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Limnology and Oceanography, Volume 40, Issue 8 (Dec., 1995), 1404-1417.

Stable URL:
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Limnol. Oceanogr., 40(8), 1995, 1404–1417

Cobalt and zinc interreplacement in marine phytoplankton: Biological and geochemical implications

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Abstract

Zinc is used extensively in the metabolism of higher organisms; cobalt's usage is minimal. We found an unusual pattern of requirement for these metals in marine phytoplankton in which the cyanobacterium *Synechococcus bacillaris* needed Co but not Zn for growth, the coccolithophore *Emiliania huxleyi* had a Co requirement that could be partly met by Zn, and the diatoms *Thalassiosira pseudonana* and *Thalassiosira oceanica* had Zn requirements that could be largely met by Co. These results indicate that Co and Zn can replace one another metabolically in the eucaryotic species. Associated with this replacement, there was up to a 700-fold increase in cellular Co uptake rates with decreasing $Zn^{'}$ ion concentration, indicating that Zn should have a major influence on biological scavenging of Co. This hypothesis is consistent with Zn and Co distributions within the oceanic nutricline which show Co depletion only after Zn has become depleted. Zn ion concentrations and Co:Zn ratios vary widely in the ocean, and these variations could influence the relative growth of diatoms and coccolithophores, with potential effects on global carbon cycles.

Zinc, an essential micronutrient, is a component of nearly 300 enzymes involved in virtually all aspects of metabolism (Vallee and Auld 1990). It typically occurs in surface oceanic waters at concentrations ≤0.1 nM (Bruland and Franks 1983), ~1/100th the levels found in coastal waters (Evans 1977). Previous experiments have shown that oceanic phytoplankton have adapted to the low levels of Zn in their environment by reducing their cellular Zn requirement, but the mechanisms by which they have done so are unknown (Brand et al. 1983; Sunda and Huntsman 1992). One possibility is the replacement of Zn in enzymatic sites by other chemically similar metals. Both cobalt and cadmium have been shown to partially replace Zn metabolically in the coastal diatom *Thalassiosira weissflogii* (Price and Morel 1990). Our previous growth limitation experiments comparing Zn requirements of oceanic and coastal species were conducted in media with added Co but not Cd. Thus, a metabolic replacement of Zn by Co could explain the low growth requirement for Zn we previously observed (Sunda and Huntsman 1992).

To test this hypothesis, we measured relationships among free ion concentrations of Co and Zn, cellular concentrations and uptake rates of these two metals, and growth rate in the coastal cyanobacterium *Synechococcus bacillaris* and three eucaryotic species we examined previously (Sunda and Huntsman 1992): the oceanic and coastal diatoms, *Thalassiosira oceanica* and *Thalassiosira pseudonana,* and the dominant oceanic coccolithophore, *Emiliania huxleyi.* The results were compared with oceanic distributions of Zn, Co, and major nutrient concentrations within the nutricline of the North Pacific and with estimates of Zn complexation and $[Zn^{2+}]$ in that oceanic region (Bruland 1989). This comparison allowed us to assess potential effects of Zn and Co on the growth of phytoplankton and to assess the role of phytoplankton in regulating concentrations of these two metals via biological uptake and regeneration.

Methods

Our methods are comparable to those used in previous experiments on trace metal-phytoplankton interactions (Sunda and Huntsman 1992, 1995*a*). Axenic cultures were obtained from the Provasoli-Guillard Center for the Culture of Marine Phytoplankton, Bigelow Laboratory, and were maintained by means of sterile technique until needed. Experiments were conducted in filtered (0.4 μm) 36‰ salinity Gulf Stream water enriched with 32 μM $NaNO_3$, 2 μM Na_2HPO_4, 40 μM Na_2SiO_3, 10 nM Na_2SeO_4, 0.4 nM biotin, and 60 nM thiamin. Media for *T. pseudonana* and *E. huxleyi* and for all but one experiment with *T. oceanica* also contained 0.074 nM vitamin B_{12}. The media contained trace metal ion buffer systems to regulate and quantify free ion concentrations of Zn, Co, and other trace metal nutrients. These buffers consisted of 0.1 mM EDTA, 100 nM $FeCl_3$, 48 nM $MnCl_2$, 40 nM $CuCl_2$, 100 nM $NiCl_2$, and different concentrations of $CoCl_2$ and $ZnCl_2$. The media were equilibrated 24 h before use.

Free trace metal ion concentrations were computed from total metal concentrations and the extent of metal complexation by EDTA and inorganic ions. Total metal concentrations equaled the estimated background concentration (0.9 and 0.1 nM for Zn and Co) plus amounts added with radiotracer solutions and as chloride salts. Computed ratios of $[Zn^{2+}]$ and $[Co^{2+}]$ to total concentrations of these metals were $10^{-3.99}$ and $10^{-3.63}$, respectively. Computed values for log $[Mn^{2+}]$, $[Cu^{2+}]$, and $[Ni^{2+}]$ were −8.54, −13.52, and −12.89. The computed mean total concentration of dissolved ferric hydrolysis species was 0.25 nM (Sunda and Huntsman 1995*b*).

Acknowledgments

We thank Lisa Lowrey and Alexander Sharp for technical assistance.

This work was funded by grants from the Office of Naval Research.

Algal cells were grown at 20°C and pH 8.2±0.1 under fluorescent lighting (500 μmol quanta m^{-2} s^{-1} PAR on a 14/10 L/D cycle). Following culture inoculation, we measured cell concentrations and total cell volumes daily with a multichannel electronic particle counter (Coulter, model TAII). These measurements were not possible for the much smaller *Synechococcus,* whose relative cell biomass was instead quantified with ^{14}C radiotracer (Welschmeyer and Lorenzen 1984). Specific growth rates of cultures were computed from linear regressions of ln cell volume (or fixed carbon) vs. time for the exponential phase of growth.

Cell Zn and Co concentrations were measured during exponential growth with ^{65}Zn and ^{57}Co radiotracers 9–10 cell divisions after culture inoculation. Cells were filtered onto 1-μm-pore Nuclepore filters (0.4 μm for *S. bacillaris*), and filtrates were passed through a set of blank filters. Experimental and blank filters were counted for ^{65}Zn and ^{57}Co emissions on a gamma spectrometer. For eucaryotic species, the fraction of radiotracer in the cells was multiplied by the total metal concentration and divided by the total cellular volume to yield cellular metal concentrations in units of mol (liters cell vol.)$^{-1}$. These values were converted to molar metal:carbon ratios by dividing them by cell C:volume ratios measured in ^{14}C-labeled cultures without radiolabeled metals. The C:cell volume ratios used for this conversion were 11, 14, and 16 mol C $liter^{-1}$ for *T. oceanica, T. pseudonana,* and *E. huxleyi.* For *Synechococcus* Co:C ratios were determined directly by dual labeling of the cultures with ^{57}Co and ^{14}C.

Steady state cellular Zn or Co uptake rates were determined by multiplying cellular metal concentrations by specific growth rates (Sunda and Huntsman 1992).

Free Zn ion concentrations were estimated for North Pacific seawater to relate our culture data for cellular Zn uptake and algal growth rate to oceanographic data for Zn concentrations. Estimates of $[Zn^{2+}]$ in seawater were based on Zn complexation data of Bruland (1989) for the central North Pacific was computed from the equation

$$[\text{dissolved Zn}] = \frac{[Zn^{2+}]}{0.66} + \frac{1.2 \times 10^{-9}[Zn^{2+}]10^{11.0}}{[Zn^{2+}]10^{11.0} + 1}; \quad (1)$$

0.66 is the ratio of $[Zn^{2+}]$ to dissolved inorganic Zn species (Byrne et al. 1988), 1.2×10^{-9} M is the concentration of the natural organic ligand that strongly complexes Zn, and $10^{11.0}$ M^{-1} is the conditional stability constant for Zn complexation by that ligand.

Results

Effects on growth rate and cell physiology—In an initial series of experiments, we examined Zn limitation in the absence of added inorganic Co and Co limitation in the absence of added Zn (Fig. 1). The results revealed an intriguing pattern of Co and Zn requirement and substitution for one another among the four species tested. *T. pseudonana, E. huxleyi,* and *S. bacillaris* were unable to grow in the absence of added Zn and inorganic Co, while the growth rate of *T. oceanica* was reduced by 36±5% relative to maximum rates (Fig. 1A,C). Maximum growth of *T. pseudonana* was achieved at free Zn ion concentrations ($[Zn^{2+}] \geq 10^{-11}$ M. Additions of Co alone also stimulated growth but yielded maximum growth rates that were only 60% of those with added Zn, similar to previous results with a related coastal diatom, *T. weissflogii* (Price and Morel 1990). Co showed an even greater capacity to replace Zn in *T. oceanica,* whose maximum growth rate with added Co was 75–80% of that with added Zn. *E. huxleyi* showed an opposite pattern, exhibiting a primary requirement for Co that was only partially (~70%) replaceable by Zn (Fig. 1A,C). *Synechococcus* also had a Co requirement, but it showed no measurable requirement for Zn nor any ability to metabolically replace Co with Zn.

Plots of growth rate vs. cellular Co or Zn revealed a striking similarity in the Co requirement of the coccolithophore and the Zn requirement of the coastal diatom (Fig. 1B,D). In the absence of added inorganic Co, *T. pseudonana* required a Zn:C ratio of 1.8 μmol^{-1} mol^{-1} to achieve a specific growth rate of 0.8 d^{-1}—the same as the Co:C ratio needed to support equivalent growth in *E. huxleyi* without added Zn. The oceanic diatom, on the other hand, required a Zn:C ratio of only 0.4 μmol^{-1} mol^{-1} to support the same growth rate in the absence of added inorganic Co—22% of the Zn:C ratio needed by its coastal congener (Fig. 1D). The requirement for cellular Co was relatively low in *Synechococcus*; a Co:C ratio of only 0.12 μmol mol^{-1} supported a specific growth rate of 0.5 d^{-1}—the maximum growth rate for this species (Fig. 1B).

