

Not all eukaryotic algae can replace zinc with cobalt: *Chaetoceros calcitrans* (Bacillariophyceae) versus *Emiliana huxleyi* (Prymnesiophyceae)

Abstract—Zinc and cobalt are essential trace metals for phytoplankton growth and can substitute for each other metabolically in some eukaryotic algal species, especially in some marine diatoms and coccolithophorids. In the present study, it is reported that in the marine diatom *Chaetoceros calcitrans*, Co cannot replace Zn: in contrast, Co substitution of Zn was found in *Emiliana huxleyi*. It is concluded that Co can substitute for Zn but that the phenomenon is species/genera-specific. Differing ability to substitute Zn with Co can be regarded as an additional factor affecting phytoplankton biomass and/or species composition.

Phytoplankton and trace metals clearly have an interactive relationship: not only can the availability of trace elements influence biomass and succession of phytoplankton, but the phytoplankton can also change trace metal concentrations through selective uptake (Sunda 1989; Bruland et al. 1991). Extremely low concentrations of available trace elements can limit primary production in the world's oceans. The importance of iron in primary production is well documented (Martin and Fitzwater 1988; de Baar et al. 1990; Hutchins 1995). Similarly, zinc and cobalt are important for proper functioning of phytoplankton cells (Sunda and Huntsman 1992; Morel et al. 1994). It has been reported that some of the trace metals can substitute for each other metabolically. Limitation of one trace metal can thus, under certain conditions, partly be compensated for by the supply of a closely related trace metal. Cadmium and cobalt can substitute for zinc in some phytoplankton species (Price and Morel 1990; Sunda and Huntsman 1995). While in some eukaryotic species (e.g., *Emiliana huxleyi*), Co can only partially substitute for Zn, in others, Co can largely meet Zn requirements (e.g., *Thalassiosira pseudonana* and *Thalassiosira oceanica*) (Sunda and Huntsman 1995). The ability to (partially) replace trace metals could be an additional factor structuring phytoplankton biomass and species composition.

In the present paper, we describe and discuss Co substitution for Zn in the diatom *Chaetoceros calcitrans* (Bacillariophyceae) and in the coccolithophorid *E. huxleyi* (Prymnesiophyceae).

C. calcitrans (CCMP 1315) was chosen as a test species because members of the genus *Chaetoceros* are common spring- and summer-bloom species in temperate waters. *E. huxleyi* (PML92/011) was selected as reference species, for which Co substitution for Zn has been described (Sunda and Huntsman 1995). Unialgal cultures were grown under batch conditions at 15°C and a 16:8 h light:dark (LD) regime. The light intensity was between 100 and 130 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$. Trace metal clean conditions were used throughout. Aged natural seawater collected in the Gulf of Biscay was prepared for use as growth medium. Filter sterilization (0.07 μm nominal size cutoff) was followed by addition of phos-

phate ($6.5 \times 10^{-6} \text{ M}$), silicate (only *C. calcitrans*, $100 \times 10^{-6} \text{ M}$), and nitrate ($100 \times 10^{-6} \text{ M}$) under trace metal clean and sterile conditions. A mix of biotin ($0.16 \times 10^{-9} \text{ M}$), thiamine-HCl ($59 \times 10^{-9} \text{ M}$), and vitamin B₁₂ ($0.59 \times 10^{-9} \text{ M}$) was added. Finally, copper, molybdenum, and selenium were added, giving final concentrations of $40 \times 10^{-9} \text{ M}$, $1.43 \times 10^{-9} \text{ M}$, and $100 \times 10^{-9} \text{ M}$, respectively, and a trace element mixture (KBr: $92 \times 10^{-6} \text{ M}$, SrCl₂·6H₂O: $13 \times 10^{-6} \text{ M}$, AlCl₃: $0.1 \times 10^{-6} \text{ M}$, LiCl: $0.07 \times 10^{-6} \text{ M}$, KI: $0.06 \times 10^{-6} \text{ M}$, H₃BO₃: $3.23 \times 10^{-6} \text{ M}$, and RbCl: $0.25 \times 10^{-6} \text{ M}$, final concentrations). Routine measurements of background levels of Zn, Co, Fe, and Cd showed that the concentrations were $10.6 \pm 3.0 \times 10^{-9} \text{ M}$, $61 \pm 10 \times 10^{-12} \text{ M}$, $120 \pm 16 \times 10^{-12} \text{ M}$, and $8.0 \pm 0.5 \times 10^{-9} \text{ M}$, respectively. The experiments were done either with variable ethylenediamine-tetraacetic acid (EDTA) concentrations and fixed trace metal concentrations or with variable metal concentrations and a fixed EDTA concentration.

