the dependence of ε_P on μ/C_e (r^2 = 0.871) is only slightly higher than that obtained for the dependence of ε_P on $1/C_e$ (r^2 = 0.856). Omission of the highest-C_e data point (C_e = 274.1 μmol kg^{-1}) results in threefold ranges of variation for both μ and C_e and yields a greater difference when μ is included in the abscissa (r^2 of 0.823 versus 0.759). These experimental results provide additional evidence that μ and C_e, among other factors (e.g., cell geometry), are important in explaining variations in ε_P. The limited data set obtained for clones BT6 and B92/11 suggests that the calcification process does not strongly affect the expression of photosynthetic fractionation (ε_P).

Table 2. Isotopic Characterization of Bulk Carbon Biomass of *E. huxleyi* Clones BT6 (Noncalcifying) and B92/11 (Calcifying) Grown in Chemostat Culture

E. huxleyi Clone	μ, d^{-1}	C_e, μmol kg^{-1}	δ_e, ‰	δ_p, ‰	ε_P, ‰
BT6	0.40	29.1	-16.8	-38.6	22.7
BT6	0.50	20.6	-15.4	-35.6	20.9
BT6	0.50	21.4	-15.0	-36.4	22.2
BT6	0.50	274.1	-22.9	-46.6	24.9
BT6	0.60	20.8	-14.2	-34.8	21.3
B92/11	0.20	12.1	-14.1	-34.9	21.5
B92/11	0.40	11.3	-14.0	-31.9	18.5
B92/11	0.40	12.2	-10.5	-29.3	19.4
B92/11	0.60	9.6	-13.9	-30.6	17.2

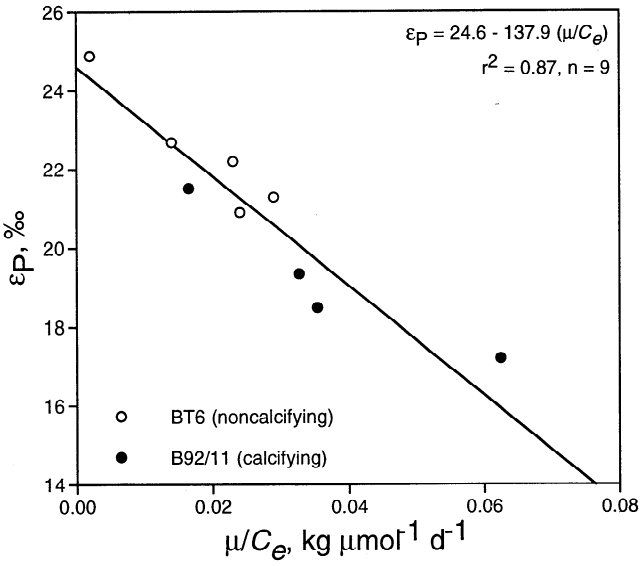

Figure 1. Isotopic fractionation as a function of μ/C_e for laboratory chemostat culture experiments using *Emiliania huxleyi*, clone BT6 (noncalcifying), and clone B92/11 (calcifying). Data are summarized numerically in Table 2. The line and equation represent a geometric mean regression analysis (reduced major axis).

The intercept is representative of the maximum isotopic fractionation (ε_f) and is similar to the "consensus value" of 25‰ that emerges from a variety of recent investigations [*Hayes*, 1993; *Laws et al.*, 1995]. The value of ε_f is the flux-weighted average of the isotope effects characteristic of all carbon-fixation reactions active in the cell. These include reactions catalyzed by ribulose bisphosphate carboxylase-oxygenase (RUBISCO) and by the β-carboxylases, phosphoenolpyruvate carboxylase (PEPC) and phosphoenolpyruvate carboxykinase (PEPCK). Estimating the relative importances of these pathways and referring to known isotope effects, *Goericke et al.* [1994] calculate the likely range of ε_f for marine phytoplankton to be 25-28‰. Adopting the minimum value because it is most representative of laboratory and field studies of alkenone-producing algae, the equation shown in Figure 1 can be rearranged to provide an equation for the calculation of growth rates of natural populations of alkenone-producing algae:

$$\mu_{CC} = (25 - \varepsilon_P)C_e/138 \quad (1)$$

where μ_{CC} is the growth rate under conditions of continuous culture (i.e., 24:0 light:dark cycle). To correct for the effects of day length and respiration on growth rate, we apply the following approximation:

$$\mu \approx [\mu_{CC}/(24/t_p)]\,0.8 \quad (2)$$

where μ refers to the 24-hour average growth rate, t_p is the day length or photoperiod in hours, and the factor 0.8 adjusts the

growth rate for dark respiration (see discussion by *Laws et al.* [1995]).