In addition to limiting growth rate, low $[Zn^{2+}]$ and $[Co^{2+}]$ also affected cellular chlorophyll concentrations and cell size (Table 1). As with growth rate, there were marked differences in the responses among species. The most pronounced effects were observed in *E. huxleyi,* where decreases in $[Co^{2+}]$ caused up to a 3-fold increase in the mean volume per cell and decreases in $[Co^{2+}]$ and $[Zn^{2+}]$ caused up to 4-fold decreases in Chl *a* concentrations. For the two diatoms, the omission of Zn and Co caused only 15–20% decreases in Chl *a* levels; for *Synechococcus,* such omission caused a slight increase in Chl *a*:C ratios. For *T. pseudonana,* the omission of Zn and inorganic Co caused cell size to decrease by up to 40%—the opposite of the response in *E. huxleyi.* The differences in cell size and Chl *a* responses among the species suggest differences in the underlying metabolic mechanisms responsible for Zn-Co limitation of growth rate.

Experiments were also conducted to investigate relationships among growth rate, metal uptake, and $[Zn^{2+}]$ for *E. huxleyi* and *T. oceanica* at higher $[Co^{2+}]$ ($10^{-12.0}$ and $10^{-11.0}$ M) and relationships among growth rate, Co uptake, and $[Co^{2+}]$ for *E. huxleyi* at higher $[Zn^{2+}]$ ($10^{-12.0}$ and $10^{-10.4}$ M). The results clearly show the ability of Co and Zn to replace one another metabolically (Figs. 2,3; Table 1). For example, in the absence of added Zn, a Co:

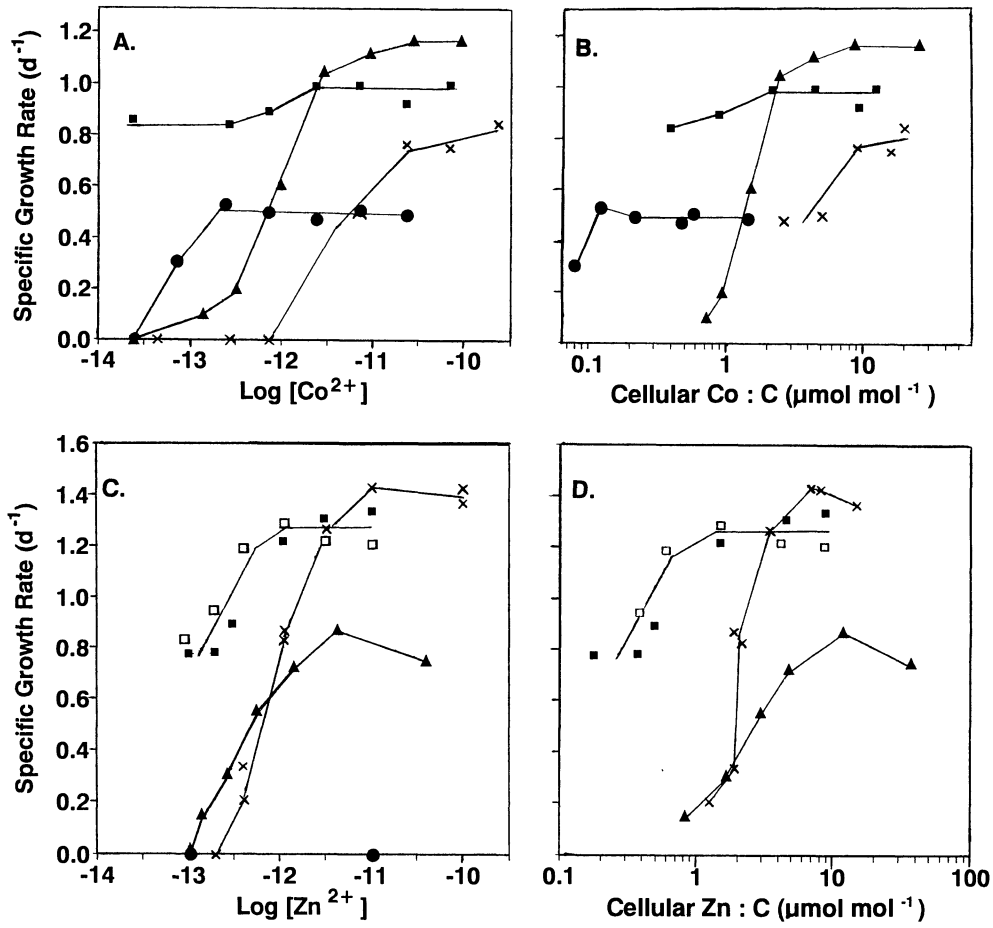


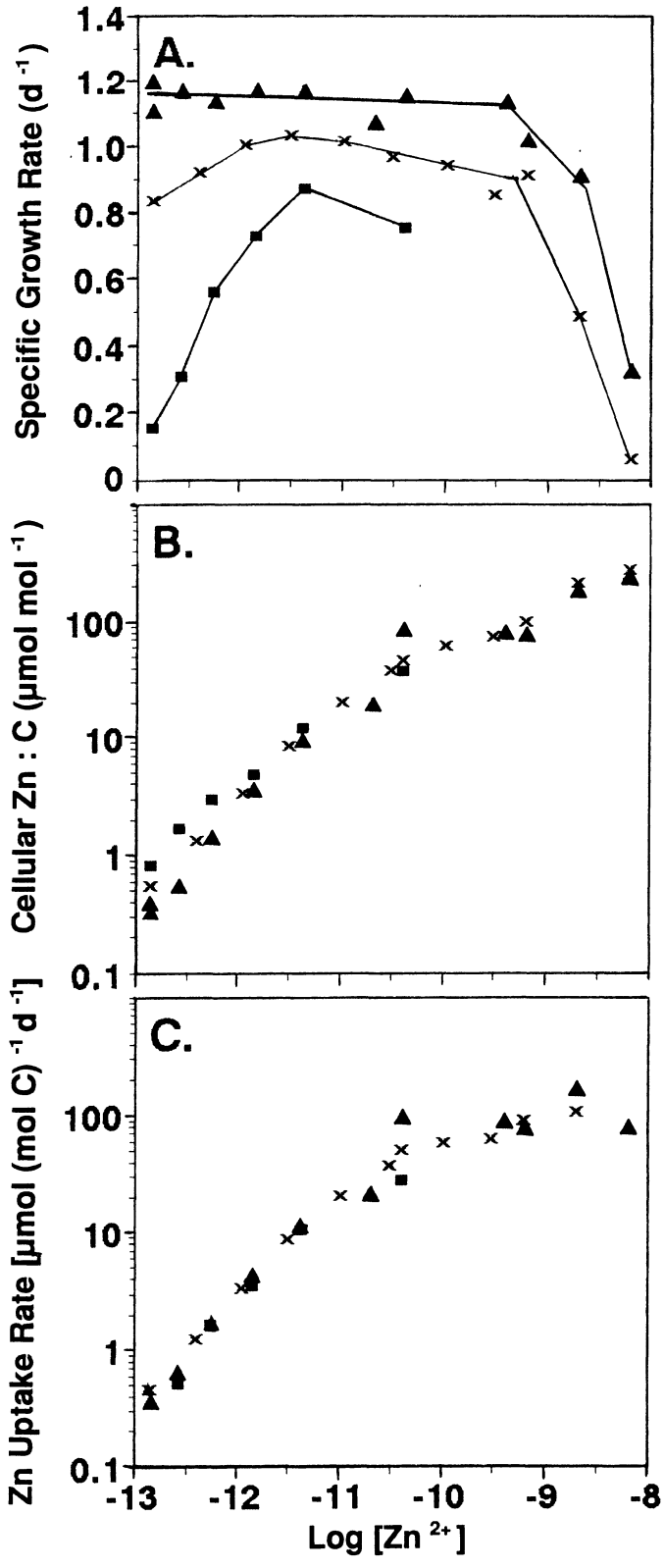
Fig. 1. A, B. Specific growth rate of *Synechococcus bacillaris* (●), *Emiliania huxleyi* (▲), *Thalassiosira oceanica* (■ □), and *Thalassiosira pseudonana* (×) as functions of log[Co^{2+}] and cellular Co : C in media without added Zn (estimated log[Zn^{2+}] = −13.0). C, D. Growth rate of same isolates as functions of log[Zn^{2+}] and cellular Zn : C mole ratio in media with no added inorganic Co (estimated log[Co^{2+}] = −13.6).

C ratio of 2.3 µmol mol^{-1} was required to achieve a specific growth rate of 1.0 d^{-1} in *E. huxleyi,* while at a [Zn^{2+}] of $10^{-10.4}$ M (4×10^{-11} M), a ratio of only 0.04 µmol mol^{-1} (a 60th) was needed to achieve the same growth rate (Fig. 3D).

Although intermediate levels of Zn replaced Co nutritionally in *E. huxleyi,* higher levels became toxic. The toxicity was inversely related to [Co^{2+}] (Fig. 2A) and was associated with growth rate inhibition, markedly depressed Co uptake rates (Fig. 4A), and up to 4-fold increases in mean volume per cell. The cell-size response was similar to that observed with Co deficiency (Table 1). These observations suggest that the Zn toxicity is related to an induced Co deficiency brought on by suppression of Co uptake by elevated [Zn^{2+}] and by competition between the two metals for internal metabolic sites. Similar toxic metal-nutrient metal antagonisms have been observed in phytoplankton between Cu and Mn, Zn and Mn, Cd and Mn (Sunda and Huntsman 1983, in press), and Cd and Fe (Harrison and Morel 1983).