Algae were grown in a range of Zn concentrations. Where a change in the growth rates was observed, additions of Zn and/or Co in separate experiments were used to determine which limitation existed, based on whether addition could relieve growth rate limitation. Further, in the case of Co addition, it was determined whether or not substitution for Zn took place. Equimolar Co substitution of Zn was assumed; therefore, concentrations were adjusted so that Zn²⁺ and Co²⁺ were approximately equal. Specific growth rates were determined based on cell counts made using a Coulter Epics XL flow cytometer. The maximum growth rates (μ_{max}) and the half saturation value for growth (K_m) in relation to the metal concentrations were estimated by a SYSTAT nonlinear fitting using a least-square fit with the simplex algorithm (see Wilkinson et al. 1992).

Speciation calculations of Zn, Co, and Fe in the growth medium were made using MINEQL⁺ (Secher and McAvoy 1992). We assumed that all dissolved species were in equilibrium and that the alpha coefficients ($K' [\text{L}^x]$) governed the distribution over the chemical species. Given the relatively high Zn concentrations ($\times 10^{-9} \text{ M}$) in the medium, high concentrations ($\times 10^{-3} \text{ M}$) of EDTA had to be used to obtain low enough Zn concentrations to induce limitation. Consequently, relatively high concentrations ($\times 10^{-6} \text{ M}$ range) of Fe had to be added to prevent Fe limitation in the experiments. Additions of Fe were chosen so that Fe³⁺ concentrations were approximately constant and nonlimiting for growth. With the high concentration of EDTA, the natural Zn-complexing ligands (Donat and Bruland 1990; Bruland et al. 1991; Ellwood and van den Berg 2000) had no effect on the Zn²⁺ concentrations, whereas natural Co-binding ligands (Zhang et al. 1990) played only a minor role (and only in the case of no addition of Co). However, the natural

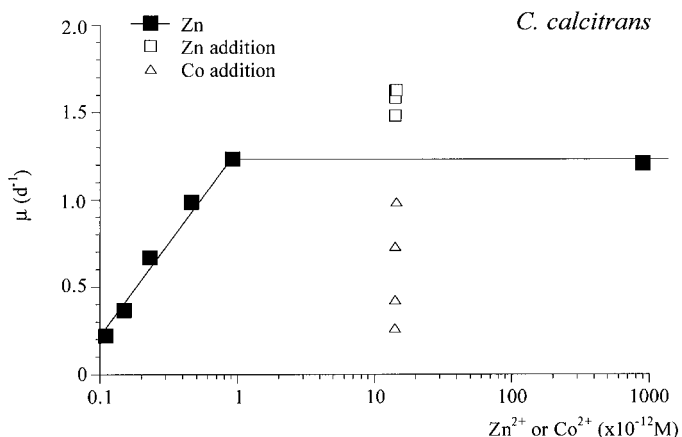


Fig. 1. Specific growth rates (d^{-1}) versus Zn^{2+} and effects of Zn (Zn^{2+} to 14×10^{-12} M) and Co additions (Co^{2+} to 14×10^{-12} M) in *C. calcitrans*. See Table 1 for metal speciation details.

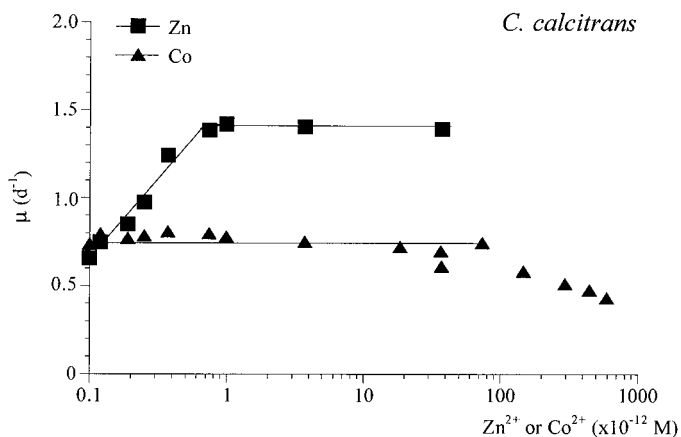


Fig. 2. Specific growth rates (d^{-1}) versus Zn^{2+} and Co^{2+} in *C. calcitrans*. EDTA concentrations were fixed ($1,100 \times 10^{-6}$ M). See Table 2 for metal speciation details.