Uncertainties in calculated growth rates can be estimated by consideration of the propagation of errors associated with the terms and coefficients in equation (1). When populations of alkenone-producing algae are compared, the uncertainties to be considered are those in C_e and ε_P. Even if a standard deviation of 0.1 μmol kg^{-1} is conservatively assigned to C_e, uncertainties contributed by ε_P are more important because the standard deviation for that parameter is approximately 0.37‰ (determined by adding in quadrature the standard deviations of δ_e, 0.2‰; $\delta_{37:2}$, 0.3‰; and $\Delta\delta$, the isotopic difference between alkenones and total biomass, 0.1‰). The standard deviation calculated for μ_{CC} is then roughly proportional to C_e, with minor variations due to changes in ε_P. For the present data, the standard deviation found for μ_{CC} ranges from 0.03 d^{-1} at low values of C_e to 0.05 d^{-1} at values of C_e near 20 μmol kg^{-1}. Exceptionally, the standard deviation of μ_{CC} approaches 0.08 d^{-1} for C_e near 30 μmol kg^{-1}.

Estimation of rates of growth by application of equations (1) and (2) is of considerable interest since conventional methods for determination of μ [*Redalje and Laws*, 1981; *Landry and Hassett*, 1982] are restricted to the present-day ocean. Since molecular-isotopic techniques [*Jasper and Hayes*, 1990; *Jasper et al.*, 1994] allow the determination of ε_P for specific algal taxa, it should be possible to estimate group-specific rates of growth for both modern and ancient oceans if C_e is known. When comparisons between groups are made, it will be necessary to consider uncertainties in the coefficients used to relate μ, ε_P, and C_e for each group (e.g., the coefficients 25 and 138 in equation (1)).

Natural populations. Table 3 reports values of δ_e, $\delta_{37:2}$, C_e, and ε_P determined for upper oceanic waters of the equatorial Pacific, Peru margin, Santa Monica Basin, and North Atlantic Ocean. Specific growth rates (μ, d^{-1}) of alkenone-producing haptophytes were estimated using equations (1) and (2). These growth rates varied fivefold, with values ranging from 0.2 to 1.0 d^{-1}. Highest rates were estimated for waters of the Peru upwelling system (0.5-1.0 d^{-1}), and lowest values were found in the equatorial Pacific, the Santa Monica Basin, and at the Bermuda Atlantic Time-series Study (BATS) site (0.2-0.5 d^{-1}). Calculated growth rates from Peru and Santa Monica Basin are related to intensity of upwelling. Upwelling rates along the Peru margin were higher at inshore stations, as indicated by low sea-surface temperatures and high nutrient concentrations (Table 1). Likewise, alkenone-based estimates of μ are highest for samples collected at the more shoreward stations, although concentrations of alkenones are lowest in these samples. This suggests that even though haptophyte abundances are low in the diatom-dominated near-shore waters, growth rates were highest in the most nutrient-rich waters.

Observed variations in growth rates in the Santa Monica Basin were well correlated with the strength of upwelling as estimated from hydrographic data and with expectations based on long-term observations. Specifically, weak upwelling was indicated at the time of collection of sample 21, when concentrations of nitrate and nitrite were less than 1 μM above a depth of 45 m. Samples 17, 19, and 20 were collected while upwelling delivered nitrate and nitrite to surface water at moderate rates, with concentrations exceeding 1 μM at depths greater than 30 m. At the times of collection of samples 18 and 22, upwelling was strong, and concentrations of nitrate and nitrite exceeded 1 μM at all depths below 15 m. For the years 1975-1988, observations from the California Coastal Ocean Fisheries Project indicate that areal average photosynthetic production regularly peaked at values greater than 1000 mg C m^{-2} d^{-1} during May (sample 18) and sometimes in April (sample 22), whereas minimal values, ~350 mg C m^{-2} d^{-1}, were usually observed during November and February, at the time of collection of samples 20 and 21 [*Eppley*, 1992].

Calculated growth rates at the BATS station also parallel changes in primary production. Winter overturn delivers cold, nutrient-rich waters to the surface, usually during the months of January and/or February. This mixing event is typically followed by thermal stratification and elevated rates of primary production (see *Bates et al.* [1996b], Figure 2c). Elevated specific growth rates of the alkenone-producing haptophytes are also observed during this period (Table 3, samples 33 and 34).

The growth rates given in Table 3 are within the range determined for laboratory cultures of alkenone-producing haptophytes. *Brand and Guillard* [1981] reported that the maximum growth rate of the alkenone-producing coccolithophorids *E. huxleyi* and *G. oceanica* is 1.7-1.9 d^{-1} when grown on a 14:10 light:dark cycle. Table 4 provides a summary of available growth rate measurements for natural populations of *E. huxleyi* and haptophytes. These rates were determined using the dilution method [*Landry and Hassett*, 1982] or ^{14}C-labeling techniques [*Gieskes and Kraay*, 1989] and lead to values of μ ranging from 0.1 to 0.8 d^{-1}. Since the dilution and ^{14}C-labeling techniques are based on the disappearance and specific radioactivity of 19'-hexanoyloxyfucoxanthin (hex-fucoxanthin), these rates pertain only to haptophytes which possess this carotenoid. *Holligan et al.* [1993] used the dilution method to estimate a growth rate of 0.4 d^{-1} for an *E. huxleyi* bloom sampled south of Iceland (61°N, 22°W).