Controls on cellular uptake of Zn and Co—Inverse relationships between Co uptake rate and [Zn^{2+}] were observed in all three eucaryotic species (Figs. 4, 5). For these species, the curves for Co uptake rate vs. [Zn^{2+}] exhibited low values at high [Zn^{2+}], large (up to 700-fold in *T. oceanica*) increases in uptake rate as [Zn^{2+}] was decreased within an intermediate range, and nearly constant and identical maximum rates at [Zn^{2+}] $< 10^{-12}$ M. The curves for the two diatoms were sigmoidal in shape and were similar to one another at [Zn^{2+}] $\leq 10^{-11}$ M. By contrast, the curve for *E. huxleyi* had a more gradual slope and was shifted appreciably to the right on the [Zn^{2+}] axis (Fig. 5). As a result, *E. huxleyi* had substantially higher Co uptake rates than the diatoms at higher [Zn^{2+}], in accord with its metabolic preference for Co.

In contrast to the large effect of [Zn^{2+}] in repressing Co uptake, increases in [Co^{2+}] had no effect on Zn uptake in the diatoms and only a small effect in *E. huxleyi.* The relationships between uptake rate and [Zn^{2+}] measured here at [Co^{2+}] of $\sim 10^{-13.6}$ and 10^{-12} M in *T. oceanica* and $\sim 10^{-13.6}$ M in *T. pseudonana* were indistinguishable



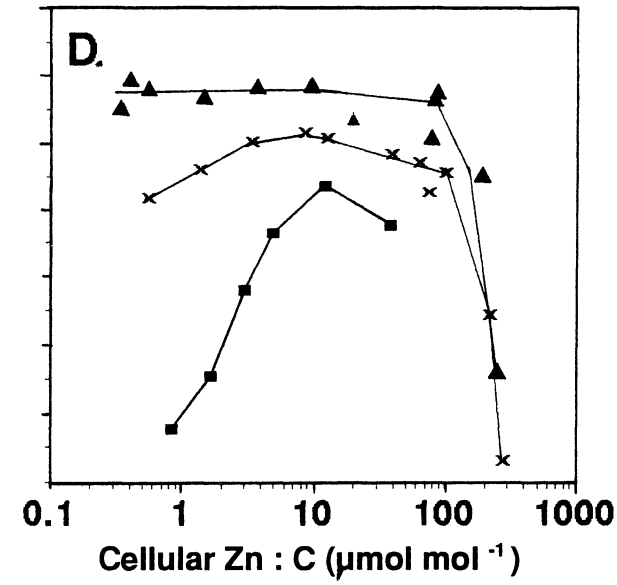


Fig. 2. Relationships among specific growth rate, log[Zn^{2+}], cellular Zn : C, and Zn uptake rate for *Emiliania huxleyi* grown at [Co^{2+}] of $\sim 10^{-13.6}$ M (■, no added inorganic Co), $10^{-12\,00}$ M (×), and $10^{-11\,03}$ M (▲). A, B, and C. Specific growth rate, cellular Zn : C ratio, and carbon-normalized cellular Zn uptake rate as functions of log[Zn^{2+}]. D. Specific growth rate as a function of cellular Zn : C ratio.

from those measured previously in these species at a [Co^{2+}] of $10^{-10.6}$ M (Fig. 6). Also, in the present experiments, an increase in [Co^{2+}] from $10^{-11.6}$ to $10^{-9.6}$ M had little effect on Zn uptake rate in *T. pseudonana*; if anything, it caused Zn uptake rates to increase slightly (Table 1, Exp. 145). Increasing [Co^{2+}] did depress Zn uptake rates in *E. huxleyi,* but the effect was minor and amounted to a decrease of only 12% at [Co^{2+}] of $10^{-10.5}$ M and 40% at [Co^{2+}] of $10^{-10.0}$ M (Table 1, Exp. 132b).

Zn uptake was quantified only in the three eucaryotic species, and uptake at [Zn^{2+}] $>10^{-10}$ M was determined only in *E. huxleyi* and *T. oceanica.* For *T. oceanica,* the relationship between steady state Zn uptake rate and [Zn^{2+}] was sigmoidal, with minimum slopes within the [Zn^{2+}] range of $10^{-10.5}$–10^{-10} M and increasing slopes above and below this range (Fig. 4B). For *E. huxleyi,* Zn uptake rates increased with [Zn^{2+}] up to 10^{-10} M and were constant above this value (Fig. 2C).

At [Zn^{2+}] $\leq 10^{-11.5}$ M, all three eucaryotic species showed similar proportionality between Zn uptake rate and [Zn^{2+}] (Fig. 7D). Also, at low [Zn^{2+}], relationships between cellular Co uptake rate and [Co^{2+}] for the eucaryotic species were similar to one another (Fig. 7A,C) and quantitatively similar to their Zn counterparts (Fig. 7B,D). Co uptake rates in *Synechococcus* were lower than those in the eucaryotic species (Fig. 7C).

Discussion

Regulation of cellular Zn and Co—The relationships between cellular Zn uptake rates and [Zn^{2+}] observed in the eucaryotic species were almost identical to those determined previously for the same species (Sunda and Huntsman 1992; *see Fig. 6B*). Short-term Zn uptake experiments conducted with *E. huxleyi* (clone BT-6) (Sunda

Table 1. Effect of variations in $[Zn^{2+}]$ and $[Co^{2+}]$ on cellular Zn : C and Co : C ratios (μmol mol C^{-1}), Chl *a* (mmol mol C^{-1}), mean cell volume (μm^3), specific growth rate (μ, d^{-1}), steady state cellular Zn and Co uptake rates [V_{Zn} and V_{Co}, μmol (mol C)$^{-1}$ d^{-1}], and ratios of cellular uptake rate to the maximum diffusion rate (V/ρ).

Exp.	log $[Zn^{2+}]$	log $[Co^{2+}]$	Zn : C	Co : C	Chl *a*	Cell vol.	μ	V_{Zn}	V/ρ*	V_{Co}	V/ρ*
					Emiliania huxleyi						
129a	−12.85	−13.6	0.83		0.052	42.7	0.15	†			
	−12.57	−13.6	1.67		0.098	33.6	0.31	0.52	0.49		
	−12.25	−13.6	2.98		0.100	29.8	0.56	1.66	0.70		
	−11.84	−13.6	4.81		0.193	29.0	0.73	3.50	0.56		
	−11.36	−13.6	12.0		0.196	29.2	0.87	10.5	0.56		
129b	−12.85	−11.03	0.33		0.192	21.7	1.10	0.368	0.50		
	−12.57	−11.03	0.54		0.211	20.4	1.16	0.629	0.43		
	−12.25	−11.03	1.43		0.198	21.2	1.13	1.62	0.55		
	−11.85	−11.03	3.62		0.200	19.6	1.17	4.23	0.53		
	−11.36	−11.03	9.12		0.207	19.9	1.17	10.7	0.44		
133	−13.0	−12.00		1.61	0.117	27.6	0.82			1.33	
	−12.85	−12.00	0.55	1.42	0.150	26.7	0.83	0.462	0.71	1.18	0.28
	−12.40	−12.00	1.35	1.44	0.179	23.7	0.92	1.25	0.63	1.33	0.29
	−11.95	−12.00	3.37	1.02	0.189	22.2	1.01	3.38	0.59	1.02	0.21
	−11.50	−12.00	8.43	0.97	0.238	21.8	1.03	8.70	0.53	0.999	0.20
	−10.98	−12.00	12.5	0.64	0.217	23.3	1.02	20.8	0.40	0.650	0.139
	−10.51	−12.00	38.4	0.38	0.182	24.4	0.98	37.2	0.25	0.363	0.082
	−9.99	−12.00	62.5	0.151	0.154	30.6	0.94	59.0	0.14	0.143	0.037
	−9.51	−12.00	73.7	0.062	0.176	37.2	0.85	63.0	0.056	0.0528	0.0154
	−9.99	−10.00	37.5	3.64	0.179	23.4	1.03	38.6	0.074	3.75	0.0080
167	−9.20	−12.00	100	0.0412	0.286	29.6	0.92	91.7	0.034	0.0378	0.0094
	−8.70	−12.00	217	0.0151	0.256	46.2	0.49	105.9	0.0167	0.00735	0.0025
	−8.20	−12.00	279	0.0100	0.207	86.8	0.06	†		†	
	−10.70	−11.03	19.4	2.22	0.208	22.1	1.07	20.7	0.20	2.37	0.052
	−9.20	−11.03	77.8	0.173	0.264	22.8	1.02	78.2	0.024	0.176	0.0040
	−8.70	−11.03	185	0.0600	0.227	33.3	0.91	168.0	0.021	0.0546	0.00158
	−8.20	−11.03	243	0.0190	0.178	79.0	0.33	79.8	0.0057	0.00621	0.00032
132a	−13.0	−13.6					0				
	−13.0	−12.86		0.722	0.066	35.4	0.10			†	
	−13.0	−12.49		0.941	0.055	35.3	0.20			†	
	−13.0	−12.01		1.52	0.141	31.3	0.61			0.925	0.244
	−13.0	−11.54		2.49	0.201	24.1	1.05			2.59	0.197
	−13.0	−11.03		4.38	0.205	21.3	1.12			4.89	0.104
	−13.0	−10.55		8.71	0.205	20.6	1.17			10.2	0.071
	−13.0	−10.03		25.6	0.205	20.7	1.17			30.1	0.063
132b	−10.39	−13.37	37.3	0.017	0.134	41.1	0.75	28.1	0.20	0.013	0.093
	−10.39	−12.86	44.6	0.064	0.194	29.5	0.99	44.4	0.25	0.064	0.116
	−10.39	−12.49	42.2	0.123	0.197	26.9	1.07	45.1	0.24	0.131	0.094
	−10.39	−12.01	46.5	0.386	0.206	23.6	1.08	50.5	0.25	0.418	0.092
	−10.39	−11.54	44.3	0.776	0.199	22.4	1.11	49.3	0.23	0.863	0.063
	−10.39	−11.03	41.5	1.86	0.196	22.9	1.15	47.7	0.23	2.14	0.048
	−10.39	−10.55	38.6	3.95	0.206	21.3	1.14	44.0	0.20	4.49	0.032
	−10.39	−10.03	25.9	7.44	0.200	21.1	1.16	30.2	0.139	8.67	0.0184
173	−12.00	−13.52		0.073	0.166	32.2	0.61				
	−12.00	−13.00		0.257	0.271	26.9	0.93			0.238	0.56
	−12.00	−12.50		0.739	0.324	26.5	1.06			0.780	0.57
	−12.00	−11.00		4.35	0.305	25.1	1.20			5.20	0.112
					Thalassiosira oceanica						
141	−13.0	−13.6			0.148	82.5	0.83				
	−12.72	−13.6	0.38		0.208	87.8	0.95	0.360	0.63		
	−12.40	−13.6	0.59		0.201	84.5	1.19	0.708	0.58		
	−11.95	−13.6	1.50		0.242	84.1	1.29	1.93	0.56		
	−11.50	−13.6	4.14		0.244	84.2	1.22	5.06	0.52		
	−10.98	−13.6	8.59		0.170	86.8	1.21	10.4	0.33		
165	−13.0	−13.6	0.18		0.209	102	0.78	0.138	0.50		
	−12.70	−13.6	0.37		0.207	122	0.78	0.292	0.61		