Zn- and Co- (and Fe) binding ligands were always taken into account in the speciation calculations.

Zn limitation—The essential function of zinc for growth in the two marine phytoplankton species was clearly demonstrated. Irrespective of the experimental setup (fixed or variable Zn or EDTA concentrations), increasing Zn^{2+} concentrations caused increased growth rates in *C. calcitrans* (Figs. 1, 2; Tables 1, 2) to a maximum of 1.41 d^{-1} . A K_m of 0.19×10^{-12} M for Zn^{2+} was calculated. Similarly, with increasing Zn^{2+} concentrations, growth rates of *E. huxleyi* increased to a maximum growth rate of 0.68 d^{-1} (Fig. 3;

Table 3). A similar response to variations in increasing Zn^{2+} concentrations has been reported for many other marine phytoplankton species (Brand et al. 1983; Sunda and Huntsman 1995). Given the important role of Zn in many enzymes, this response is to be expected (Vallee and Galde 1984). The $K_m(\text{Zn}^{2+})$ values for growth as reported here for *C. calcitrans* are in good agreement with those reported by Sunda and Huntsman (1992, 1995) for *E. huxleyi* and *T. pseudonana*.

Relief of Zn limitation—Zn or Co addition: Restorations of the growth rates to values greater than the maximum

Table 1. Speciation calculations for experiments with *C. calcitrans*. In the MINEQL⁺ calculations, natural Zn-binding ligands were assumed to be present with a concentration of 10×10^{-9} M and with a log K' of 10.5 (Bruland 1989; Donat and Bruland 1990; Ellwood and van den Berg 2000). For natural Co-binding ligands, a concentration of 0.4×10^{-9} M and a log K' of 15 was used (Zhang et al. 1990). Background Fe_{diss} was 8.5×10^{-9} M; Zn_{diss} was 8.4×10^{-9} M; Co_{diss} was 0.06×10^{-9} M; pH in medium at start of experiment was 8.03. Zn or Co additions were done on day 3 to subsamples of the original series ($125\text{--}750 \times 10^{-6}$ M EDTA). All concentrations are given in M, with multiplication factor ($\times 10^{-6} = \mu\text{M}$, $\times 10^{-9} = \text{nM}$, $\times 10^{-12} = \text{pM}$, $\times 10^{-21}$).

EDTA ($\text{M} \times 10^{-6}$)	Fe added ($\text{M} \times 10^{-9}$)	Fe^{3+} ($\text{M} \times 10^{-21}$)	Zn added ($\text{M} \times 10^{-9}$)	Zn^{2+} ($\text{M} \times 10^{-12}$)	Co added ($\text{M} \times 10^{-9}$)	Co^{2+*}	$\text{Zn}^{2+} + \text{Co}^{2+}$ ($\text{M} \times 10^{-12}$)
Zn†							
125	125	1.32	0	0.92	0	0.30	0.92
250	250	1.28	0	0.46	0	0.27	0.46
500	500	1.25	0	0.23	0	0.26	0.23
750	750	1.25	0	0.15	0	0.24	0.15
1,000	1,000	1.24	0	0.11	0	0.32	0.11
Zn addition‡							
125	125	1.33	125	14.48	0	0.3	14.48
250	250	1.28	250	14.03	0	0.27	14.03
500	500	1.25	500	13.80	0	0.26	13.80
750	750	1.25	750	13.73	0	0.24	13.73
Co addition§							
125	125	1.3	0	0.92	62.5	13.55	14.47
250	250	1.2	0	0.46	125	13.55	14.01
500	500	1.2	0	0.23	250	13.54	13.77
750	750	1.2	0	0.15	375	13.54	13.69

* For Zn and Zn addition, Co^{2+} uses $\text{M} \times 10^{-15}$, whereas for Co addition, Co^{2+} uses $\text{M} \times 10^{-12}$.