While many of the common oceanic haptophytes possess hex-fucoxanthin (e.g., *Emiliania*, *Gephyrocapsa*, *Chrysochromulina*, *Phaeocystis*, *Corymbellus*, and *Imantonia*), only certain coccolithophorids (i.e., *E. huxleyi* and *G. oceanica*) are known to produce alkenones [*Conte et al.*, 1994; *Jeffrey and Wright*, 1994; *Thomsen et al.*, 1994]. Thus comparisons of haptophyte growth rates based on different molecular techniques should be made cautiously. Although growth rates determined for alkenone- and hex-fucoxanthin-containing haptophytes in equatorial Pacific waters were similar during boreal spring 1992 (0.3-0.4 d^{-1}, Tables 3 and 4), microscopic analysis revealed that coccolithophorids comprised only a small percentage (≤4%) of the total haptophytes [*Thomsen et al.*, 1994]. The alkenone- and hex-fucoxanthin-based techniques thus sampled different haptophyte populations, and the agreement between them in this case is coincidental. Notably, results obtained for the equatorial Pacific during boreal fall 1992 show that growth rates of alkenone- and hex-fucoxanthin-containing haptophytes can differ by a factor of ~2 (0.4 versus 0.7 d^{-1}, Tables 3 and 4). Future calibrations of isotopically based estimates of growth rates should be based on dilution experiments performed in conjunction with species-specific oligonucleotide or immunochemical probes. A single probe could be used for both *E. huxleyi* and *G. oceanica* since they are immunologically and genetically similar [*Medlin et al.*, 1996].

Correlation of estimated growth rates with oceanographic conditions. Prior studies of relationships between ε_P and C_e in natural settings [e.g., *Rau et al.*, 1992; *Francois et al.*, 1993; *Jasper et al.*, 1994] have not included quantitative treatments of growth rate. Observed variations have instead been discussed in

Table 3. Isotopic Fractionation Imposed by Natural Populations of Alkenone-Producing Haptophytes (Table 1) and Calculated Growth Rates

N	δ_e, ‰	$\delta_{37:2}$, ‰	C_e, µmol kg^{-1}	ε_p,[a] ‰	t_p,[b] hour	µ,[c] d^{-1}	b,[d] ‰ µmol kg^{-1}
			Equatorial Pacific at 140°W (TT007/TT008)				
1	-6.68	-27.0	10.76	16.70	12.2	0.3	89
2	-6.57	-25.3	10.96	15.05	12.1	0.3	109
3	-6.71	-25.6	12.42	15.22	12.0	0.4	121
4	-6.56	-24.8	12.05	14.54	12.0	0.4	126
5	-6.70	-24.9	11.32	14.50	12.0	0.3	119
6	-6.57	-24.5	10.90	14.22	12.0	0.3	118
7	-6.51	-25.4	10.60	15.22	12.1	0.3	104
			Equatorial Pacific at 140°W (TT011)				
8	-7.29	-26.1	13.78	15.14	12.0	0.4	136
9	-7.27	-27.0	13.47	16.10	12.0	0.4	120
10	-7.28	-26.5	13.61	15.57	12.0	0.4	128
11	-7.17	-26.4	12.55	15.58	11.9	0.3	118
12	-7.18	-25.3	12.68	14.43	11.9	0.4	134
13	-7.02	-25.2	12.51	14.49	11.9	0.4	131
14	-7.02	-26.4	12.40	15.73	11.9	0.3	115
15	-6.65	-25.3	9.92	14.97	11.9	0.3	99
16	-6.65	-26.7	9.80	16.42	11.9	0.2	84
			Santa Monica Basin, 38 km Offshore Southern California				
17	-8.23	-30.3	15.24	18.56	12.0	0.3	98
18	-7.85	-28.2	17.22	16.76	12.0	0.4	142
19	-7.44	-29.8	13.60	18.85	12.0	0.2	84
20	-8.14	-28.4	15.99	16.66	11.9	0.4	133
21	-7.49	-28.9	14.09	17.86	11.9	0.3	101
22	-7.41	-26.6	16.50	15.54	11.9	0.4	156
			Peru Upwelling Zone				
23	-9.67	-24.6	28.68	11.16	10.4	1.0	397
24	-8.75	-19.1	10.60	6.45	10.0	0.5	197
25	-8.64	-21.8	16.19	9.33	10.8	0.7	254
26	-8.56	-23.0	16.20	10.65	11.7	0.7	233
27	-8.28	-19.2	12.57	7.03	13.0	0.7	226
28	-8.23	-22.2	17.91	10.15	14.0	0.9	266
29	-8.83	-21.4	17.38	8.72	13.8	0.9	283
30	-9.05	-20.7	13.22	7.78	13.1	0.7	228
			Bermuda Atlantic Time-series Study Site, 31°50'N 64°10'W				
31	-7.03	-25.4	10.12	14.69	10.4	0.3	104
32	-7.10	-24.8	9.87	13.99	10.0	0.3	109
33	-7.50	-22.1	11.75	10.79	10.8	0.4	167
34	-7.47	-22.2	10.66	10.93	11.7	0.4	150
35	-7.43	-26.1	10.86	15.00	13.0	0.3	109
36	-7.10	-27.4	11.24	16.69	14.0	0.3	93
37	-6.86	-25.4	11.33	14.85	13.8	0.4	115
38	-6.68	-25.3	10.97	14.94	13.1	0.4	110
39	-6.73	-25.0	10.64	14.58	12.2	0.3	111
			IronExII, 4°-8°S 105°-110°W				
40	-7.42	-25.3	15.64	14.16	11.8	0.5	169
41	-7.25	-25.4	15.08	14.45	11.7	0.4	159
42	-7.26	-26.6	15.31	15.69	11.7	0.4	143
43	-7.23	-26.2	15.02	15.36	11.7	0.4	145
44	-7.08	-25.4	13.92	14.60	11.7	0.4	145
45	-7.09	-26.1	13.53	15.38	11.6	0.4	130
46	-7.13	-25.7	13.83	14.86	11.6	0.4	140
47	-7.06	-26.5	13.18	15.78	11.6	0.3	122
48	-7.03	-25.8	13.63	15.08	11.5	0.4	135