Table 1. Continued.

Exp.	log $[Zn^{2+}]$	log $[Co^{2+}]$	Zn : C	Co : C	Chl *a*	Cell vol.	μ	V_{Zn}	V/ρ*	V_{Co}	V/ρ*
	−12.51	−13.6	0.49		0.204	87.7	0.89	0.439	0.47		
	−11.96	−13.6	1.50		0.258	81.3	1.22	1.83	0.53		
	−11.51	−13.6	4.57		0.247	81.9	1.31	6.00	0.62		
	−10.98	−13.6	8.78		0.225	84.2	1.34	11.8	0.36		
149	−13.0	−12.00		1.44	0.177	94.5	1.15			1.65	
	−12.85	−12.00	0.15	1.26	0.210	93.4	1.15	0.175	0.43	1.46	0.53
	−12.30	−12.00	0.70	1.66	0.216	93.1	1.19	0.837	0.59	1.97	0.73
	−11.91	−11.99	1.33	1.12	0.210	97.4	1.18	1.58	0.45	1.328	0.48
	−11.49	−11.99	3.34	0.656	0.217	89.4	1.24	4.15	0.46	0.816	0.30
	−10.98	−11.99	7.06	0.0454	0.194	93.1	1.27	8.98	0.29	0.0578	0.020
	−10.51	−11.98	11.5	0.00892	0.222	90.9	1.29	14.8	0.166	0.0115	0.0040
	−9.99	−12.00	13.5	0.00262	0.210	88.4	1.28	17.3	0.058	0.00335	0.00121
	−9.51	−12.00	21.3	0.00208	0.216	91.9	1.31	27.9	0.030	0.00272	0.00097
	−8.99	−12.00	42.3	0.00197	0.205	91.9	1.31	55.5	0.019	0.00258	0.00094
150a‡	−13.0	−13.6			0.144	102	0.75				
	−12.71	−13.6	0.38		0.155	100	0.90	0.345	0.65		
	−12.36	−13.6	0.80		0.180	97.3	0.90	0.654	0.58		
	−11.97	−13.6	1.72		0.178	96.9	1.03	1.77	0.59		
	−11.51	−13.6	4.41		0.175	101	1.05	4.65	0.55		
	−10.98	−13.6	8.23		0.176	101	1.05	8.63	0.30		
	−10.51	−13.6	10.6		0.181	101	1.09	11.5	0.136		
150b‡	−13.0	−12.58		0.396	0.183	93.8	0.84			0.333	0.47
	−13.0	−12.14		0.889	0.143	95.4	0.89			0.795	0.41
	−13.0	−11.62		2.20	0.163	91.8	0.99			2.18	0.33
	−13.0	−11.15		4.48	0.167	91.4	0.99			4.45	0.23
	−13.0	−10.63		9.33	0.163	94.2	0.92			8.63	0.136
	−13.0	−10.15		12.4	0.195	87.5	0.99			12.3	0.062
					Thalassiosira pseudonana						
144	−13.0	−13.49					0				
	−12.70	−13.52					0				
	−12.39	−13.52	1.90	0.0811	0.170	31.9	0.33	0.635	0.33	0.0272	0.21
	−12.39	−13.52	1.25	0.0646		41.2	0.21	†		†	
	−11.94	−13.52	2.19	0.0363	0.168	42.4	0.83	1.82	0.41	0.0302	0.28
	−11.94	−13.52	1.89	0.0359	0.150	33.9	0.97	1.85	0.36	0.0350	0.28
	−11.94	−13.6				43.2	0.87				
	−11.49	−13.49	3.40	0.0107	0.184	49.3	1.27	4.32	0.39	0.0136	0.129
	−10.99	−13.49	6.86	0.00101	0.195	55.5	1.43	9.84	0.30	0.00145	0.0149
	−9.99	−13.49	8.12	0.00047	0.197	56.0	1.43	11.6	0.035	0.00067	0.006
145	−12.99	−13.35					0				
	−12.99	−12.55					0				
	−12.99	−12.13					0				
	−12.99	−11.63	0.29	2.68	1.28	41.9	0.48	0.141	0.36	1.28	0.149
	−12.99	−11.15	0.28	5.04	2.51	48.6	0.50	0.138	0.38	2.51	0.108
	−12.99	−10.63	0.22	9.07	6.95	48.8	0.77	0.171	0.48	6.95	0.090
	−12.99	−10.16	0.23	15.8	11.8	54.6	0.75	0.173	0.52	11.8	0.056
	−12.99	−9.63	0.19	20.0	17.0	48.0	0.85	0.158	0.44	17.0	0.022
					Synechococcus bacillaris						
152‡	−13.0	−13.6					0				
	−13.0	−13.15		0.080	0.228		0.30			0.0241	
	−13.0	−12.63		0.124	0.134		0.53			0.0700	
	−13.0	−12.15		0.219	0.129		0.50			0.109	
	−13.0	−11.63		0.475	0.132		0.47			0.224	
	−13.0	−11.15		0.578	0.154		0.51			0.293	
	−13.0	−10.63		1.43	0.132		0.49			0.699	
	−10.98	−13.6					0				

* V/ρ gives the ratio of steady state Zn or Co uptake to the maximum diffusion rate of kinetically labile inorganic species (free ions plus inorganic complexes) to the cell surface. This ratio was computed by multiplying the steady state uptake rate per cell volume

⟶

Table 1. Footnote continued.

by the mean volume per cell to give the uptake rate per cell. This value was then divided by the maximum diffusion rate per cell (ρ), computed from the equation $\rho = 4\pi rD[M']$ by assuming that the cells are approximately spherical (Hudson and Morel 1990). The cell radius, r, was computed from the mean cell volume by using the relation between the radius of a sphere and its volume. D is the diffusion rate constant for inorganic species at 20°C (6.1 and 6.4×10^{-6} cm^2 s^{-1} for Co and Zn, Li and Gregory 1974), and $[M']$ is the concentration of dissolved inorganic species computed by dividing the free ion concentration, $[M^{2+}]$, by values for $[M^{2+}]/[M']$ (0.67 for Co and 0.66 for Zn, Byrne et al. 1988).

† Insufficient cell divisions at constant growth rate for steady state to be established.

‡ No vitamin B_{12} added in this experiment.

and Huntsman 1992) and with *T. pseudonana* and *T. oceanica* (unpubl. data) indicate that cellular Zn is actively regulated by an inducible high-affinity uptake system whose capacity for uptake increases as $[Zn^{2+}]$ in the medium is decreased. The half-saturation constant for this high-affinity system for *E. huxleyi* ($10^{-9.6}$ M; Sunda and Huntsman 1992) is similar to that for *T. pseudonana* ($10^{-9.8}$ M; unpubl. data). Both constants fall within the $[Zn^{2+}]$ range where relatively constant cellular transport rates are maintained (i.e. $10^{-10.5}$–$10^{-9.5}$ M for *T. pseudonana* and $10^{-10.5}$–$10^{-8.1}$ M for *E. huxleyi*). At $[Zn^{2+}]$ below the half-saturation constant, nearly constant uptake rates are maintained by negative feedback regulation of transport capacity, while at $[Zn^{2+}]$ above these values, constant rates are primarily related to saturation of the uptake system.

At $[Zn^{2+}] < 10^{-11}$ M, regulation of cellular Zn transport is no longer possible because Zn uptake by the cells approaches the physical limits imposed by diffusion of kinetically labile inorganic species (Zn^{2+}, $ZnCl^+$, and $ZnCO_3$) to the cell surface (Sunda and Huntsman 1992; Hudson and Morel 1993). Within this $[Zn^{2+}]$ range, uptake rates were 40–60% of the computed maximum diffusion rates (Table 1; Fig. 4). As dictated by diffusion, uptake rates became proportional to the concentration of inorganic Zn species and therefore proportional to $[Zn^{2+}]$.