[a]Values of ε_p for natural populations are based on $\delta_{37:2}$ and δ_e assuming a depletion in ^{13}C relative to carbon biomass of 4‰.
[b]Day length or photoperiod.
[c]Growth rate calculated using equations (1) and (2).
[d]$b = (25 - \varepsilon_p)C_e$; see text.

Table 4. Variability of Specific Growth Rates (μ) Determined for Natural Populations of *Emiliania huxleyi* and 19'-Hexanoyloxyfucoxanthin-Containing Haptophytes

Study Site and Method	Depth, m	μ, d^{-1}	Reference
	Emiliania huxleyi		
North Atlantic Ocean (dilution method)	3	0.4	*Holligan et al.* [1993]
	Haptophytes		
Equatorial Pacific (TT007, 3°N-3°S, dilution method)	10-20	0.3	*Latasa et al.* [1997]
	40-50	0.3	*Latasa et al.* [1997]
Equatorial Pacific (TT011, 3°N-3°S, dilution method)	10-20	0.6	*Latasa et al.* [1997]
	40-50	0.8	*Latasa et al.* [1997]
Subarctic Pacific (^{14}C labeling method)	10	0.2-0.3	*Welschmeyer et al.* [1991]
Subarctic Pacific (dilution method)	10	0.1-0.4[a]	*Strom and Welschmeyer* [1991]
	30	0.2	*Strom and Welschmeyer* [1991]
Eastern Indonesian waters (^{14}C labeling method)	5-10	0.1-0.8[b]	*Gieskes and Kraay* [1989]

[a]Range of measurable specific growth rates.
[b]Range of measurable specific growth rates after 9 hours of incubation.

terms of a relationship in which ε_P varies inversely with C_e, with the intercept on the ε_P axis being given by ε_f, the isotopic fractionation that would be observed in the absence of any limitations imposed by the supply of dissolved CO_2 to the site of enzymatic fixation. The slope of the relationship quantifies that rate at which ε_P decreases as concentrations of CO_2 become smaller. The numerical value is derived empirically, but it has been shown theoretically by *Rau et al.* [1996] to depend on a variety of physiological and environmental controls (see discussion below). Here the slope is assigned the symbol b, and the b/C_e term is subtracted from ε_f. With this formulation, increased sensitivity to variations in the concentration of dissolved CO_2 is associated with increased values of b:

$$\varepsilon_P = \varepsilon_f - b/C_e \quad (3)$$

The development of this "hyperbolic form," largely by *Rau et al.* [1992], *Francois et al.* [1993], and *Goericke et al.* [1994], has been reviewed by *Hayes* [1993]. More recently, the physiological basis of the relationship has been clarified by the observations of *Laws et al.* [1995]. Comparison of equation (3) with the expression shown in Figure 1 shows that, for a given organism, b should be linearly related to μ. Indeed, both *Francois et al.* [1993] and *Jasper et al.* [1994] concluded that b was related to rates of growth and, by extension, the supply of the limiting nutrient under saturating growth irradiances ($E \geq E_k$). Extending this line of thought, *Fluegge* [1994] showed that the slope of ε_P-$1/C_e$ relationships was correlated with the concentration of dissolved phosphate and proposed that nutrient controls should be taken into account in calibrations of ε_P-C_e relationships.