As $[Zn^{2+}]$ was decreased in our experiments, there was a concomitant dramatic increase Co uptake rates. This increase continued until Co rates (like those of Zn) approached diffusion-limited values (Fig. 4). Thus, diffusion limitation in similarly sized cells appears to account for the similarity in Co and Zn uptake rates among the three eucaryotic species at low $[Zn^{2+}]$ and $[Co^{2+}]$ (Fig. 7C,D). Evolutionary forces have pushed Co and Zn uptake rates in these species to their maximum physical limits.

The induction of the high-affinity Zn uptake system and the concomitant increase in Co uptake at low $[Zn^{2+}]$ suggests that both metals are taken up by the same transport system. If this is true, the increase in Co uptake rates with decreasing $[Zn^{2+}]$ would be accounted for by negative feedback increases in the capacity (or affinity) of the transport system and by decreased competition between the two metals for binding to uptake sites. We cannot, however, rule out the involvement of more than one inducible transport system, each having different relative affinities for Zn and Co.

Unlike in the eucaryotic species, Co uptake does not seem to be physically limited by diffusion in *Synechococcus*. Despite its small diameter (~0.7 μm), which increases diffusion rates per unit of cell volume, Co uptake rates in this species in media without added Zn were a third to a tenth of rates in the eucaryotic species (Fig. 7C). These low rates probably reflect the low Co requirement for growth in *Synechococcus* and the apparent absence of a Zn requirement (Fig. 1B).

Under steady state conditions, cellular metal concentration equals the net uptake rate, V_M, divided by the specific growth rate, μ:

$$[\text{cell metal}] = V_M/\mu. \quad (2)$$

Because of this relationship, limitation of growth rate by low $[Zn^{2+}]$ or $[Co^{2+}]$ or inhibition by high $[Zn^{2+}]$ causes enhancement of cellular metal concentrations over that which would occur at constant growth rate. For example, the limitation of growth rate with decreasing $[Zn^{2+}]$ or $[Co^{2+}]$ caused relationships between cellular metal and free metal ion concentration to have progressively decreasing slopes with increasing growth limitation (Fig. 3A,B; Fig. 6A, *T. pseudonana*). The relation in Eq. 2 also seems to account for the 3-fold increase in cellular Zn : C in *E. huxleyi* associated with a 3-fold decrease in growth rate due to Zn toxicity (Fig. 2). These changes occurred over the $[Zn^{2+}]$ range of $10^{-9.2}$–$10^{-8.2}$ M, where Zn uptake rates were constant, apparently due to saturation of the Zn uptake system, as discussed earlier.

Because of the inverse relationship between cellular metal and growth rate, limitation of growth rate by one nutrient metal should increase cellular concentrations of other nutrient metals provided that their uptake rates are not affected. In *E. huxleyi,* Co limitation of growth increased cellular Zn concentrations (Fig. 2B) and limitation of growth rate by Zn had a similar effect on cellular Co (Fig. 3B). At $[Zn^{2+}]$ of $10^{-12.6}$ M, a decrease in $[Co^{2+}]$ from 10^{-11} M to $10^{-13.6}$ M strongly limited growth rate and, as a result, increased cellular Zn by 3-fold (Fig. 2B).

Metabolic interreplacement of Zn and Co—The interreplacement of Zn and Co in the diatoms and the coccolitophore indicates that each can fulfill similar metabolic functions, which is consistent with in vitro studies that show functional substitution of Co for Zn in many metalloenzymes (Vallee and Galdes 1984). Of greater relevance are in vivo studies with the diatom *T. weissflogii* that document Co-Zn substitution in carbonic anhydrase (Morel et al. 1994). The only absolute requirement for Co in eucaryotic cells (including phytoplankton) is as a cofactor in vitamin B_{12} (da Silva and Williams 1991). The Co stimulation of growth in our eucaryotic species

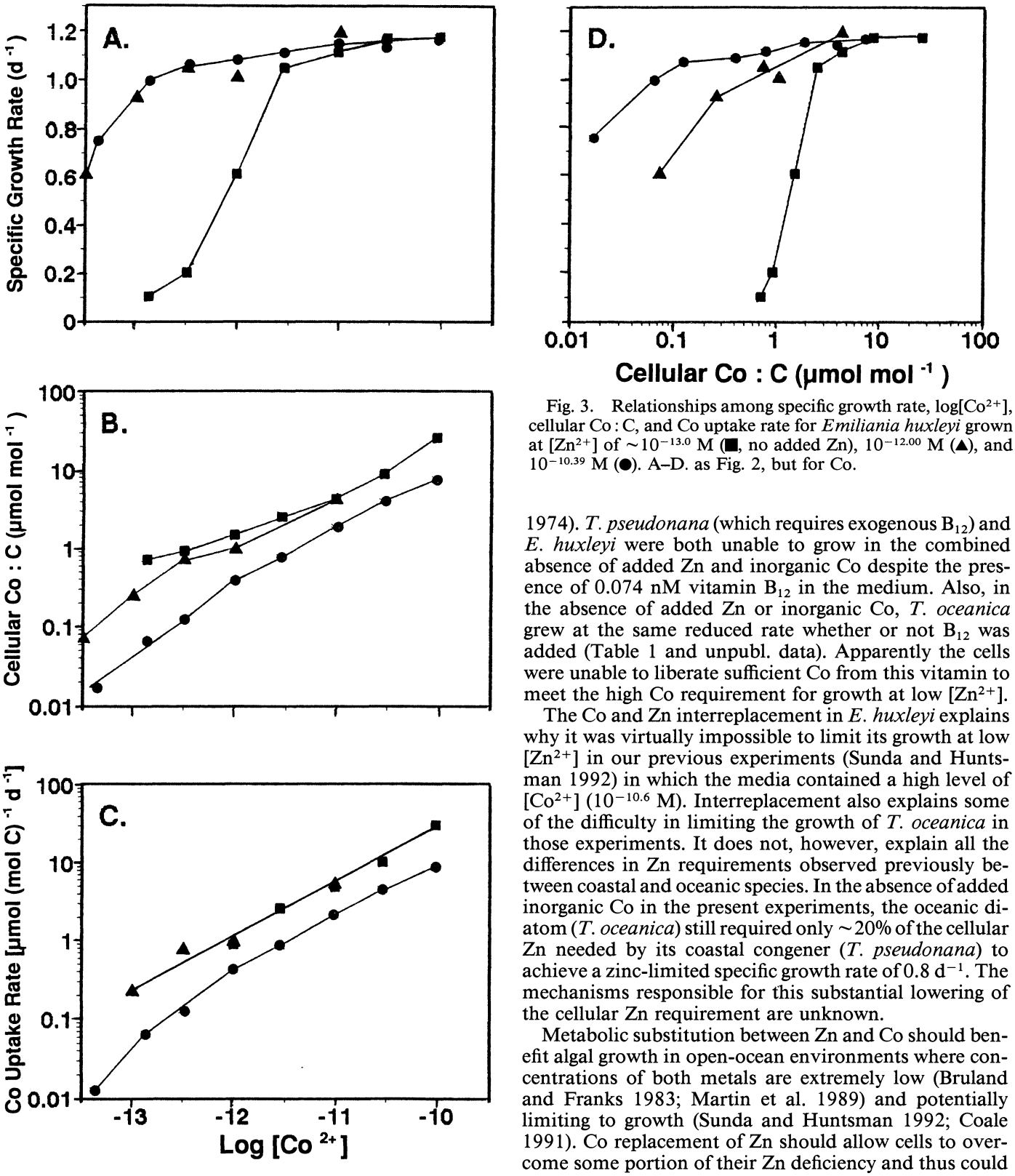


Fig. 3. Relationships among specific growth rate, log[Co^{2+}], cellular Co : C, and Co uptake rate for *Emiliania huxleyi* grown at [Zn^{2+}] of ~$10^{-13.0}$ M (■, no added Zn), $10^{-12.00}$ M (▲), and $10^{-10.39}$ M (●). A–D. as Fig. 2, but for Co.

clearly is not related to a need for this vitamin because the Co requirement for growth in *E. huxleyi* and *T. pseudonana* is ~100 times the B_{12} concentrations reported in these species (Carlucci and Bowes 1972; Swift and Taylor 1974). *T. pseudonana* (which requires exogenous B_{12}) and *E. huxleyi* were both unable to grow in the combined absence of added Zn and inorganic Co despite the presence of 0.074 nM vitamin B_{12} in the medium. Also, in the absence of added Zn or inorganic Co, *T. oceanica* grew at the same reduced rate whether or not B_{12} was added (Table 1 and unpubl. data). Apparently the cells were unable to liberate sufficient Co from this vitamin to meet the high Co requirement for growth at low [Zn^{2+}].

The Co and Zn interreplacement in *E. huxleyi* explains why it was virtually impossible to limit its growth at low [Zn^{2+}] in our previous experiments (Sunda and Huntsman 1992) in which the media contained a high level of [Co^{2+}] ($10^{-10.6}$ M). Interreplacement also explains some of the difficulty in limiting the growth of *T. oceanica* in those experiments. It does not, however, explain all the differences in Zn requirements observed previously between coastal and oceanic species. In the absence of added inorganic Co in the present experiments, the oceanic diatom (*T. oceanica*) still required only ~20% of the cellular Zn needed by its coastal congener (*T. pseudonana*) to achieve a zinc-limited specific growth rate of 0.8 d^{-1}. The mechanisms responsible for this substantial lowering of the cellular Zn requirement are unknown.