Given ε_f, b can be calculated from any pair of observations of ε_P and C_e. Here we adopt $\varepsilon_f = 25‰$ because it approximates the intercept found by regression analysis of the data summarized in Table 2 and because it is within ~0.5‰ of intercepts based on all other available sets of C_e, ε_P pairs [*Laws et al.*, 1995; *Francois et al.*, 1993; *Goericke et al.*, 1994]. Accordingly, $b = (25 - \varepsilon_P)C_e$. The resulting values of b for the present C_e, ε_P pairs are reported in the last column of Table 3. These values summarize a quantitative relationship between isotopic fractionation and the concentration of dissolved CO_2. They differ in principle from the accompanying estimates of μ, which pertain specifically to the estimated rate of growth.

The relationship between b and the concentration of soluble reactive phosphate (SRP) is shown graphically in Figure 2a. With the exception of the BATS site, b was found to be highly correlated with the concentration of SRP ($r^2 = 0.95$). When the diversity of locations and laboratories is considered, the correlation is striking. Comparison of equation (3) with the equation for the line in Figure 1 shows that the coefficients b and 138μ are functionally equivalent and therefore that b is directly proportional to growth rate. The correlation relating b and $[PO_4]$ therefore shows that for environments with nonzero concentrations of phosphate, the rate of growth of alkenone-producing algae is linearly related to $[PO_4]$.

The mechanism underlying the relationship summarized by Figure 2a is, however, unclear. The concentrations of SRP were in most cases well above the levels associated with phosphate-limited growth [*Perry*, 1972; *Townsend et al.*, 1994]. Apparently, *E. huxleyi* has a low phosphorus requirement since it exhibits a strong "phosphate sparing effect" when grown under P limitation in chemostat culture (C:P = ~600 mol:mol at a growth rate of 50% μ_{max} [*Paasche and Brubak*, 1994]). Furthermore, *E. huxleyi* can effectively outcompete other phytoplankton species when grown at elevated N:P ratios [*Riegman et al.*, 1992]. It is therefore unlikely that phosphorus limitation is responsible for the correlation observed in Figure 2a. Values of b were also correlated with concentrations of nitrate plus nitrite ($r^2 = 0.72$); however, the correlation was not as strong as that obtained with phosphate. Nitrogen limitation also seems unlikely since inorganic nitrogenous nutrient concentrations at most Pacific

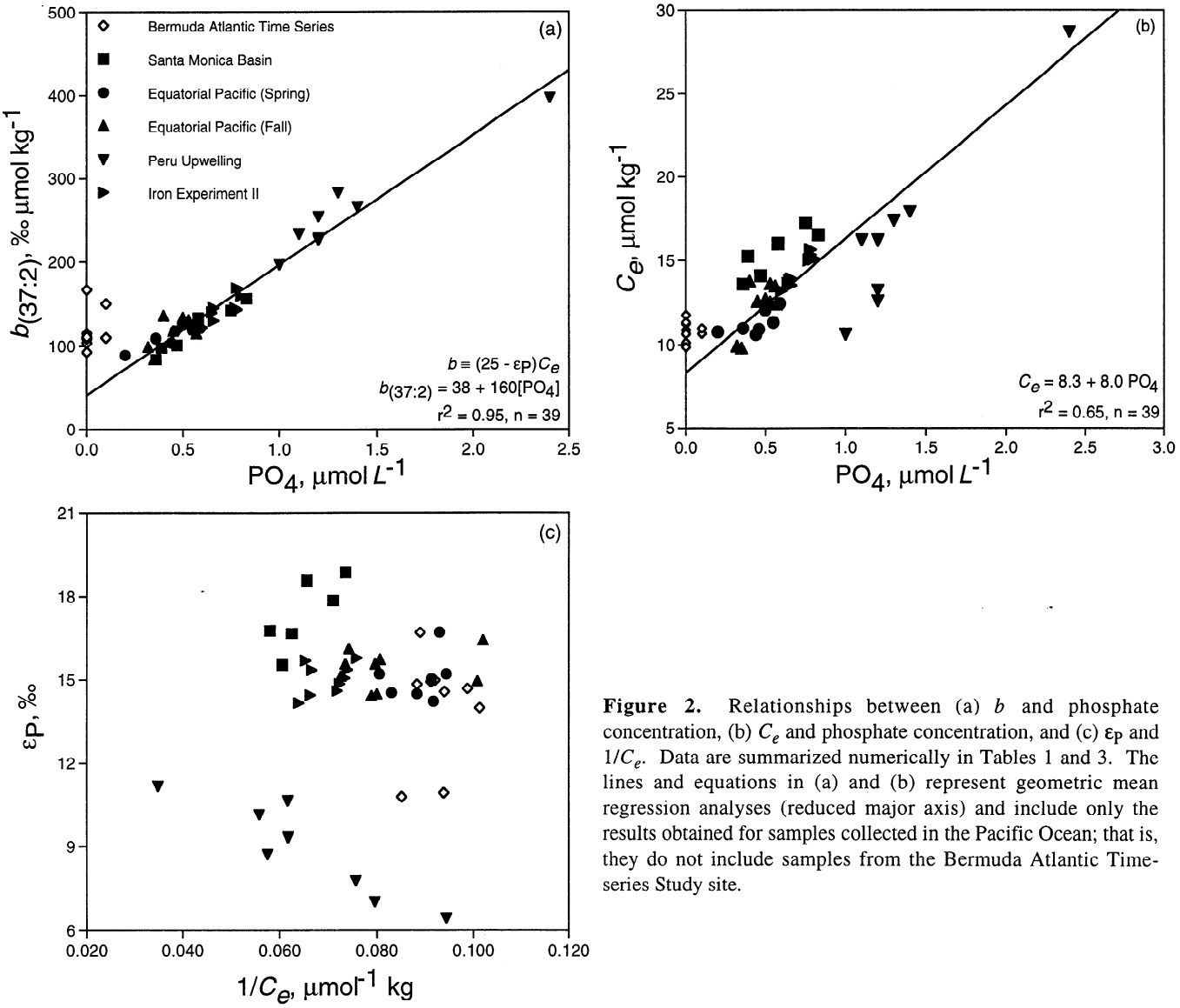

Figure 2. Relationships between (a) b and phosphate concentration, (b) C_e and phosphate concentration, and (c) ε_P and $1/C_e$. Data are summarized numerically in Tables 1 and 3. The lines and equations in (a) and (b) represent geometric mean regression analyses (reduced major axis) and include only the results obtained for samples collected in the Pacific Ocean; that is, they do not include samples from the Bermuda Atlantic Time-series Study site.

Ocean stations were well above the K_s determined for *E. huxleyi* (≤0.5 µM N [*Eppley et al.*, 1969]). Furthermore, light limitation is not considered to be important as a controlling factor since field samples were collected from near-surface waters.

As shown by *Rau et al.* [1996, equation (15)], ε_P can vary as a function of μ, C_e, ε_f, cell radius, membrane permeability, and temperature. Effects of temperature are not likely to be important here, since *Rau et al.* [1996] predicted that even a 30°C increase in temperature would lead to only a small change in ε_P. Of the remaining factors, variations in cell radius, ε_f, and membrane permeability are not explicitly accounted for in equation (3) and Figure 2a. We assume, however, that variations in ε_f and membrane permeability are likely to be species-dependent and thus can be treated as constants in the context of this study. Unfortunately, we were unable to make measurements of cell radius variability and evaluate its influence on ε_P. *Hallegraeff* [1984] reported that the coccosphere diameters of *E. huxleyi* and *G. oceanica* sampled in Australian waters overlapped and had values ranging from 5 to 10 µm and 5 to 15 µm, respectively. These values correspond to a threefold range in the magnitude of coccosphere radius (2.5-7.5 µm). It should be noted, however, that coccosphere radii do not equate directly to cell radii since the former includes a variable number of coccolith layers. With this caveat in mind, the model of *Rau et al.* [1996] predicts that this range of cell radii translates into a ~2‰ variation in ε_P at a growth rate of 0.5 divisions d^{-1} (see *Rau et al.* [1996], Figure 2a). This growth rate is equivalent to a specific growth rate of 0.35 d^{-1}, a value which is close to the average growth rate estimated in this study (0.4 ± 0.2 d^{-1}, Table 3). Since a 12‰ range in ε_P was observed in this study (Table 3), we conclude that cell radius variations can at most account for a small degree of the variability observed in Figure 2a.

Another possible explanation for the relationship shown in Figure 2a is that it results entirely from the natural correlation between concentrations of dissolved CO_2 and phosphate. Specifically, since b is defined as a linear function of C_e, the

correlation between C_e and [PO_4] that is observed in ocean waters could lead to a pseudocorrelation between b and [PO_4]. This point is explored in Figure 2b, which plots values of C_e and [PO_4] for this data set. The correlation is poor relative to that between b and [PO_4] (r^2 = 0.656 versus 0.952, n = 39). Moreover, it depends very strongly on inclusion of the high-C_e point from Peruvian coastal waters. If that point is omitted, the coefficient of determination for the dependence of C_e on [PO_4] is only 0.359, and that for the dependence of b on [PO_4] is 0.928. Accordingly, we conclude (1) that C_e and [PO_4] are for the most part not tightly correlated in the near-surface waters of our study sites and (2) that the weak correlation which is observed is not the primary cause of the highly significant dependence of b on [PO_4].

More plausibly, one or a combination of micronutrients is growth-rate-limiting, and concentrations of SRP are closely related to the concentration of the limiting factor(s). Of the essential micronutrients, iron, zinc, and cobalt are most often mentioned as potentially limiting factors in marine waters [*Brand et al.*, 1983; *Martin et al.*, 1991; *Sunda and Huntsman*, 1992, 1995a, b]. Iron is required for the synthesis of chlorophyll *a* and is a required cofactor for nitrate and nitrite reductases, catalase, peroxidase, superoxide dismutase, and is in the active centers of cytochromes and FeS proteins involved in photosynthetic and respiratory electron transport [*Geider and LaRoche*, 1994; *Raven*, 1988; *Sunda*, 1994]. Zinc is a required cofactor for alkaline and acid phosphatases, carbonic anhydrase, and DNA and RNA polymerases [*Morel et al.*, 1994; *Sunda*, 1994]. While cobalt is present in the active center of vitamin B_{12} and can substitute for zinc in the carbonic anhydrase of diatoms, its other cellular functions are unclear at the present time [*Sunda*, 1994; *Yee and Morel*, 1996]. Of particular relevance to this study is the recent work of *Sunda and Huntsman* [1995b], who have shown that *E. huxleyi* has a cobalt requirement which can be partially satisfied by zinc.