Metabolic substitution between Zn and Co should benefit algal growth in open-ocean environments where concentrations of both metals are extremely low (Bruland and Franks 1983; Martin et al. 1989) and potentially limiting to growth (Sunda and Huntsman 1992; Coale 1991). Co replacement of Zn should allow cells to overcome some portion of their Zn deficiency and thus could provide an adaptive strategy for growth in the open ocean. The low concentrations of Co relative to Zn in the ocean (maximum Co : Zn ratios are ~0.4), however, should limit the usefulness of this strategy, particularly for diatoms.

For an element to be biologically useful, it must have

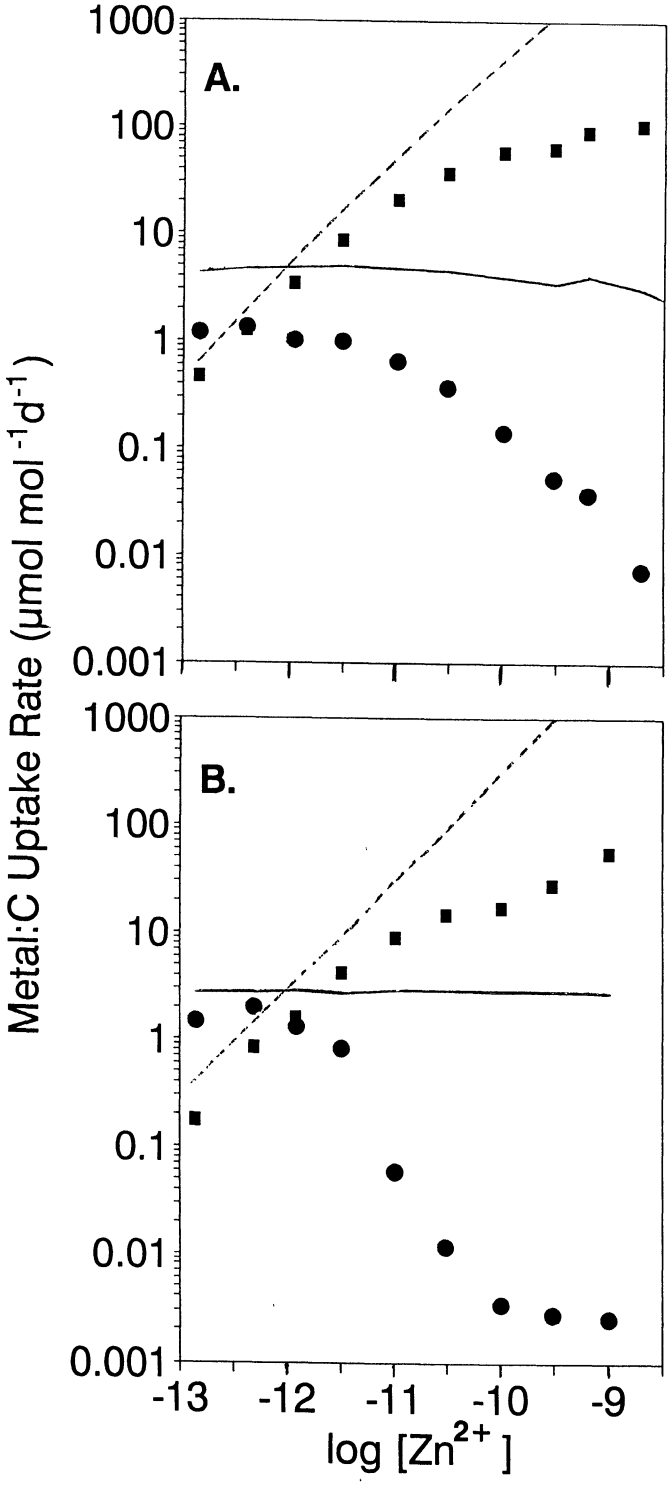


Fig. 4. Effect of $[Zn^{2+}]$ on steady state cellular Zn (■) and Co (●) uptake rates in *Emiliania huxleyi* (A) and *Thalassiosira oceanica* (B) at a $[Co^{2+}]$ of $10^{-12.0}$ M. Lines give the theoretical limiting rates for the diffusion of dissolved inorganic Co (——) and Zn (– – –) species to the cell surface.

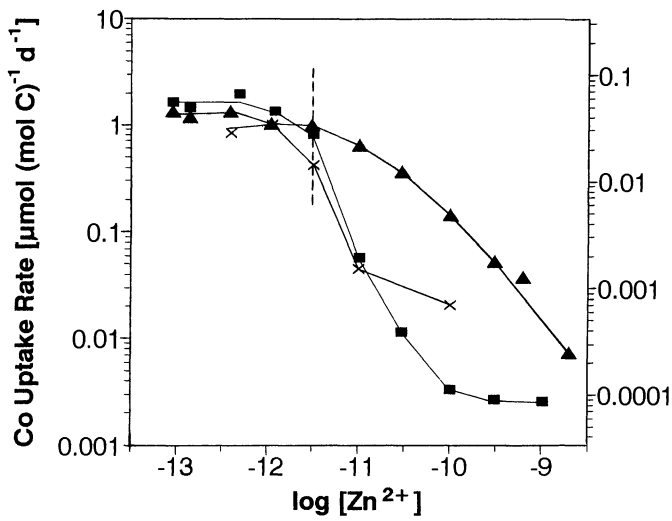


Fig. 5. Effect of $[Zn^{2+}]$ on steady state Co uptake rates. Uptake values for *Emiliania huxleyi* (▲) and *Thalassiosira oceanica* (■)(scale on left) were determined at $\log[Co^{2+}] = -12.0$. Values for *Thalassiosira pseudonana* (×, right axis) were determined at $\log[Co^{2+}] = -13.5$. Comparison is facilitated by shifting up the scale on right relative to that on left by 32, the ratio of the two $[Co^{2+}]$ values. Vertical line marks Co uptake rates at the estimated $\log[Zn^{2+}]$ (−11.5) where Co begins to be depleted in the upper nutricline of the North Pacific (*see Fig. 8*).

both the right chemical attributes to perform necessary metabolic functions and have sufficient environmental availability for cells to acquire needed amounts (da Silva and Williams 1991). The preference for Zn over Co in the marine diatoms makes sense biologically given the 2.5–500-fold higher concentrations of Zn relative to Co in the ocean (Martin et al. 1989, 1990, 1993). The low oceanic Co : Zn ratios partly reflect the low ratio (1 : 3) of the two elements in crustal rocks (Mason 1966). However, the extremely low ratios in deep seawater, which are ≤1 : 400 at depths below 1,000 m in the Pacific (Martin et al. 1989), largely reflect the insolubility of Co(III) oxide—the thermodynamically stable redox form in oxygenated seawater. Like Mn, Co is oxidatively scavenged from seawater, apparently by microbially mediated processes (Tebo et al. 1984; Lee and Fisher 1993).

Given the higher oceanic abundance of Zn over Co and the ability of Zn and Co to perform similar metabolic functions, it is puzzling that *Synechococcus* would have evolved a requirement for Co but not for Zn and that *E. huxleyi* would have evolved a metabolic preference for Co. Such preference for Co contrasts the metabolic use of these two metals in higher organisms, where Zn is by far more widely utilized (Vallee and Auld 1990; da Silva and Williams 1991).

Although we have no ready answers for the preference for Co over Zn in some species, one intriguing possibility is that this preference is vestigial and reflects the higher availability of Co relative to Zn during early evolution. In the primordial reducing ocean, the thermodynamically stable form of Co would have been the more soluble Co(II), and Zn would have been less abundant and biologically reactive than Co due to its much higher binding affinity for sulfide ions (Dyrssen and Kremling 1990). Evidence for these ideas is found in marine anoxic sulfidic basins, which have ~10-fold lower Zn concentrations

(Jacobs et al. 1985) and 10-fold higher Co concentrations (Dyrssen and Kremling 1990) than are found in overlying oxic waters.

Consistent with the above ideas, da Silva and Williams (1991) noted that there is a proliferation of Zn enzymes only in organisms that evolved after the establishment of an oxidizing environment. They further noted that biological utilization of Co shows the opposite trend. Co use remains widespread in bacterial metalloenzymes (Wackett et al. 1989), and there is an unusually high requirement (as corrinoid complexes) in methanogenic Archaebacteria (among the most primitive microorganisms, DiMarco et al. 1990). The algal Zn and Co requirements we found are consistent with these trends: *Synechococcus,* which requires Co but not Zn, is by far the most primitive alga (and evolved before the world became oxidizing), while the diatoms, which have a clear Zn requirement, are the most modern (Bold and Wynne 1978).

Relationships with oceanographic data—Previously we showed that there is a remarkable consistency among cellular Zn : C vs. $[Zn^{2+}]$ relationships measured in algal cultures, recent measurements of $[Zn^{2+}]$ and organic complexation of Zn in seawater, Zn : C ratios in field plankton samples, and Zn : C ratios derived from "Redfield" biological scavenging models for the oceanic nutricline (Redfield et al. 1963; Sunda and Huntsman 1992; Sunda 1994). A similar consistency between algal culture data and oceanographic chemical data also has been found for Cu (Sunda and Huntsman 1995*a*). Such agreement provides strong evidence that the distributions of Zn and Cu within oceanic nutriclines are controlled by phytoplankton uptake and remineralization, as occurs for major nutrients (N, P, and Si).

Comparisons of our algal Co uptake data with Co and Zn distributions within the oceanic nutricline support the hypothesis that Co concentrations, like those of Zn, are controlled by phytoplankton uptake and regeneration processes. Our culture data show that Co uptake increases dramatically with decreasing $[Zn^{2+}]$. For *T. oceanica,* Co uptake increased by 460-fold as $[Zn^{2+}]$ was decreased from 10^{-10} to 10^{-12} M—within the estimated $[Zn^{2+}]$ range for open-ocean seawater (Bruland 1989). Based on these results, we would expect to see algal scavenging of Co only in the upper nutricline, where Zn levels are greatly decreased by efficient uptake by phytoplankton. This, in fact, is what is observed at five North Pacific stations along a transect from 33.3°N, 139.1°W to 55.5°N, 147.5°W (Martin and Gordon 1988; Martin et al. 1989) (Fig. 8). At all of these stations, Co levels were either invariant or decreased slightly with depth at total $[PO_4]$ of 1.1–3.0 μM, but decreased sharply with decreasing $[PO_4]$ toward the surface as Zn concentrations fell below 0.3 nM. On the basis of Bruland's (1989) Zn complexation data for the North Pacific, 0.3 nM Zn corresponds to a $[Zn^{2+}]$ of $10^{-11.5}$ M—a value at which we see strong induction of Co uptake in the eucaryotic species (Fig. 5). At this $[Zn^{2+}]$, the Co uptake rate in *T. oceanica* is 300 times the value observed at a $[Zn^{2+}]$ of $10^{-9.5}$ M and half the maximum limiting value found at $[Zn^{2+}] \leq 10^{-12}$ M.

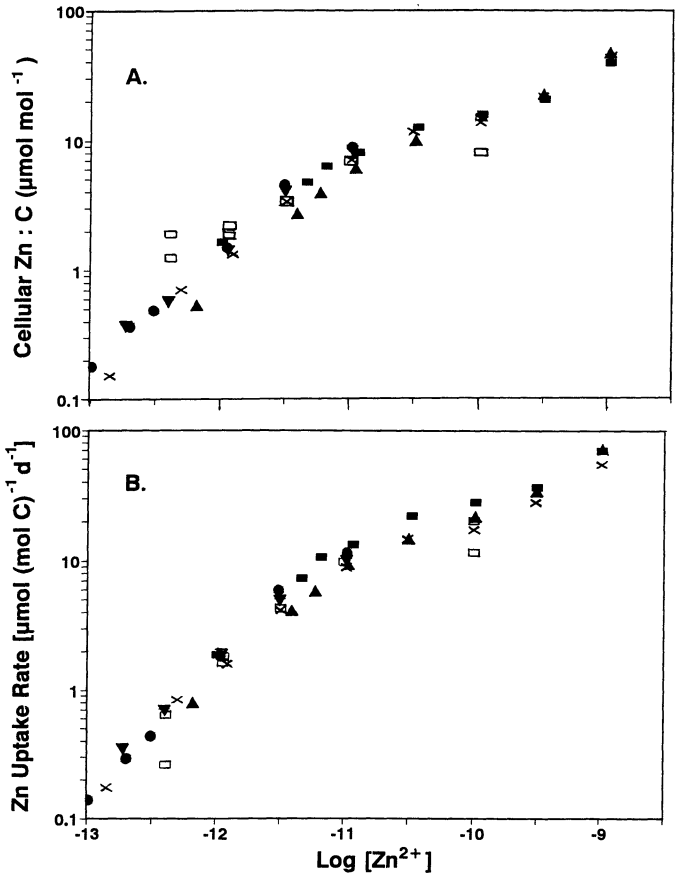


Fig. 6. Relationship between cellular Zn : C and log$[Zn^{2+}]$ and between steady state cellular uptake rate and log$[Zn^{2+}]$. Data are given for *Thalassiosira oceanica* at log$[Co^{2+}]$ of -13.6 (● ▼), -12.0 (×), and -10.6 (▲) and for *Thalassiosira pseudonana* at -13.5 (□) and -10.6 (■). Data for log$[Co^{2+}]$ of -10.6 are taken from Sunda and Huntsman (1992). Zn to cell volume data from Sunda and Huntsman (1992) were converted to Zn : C ratios by using C : cell volume ratios of 11 and 14 mol (liters cell vol.)$^{-1}$, respectively, for *T. oceanica* and *T. pseudonana.*

According to Redfield theory, if variations in Zn and PO_4 concentrations within the nutricline are due to algal uptake and remineralization, then the slope (dZn/dPO_4) of Zn vs. PO_4 plots should equal the Zn : PO_4 ratio in phytoplankton responsible for export of nutrients from the euphotic zone. By this reasoning, the mean Zn : PO_4 ratio of phytoplankton growing at intermediate to high concentrations of PO_4 (1–3 μM) and Zn (1–9 nM) in the North Pacific should be 4.3 mmol mol^{-1}, based on a linear regression (R^2 = 0.974) of the combined data of Martin et al (1989) for stations T-5 through T-8 (Fig. 8A). If we assume a Redfield C : P of 106 : 1 (here and in the following discussion), this value translates to a Zn : C ratio of 41 μmol mol^{-1}. On the basis of our culture data, this Zn : C ratio occurs in both experimental diatoms at a $[Zn^{2+}]$ of $10^{-8.9}$ M (Fig. 6A), and on the basis of Bruland's (1989) Zn complexation data in the North Pacific (Eq. 1), this $[Zn^{2+}]$ would occur at a Zn concentration of 3.1 nM, near the middle of the range (1–9 nM) over which the Zn vs. PO_4 slope was determined.

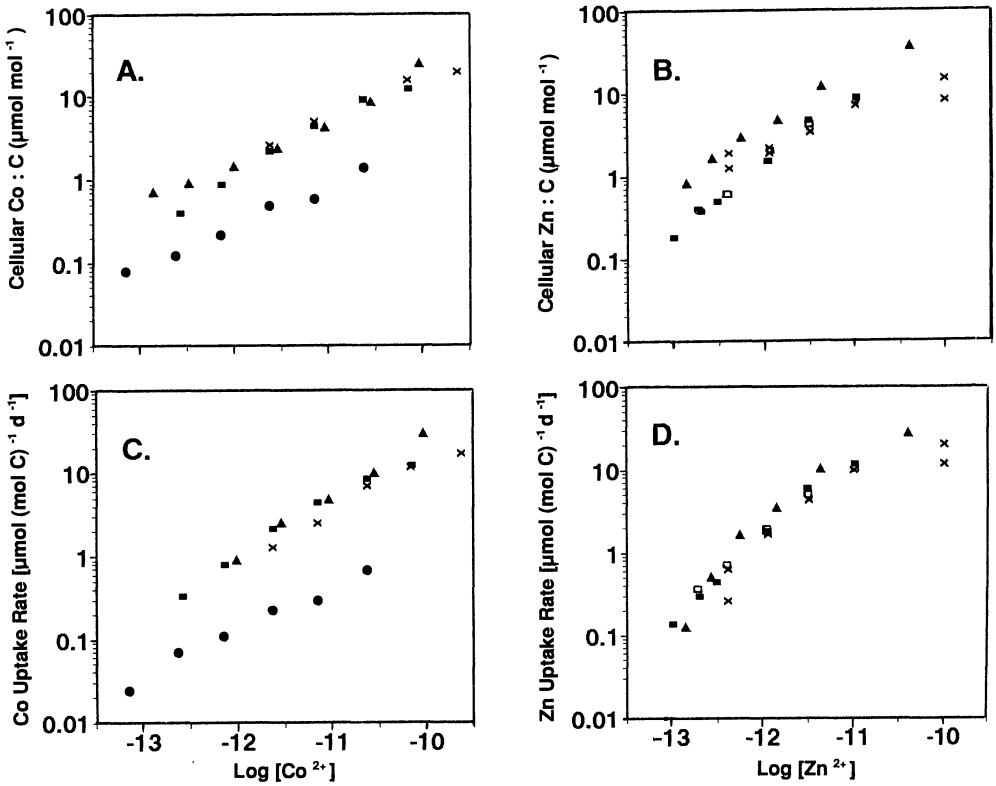


Fig. 7. A, C. Cellular Co : C and specific Co uptake rate of *Synechococcus bacillaris* (●), *Emiliania huxleyi* (▲), *Thalassiosira oceanica* (■ □), and *Thalassiosira pseudonana* (×) as functions of log[Co^{2+}] without added Zn. B, D. Cellular Zn : C and specific Zn uptake rate of same isolates (except *S. bacillaris* which did not grow) as functions of log[Zn^{2+}] in media without added inorganic Co. Growth rate data for these experiments are given in Fig. 1.

An analysis of the Zn vs. PO_4 slope (0.25 mmol mol^{-1}) at both station T-5 at depths of 50–150 m and station T-8 at 8–50 m (Table 2) reveals a similar consistency with our culture data. This Zn : PO_4 slope translates to a Zn : C of 2.4 µmol mol^{-1}, which occurs in our *E. huxleyi* and *T. oceanica* cultures at [Zn^{2+}] of $\sim 10^{-12.1}$ and $\sim 10^{-11.7}$ M (Figs. 2B, 6B). On the basis of Bruland's (1989) complexation data, these [Zn^{2+}] values would occur in North Pacific seawater at Zn levels of 0.1 and 0.2 nM, respectively—again near the middle of the range (0.06–0.3 nM Zn) over which the Zn : PO_4 slope was determined. This agreement, as before (Sunda and Huntsman 1992), provides strong evidence that Zn concentrations in the nutricline are controlled by algal uptake and regeneration.