Micronutrient effects. The concentrations of both iron and zinc are related to that of SRP in marine waters, and in both cases, the relationship is curvilinear [*Sunda*, 1994]. Relationships between nutrient-limited microalgal growth rates and concentrations of substrates are also curvilinear [*Caperon*, 1967; *Goldman*, 1977; *Sunda and Huntsman*, 1995a]. The linear relationship between the concentration of SRP and growth rates of alkenone-containing phytoplankton indicated in Figure 2a could therefore conceivably result from combination of these curvilinear relationships (i.e., the relationship between b and [PO_4] is linear because each is curvilinearly related to a third variable, namely the concentration of the limiting micronutrient, a remarkable congruence of the curves being required in order to produce this result). Concentrations of iron and zinc associated with growth-rate limitation are species-dependent and are lower for oceanic versus coastal eukaryotic species [*Brand et al.*, 1983; *Brand*, 1991; *Sunda et al.*, 1991; *Sunda and Huntsman*, 1992, 1995a, b].

For *E. huxleyi*, concentrations of dissolved inorganic iron (i.e., [Fe'], the concentration of dissolved inorganic iron species) become growth-rate limiting below ~20 pM [*Sunda and Huntsman*, 1995a]. Since total dissolved iron concentrations in the North and equatorial Pacific are ≤100 pM [*Martin and Gordon*, 1988; *Martin et al.*, 1989; *Coale et al.*, 1996a], of which ~95-99.98% is organically complexed depending on the extent of photoredox cycling [*Rue and Bruland*, 1995; *Sunda and Huntsman*, 1995a], it is possible that effects of iron availability play a role in the relationship summarized by Figure 2a. However, the IronEx II experiment [*Coale et al.*, 1996b] allowed a direct evaluation of the effects of increased iron availability on ^{13}C fractionation (approximately growth rate) in *E. huxleyi*. Following the addition of 2 nM iron on May 30, 1995, the growth rates (μ or b) calculated for alkenone-containing haptophytes (samples 42-48, Table 3) did not increase significantly relative to those measured at prerelease and control stations (samples 40-41, Table 3). These results imply that the growth rates of alkenone-containing haptophytes at the IronEx II study site are not limited by iron.

Limitation of phytoplankton growth rate by trace metal availability is complex and often involves interactions between several trace elements [*Cullen*, 1991; *Morel et al.*, 1994; *Yee and Morel*, 1996]. As mentioned above, *Sunda and Huntsman* [1995b] have recently demonstrated that *E. huxleyi* has a cobalt requirement which can be partially satisfied by zinc. In the absence of added zinc, cobalt becomes limiting at free cobalt ion concentrations below 3 pM, or at a dissolved inorganic cobalt concentration of 5 pM. Note that [Co^{++}] is equal to [Co'] x 0.65 due to the presence of inorganic complexes ($CoCl^+$, $CoOH^+$, etc.). Total dissolved cobalt concentrations for North Atlantic and North Pacific surface waters range from 18 to 300 pM and 4 to 50 pM, respectively [*Jickells and Burton*, 1988; *Martin and Gordon*, 1988; *Donat and Bruland*, 1995]. Redfield modeling suggests that in seawater, the extent of complexation of cobalt is similar to that of zinc. If so, concentrations of free cobalt would be 0.2-3.0 and <0.1-0.5 pM in surface waters of the North Atlantic and North Pacific, respectively (W. G. Sunda, personal communication, 1996). Thus the available laboratory and field data imply that the in situ growth rates of *E. huxleyi* may be limited by cobalt. In the absence of added cobalt, zinc becomes growth-rate limiting for *E. huxleyi* at free zinc ion concentrations below 3 pM [*Sunda and Huntsman*, 1995b]. Since total concentrations in North Pacific and equatorial Pacific surface waters are ≤200 pM [*Bruland*, 1980; *Donat and Bruland*, 1995; M. Gordon, personal communication, 1996, see http://dataone.whoi.edu/jg/serv/jgofs/eqpac/tt008/diss.html0 and http://dataone.whoi.edu/jg/serv/jgofs/eqpac/tt012/diss.html0], of which 98-99% is complexed [*Bruland*, 1989; *Bruland et al.*, 1991], the notion of a combined cobalt and zinc limitation is consistent with the relationship observed in Figure 2a.

Sunda and Huntsman [1995b] also showed that elevated concentrations of cobalt and high cobalt:zinc ion ratios favor the growth of *E. huxleyi*. The fact that concentrations of cobalt in surface waters of the North Atlantic are several-fold higher than those in the North Pacific might therefore explain why the points representing samples from the BATS site fall above the $b = f$[PO_4] line in Figure 2a, since smaller ε_p values are indicative of higher growth rates. Alternatively, the possibility exists that δ_e results used to calculate b for samples collected at the BATS site may not have been representative of the time of sampling.

Conclusions

Causation. Although limitations imposed by the availability of trace-metal micronutrients seem most likely to underlie the correlation found between b and [PO_4], our present inability to identify a specific mechanism leads us to two concluding points. First, whatever the mechanism, the correlation must be related to effects influencing growth rates since other potentially controlling factors (e.g., cell radius, membrane permeability, and ε_f [*Rau et*

al., 1996]) are expected to be relatively invariant for natural populations of *E. huxleyi* and *G. oceanica* (see above). Second, the linearity, precision, and generality of the relationship between b and $[PO_4]$ indicate that mechanisms directly involving phosphate itself should not be forgotten. The first conclusion is supported not only by the quantitative relationships between μ, b, and $[PO_4]$ but by logical analysis. The magnitude of any isotopic fractionation can be affected only by isotope effects and by the distribution of carbon among products within the reaction network. In this case nothing indicates that the nature of the reaction network is changing as b and $[PO_4]$ increase. For example, nothing suggests that the pathway of carbon assimilation or the mechanism of carbon fixation is changing as concentrations of phosphate increase. Accordingly, there is no reason to expect that one or more isotope effects enters or leaves the reaction network as b and $[PO_4]$ increase. The observed variations in b must therefore be related not to changes in the isotope effects but to changes in the distribution of carbon among flow pathways. For example, isotopic fractionation will be reduced whenever a larger portion of the inorganic carbon enters the cell and is retained there by the process of carbon fixation. Current evidence indicates that the relationship between b and μ reflects one between cellular carbon budgets and growth rates. Our data clearly show that for alkenone-producing algae in nonzero-PO_4 environments, μ is linearly related to $[PO_4]$.

CO_2 paleobarometry. Our results have implications for the determination of ancient ocean P_{CO_2} levels from carbon isotopic compositions of coexisting carbonates and marine organic matter. In such studies, correlations between P_{CO_2} and ε_P have been both tantalizing (e.g., *Rau* [1994]; WOCE SR-3 data of *Popp et al.* [1997]; results obtained south of the subtropical convergence by *Francois et al.* [1993]) and frustrating (e.g., PET data of *Popp et al.* [1997]; results obtained north of the subtropical convergence by *Francois et al.* [1993]). The present study reveals the origins of that dichotomy. As summarized graphically in Figure 2c, ε_P and C_e are very poorly correlated in the alkenone-based measurements reported here. However, as shown in Figure 2a, a transformation that allows simultaneous consideration of ε_P, C_e, and growth rate, namely, the plotting of b as a function of $[PO_4]$, shows convincingly that these data can be unified using the interpretative approach introduced by Laws *et al.* [1995]. In the "frustrating" cases noted above, it is possible that variations in growth rate are significant.

In alkenone-based paleo-P_{CO_2} investigations generally, if growth rate (i.e., b) were known independently, then C_e could be calculated directly from ε_P. If the BATS data are excluded, the present data set suggests that C_e can be determined to within 1.1 ± 0.8 μmol kg^{-1} (n = 39) given knowledge of ε_P and phosphate concentration. Since b can be estimated from $[PO_4]$, *Fluegge* [1994] suggested the Cd/Ca ratio in planktonic foraminifera can provide an estimate of b. The association between Cd and phosphorus in the contemporary ocean is well documented [*Boyle*, 1976; *Bruland and Franks*, 1978; *Boyle*, 1988; *Frew and Hunter*, 1992; *Saager and de Baar*, 1993; *de Baar et al.*, 1994]. *Boyle* [1988] suggested that because the Cd content of foraminiferal shells is governed by the cadmium content of seawater, Cd/Ca ratios in foraminiferal calcite may be used to infer distributions of phosphate in ancient oceans. Results of several studies of Cd/Ca ratios in benthic foraminifera in sediment cores suggest that the relationship between Cd/Ca and SRP in global ocean waters did not change appreciably between the Holocene and at least the last glacial maximum [e.g., *Boyle*, 1992; *Oppo and Rosenthal*, 1994], and thus Cd/Ca ratios may be used as a proxy for phosphate concentration over this time period.

Interpretation of the Cd/Ca signal in planktonic species is complicated (e.g., *Mashiotta et al.* [1993]; however, see also *Boyle* [1981] and *Delaney* [1989]). However, if difficulties can be overcome, we suggest, wherever both Cd/Ca and $\delta_{37:2}$ can be determined, accurate estimates of paleo-ocean P_{CO_2} can be made. In this way, it should be possible to regionally reconstruct sources and sinks of atmospheric CO_2 and to contribute significantly to an understanding of the biogeochemical dynamics of glacial-interglacial transitions.

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