The agreement between the estimated [Zn^{2+}] in seawater at which Co begins to be depleted and [Zn^{2+}] values that bring about large increases in Co uptake in our algal cultures provides evidence that Co depletion in the upper nutricline, like that of Zn, results from phytoplankton uptake. Accordingly, slopes of Co vs. PO_4 plots for the upper nutricline provide estimates of algal Co : PO_4 ratios. In near-surface waters of stations T-6, T-7, and T-8, Co : PO_4 slopes were 40, 36, and 38 µmol mol^{-1}, and Zn : PO_4 slopes were 250, 370, and 254 µmol mol^{-1} (Table 2). Based on these values, we estimate algal Co : Zn ratios of 1 : 6, 1 : 10, and 1 : 7, indicating only minor Co uptake relative to that of Zn. Co uptake is nonetheless important, especially for *E. huxleyi,* which has a specific Co require-

Table 2. Regression slopes of Zn and Co vs. PO_4 (µmol mol^{-1}) for the upper nutricline at stations T-5, T-6, and T-8 in the subarctic Pacific (data from Martin et al. 1989).

Sta.	Depth (m)	Zn (nM)	ΔZn/ΔP	R^2*	Co (pM)	ΔCo/ΔP	R^2*
T-5	50–150	0.06–0.22	251	0.986	7.9–32	39.8	0.981
T-6	50–150	0.14–0.26	370	0.923	28–40	35.5	0.994
T-8	8–50	0.06–0.32	254	0.697	25–55	38.4	0.977

* $n = 3$ in all cases.

ment for maximum growth rate and is the dominant coccolithophore in the subarctic Pacific (Okada and Honjo 1973).

Effects on algal growth rate and relative species abundances—Differences in the availability of Co and Zn could be an important factor regulating the growth and distribution of diatoms, coccolithophores, and cyanobacteria in the ocean where Zn : Co ratios can increase by 100-fold between nutrient-depleted surface waters and nutrient-rich intermediate waters (Martin et al. 1989)—the source waters for oceanic upwelling. High $[Co^{2+}]:[Zn^{2+}]$ ratios should favor the growth of *E. huxleyi,* and indeed, oceanic Co : Zn ratios (~0.4 in the North Pacific; Martin et al. 1989) are highest in mesotrophic waters where *E. huxleyi* often blooms and in oligotrophic waters where coccolithophores typically predominate over diatoms (Dymond and Lyle 1985; Robertson et al. 1994).

Elevated $[Zn^{2+}]$ favors the growth of diatoms, which dominate algal communities in nutrient-rich upwelling waters (Dymond and Lyle 1985) where both Zn concentrations and Zn : Co ratios are high (Martin et al. 1989, 1993). On the other hand, the high $[Zn^{2+}]$ and $[Zn^{2+}]:[Co^{2+}]$ ratios in nutrient-rich waters could inhibit the growth of *E. huxleyi.* For example, at station T-8 in the subarctic Pacific where active upwelling occurs, the Zn and Co concentrations were 7.2 and 0.052 nM at 150 m (below the euphotic zone) and decreased to 0.06 and 0.025 nM at 8 m (near the productivity maximum). The deeper sample had a Zn : Co molar ratio of 140, whereas the 8-m water had a ratio of 2.4. On the basis of Bruland's (1989) Zn complexation data, the estimated $[Zn^{2+}]$ in the deeper sample is $10^{-8.4}$ M—a value more than sufficient to support the maximum growth rate of the two diatoms but which could inhibit growth of *E. huxleyi* (Fig. 2A). In water from 8-m depth, the estimated $[Zn^{2+}]$ would be $10^{-12.3}$ M—a value that decreased growth rates of *T. pseudonana* by ~75% and *T. oceanica* by 10–20% in the absence of added Co (Fig. 1C) and at which Co-Zn limitation of *E. huxleyi* (rather than Zn toxicity) might come into play. From Redfield modeling of Co, Zn, and PO_4 distributions in the upper 50 m at station T-8, we estimate Co : C and Zn : C ratios of 0.36 and 2.4 μmol mol^{-1}; at these values, *E. huxleyi* should be able to grow at a slightly Co-Zn-limited rate of 1.0 d^{-1} (based on data in Figs. 2, 3). These observations suggest that high $[Zn^{2+}]:[Co^{2+}]$ ratios may initially inhibit *E. huxleyi* growth in freshly upwelled water through an antagonism of Co uptake and nutrition. Growth rates should subsequently increase in the water, with declining $[Zn^{2+}]$ and Zn : Co ratios associated with biological scavenging of Zn.

The above discussion suggests that $[Zn^{2+}]$ and $[Co^{2+}]$ in near-surface waters of the subarctic Pacific (and other oceanic regions) are low enough to limit the growth of at least some fraction of the phytoplankton community. This hypothesis is supported by the results of a shipboard incubation experiment conducted with 20-m water from station T-7 in the subarctic Pacific on the same cruise during which Martin et al. (1989) collected data on Zn and Co distributions. In this experiment, the addition of 0.75 nM Zn increased productivity by 34% relative to controls after 6 d of incubation (Coale 1991).

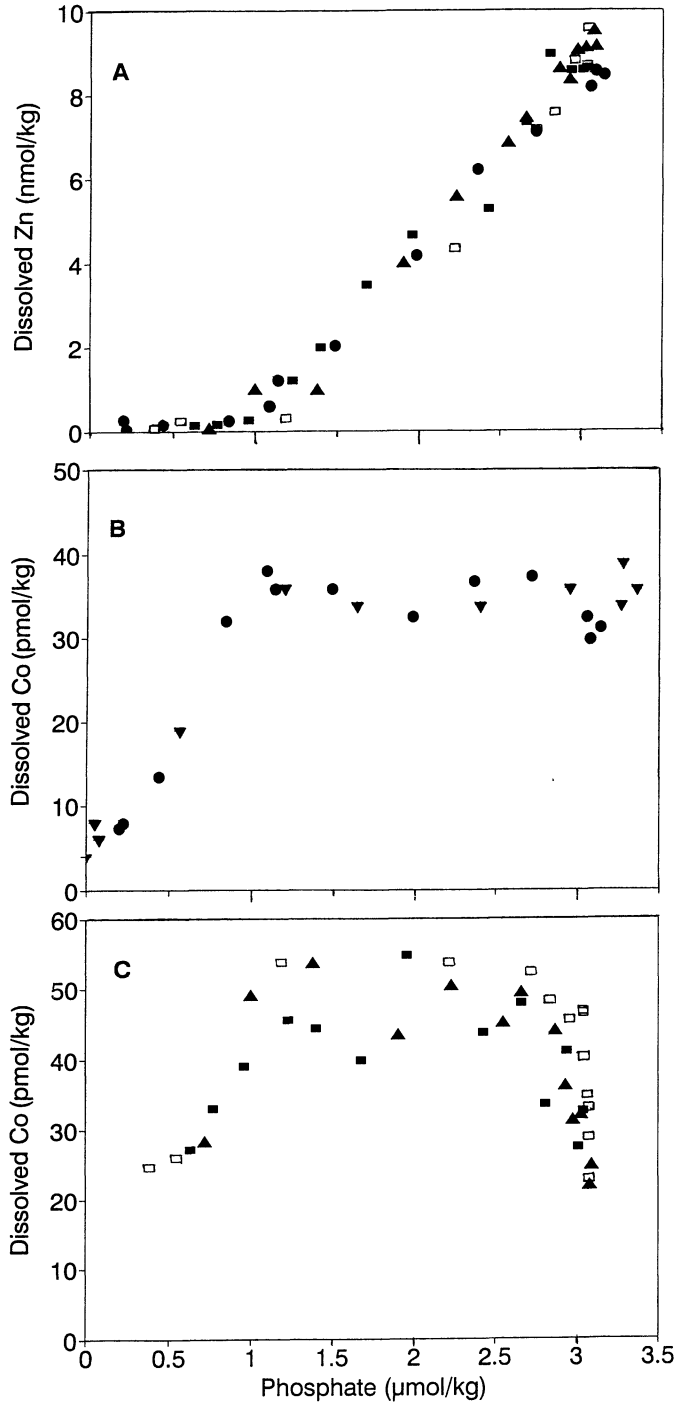


Fig. 8. Plots of dissolved Zn and Co vs. phosphate for data from the North Pacific: Station T-4 (▼; 33.3°N, 139.1°W), T-5 (●; 39.6°N, 140.8°W), T-6 (■; 45.0°N, 142.9°W), T-7 (▲; 50.0°N, 145.0°W), and T-8 (□; 55.5°N, 147.5°W). Co data for station T-4 are taken from Martin and Gordon (1988) (no Zn data were published for this station). Zn and Co data from the remaining four stations are taken from Martin et al. (1989).

It is generally recognized that diatoms photosyntheti-

cally fix carbon from dissolved CO_2, while coccolithophores, through calcification, effectively convert Ca^{2+} and HCO_3^- into reduced carbon (CH_2O) and $CaCO_3$ (Dymond and Lyle 1985; Nimer and Merrett 1992). As a result, growth of coccolithophores decreases seawater alkalinity and is accompanied by a much lower decrease in CO_2 concentrations than occurs with diatom growth, as has been documented for blooms of *E. huxleyi* (Robertson et al. 1994). Morel et al. (1994) have suggested that variations in the availability of Zn in seawater could influence relative growth of diatoms and coccolithophores and thereby affect surface concentrations and air–sea exchange of CO_2. This suggestion was based on the observed importance of the Zn enzyme, carbonic anhydrase, for CO_2 uptake and algal growth in the coastal diatom *T. weissflogii* and on the much lower need for this enzyme in coccolithophores. Our results support this hypothesis and suggest that Co concentrations and Co : Zn ratios could also be important. Zn and Co would, of course, act in concert with other potential controlling factors such as the availability of silicon (which is required only by diatoms), iron, and nitrogen. That zinc and cobalt might be important variables affecting air–sea exchange of CO_2 is an unexpected and intriguing hypothesis and one that warrants further investigation.

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Submitted: 17 January 1995
accepted: 12 July 1995
amended: 3 August 1995