† Variable EDTA; no Zn or Co added.

‡ Zn^{2+} increased to 14×10^{-12} M; no Co added.

§ Co^{2+} increased to 14×10^{-12} ; no Zn added.

Table 2. Speciation calculations for experiments with *C. calcitrans* (see Table 1 for conditions). Background Fe_{diss} was 7.6×10^{-9} M; Zn_{diss} was 9.4×10^{-9} M; Co_{diss} was 0.06×10^{-9} M; pH in the medium at the start of the experiment was 8.02. EDTA concentration was fixed at $1,100 \times 10^{-6}$ M. All concentrations are given in M, with multiplication factor ($\times 10^{-6} = \mu\text{M}$, $\times 10^{-9} = \text{nM}$, $\times 10^{-12} = \text{pM}$).

	Fe added ($\text{M} \times 10^{-9}$)	Fe^{3+} ($\text{M} \times 10^{-21}$)	Zn added ($\text{M} \times 10^{-9}$)	Zn^{2+} ($\text{M} \times 10^{-12}$)	Co added ($\text{M} \times 10^{-9}$)	Co^{2+*}	$\text{Zn}^{2+} + \text{Co}^{2+}$ ($\text{M} \times 10^{-12}$)
Zn							
1	1,100	1.2	0.0	0.12	0	2.5	0.12
2	1,100	1.2	2.5	0.15	0	2.5	0.15
3	1,100	1.2	5.0	0.18	0	2.5	0.18
4	1,100	1.2	10	0.24	0	2.5	0.24
5	1,100	1.2	20	0.36	0	2.5	0.36
6	1,100	1.2	45	0.67	0	2.5	0.67
7	1,100	1.2	75	1.04	0	2.5	1.04
8	1,100	1.2	300	3.83	0	2.5	3.83
9	1,100	1.2	3,000	37.29	0	2.5	37.29
Co (low concentrations)							
1	1,100	1.2	0	0.12	4	0.10	0.22
2	1,100	1.2	0	0.12	5	0.12	0.24
3	1,100	1.2	0	0.12	7.5	0.19	0.29
4	1,100	1.2	0	0.12	10	0.25	0.37
5	1,100	1.2	0	0.12	15	0.37	0.49
6	1,100	1.2	0	0.12	30	0.74	0.86
7	1,100	1.2	0	0.12	40	0.99	1.11
8	1,100	1.2	0	0.12	150	3.71	3.83
9	1,100	1.2	0	0.12	1,500	37.07	37.19
Co (high concentrations)							
1	1,100	1.1	0	0.12	750	18.42	18.54
2	1,100	1.1	0	0.12	1,500	36.77	36.89
3	1,100	1.1	0	0.12	3,000	73.52	73.64
4	1,100	1.1	0	0.12	6,000	147.13	147.25
5	1,100	1.1	0	0.12	12,000	294.92	295.04
6	1,100	1.1	0	0.12	18,000	443.74	443.86
7	1,100	1.1	0	0.12	24,000	593.25	593.37
Variation of Zn and Co ($\Sigma \text{Zn}^{2+} + \text{Co}^{2+} = 1.11 \times 10^{-12}$ M)							
1	1,100	1.2	0	0.12	40	0.99	1.11
2	1,100	1.2	8	0.22	36	0.89	1.11
3	1,100	1.2	16	0.32	32	0.79	1.11
4	1,100	1.2	30	0.49	25	0.62	1.11
5	1,100	1.2	50	0.74	15	0.37	1.11
6	1,100	1.2	70	0.99	5	0.12	1.11
7	1,100	1.2	80	1.11	0	0.0025	1.11

* For Zn, Co^{2+} uses $\text{M} \times 10^{-15}$, whereas for Co (low concentrations), Co (high concentrations), and variation of Zn and Co, Co^{2+} uses $\text{M} \times 10^{-12}$.

growth rate previously observed were obtained after addition of Zn to values of 14×10^{-12} M to *C. calcitrans* growing at low growth rates (Fig. 1). As in *Ditylum brightwellii* (Graneli and Haraldson 1993), *C. calcitrans* growth rates were not stimulated by increasing Co concentrations (to Co^{2+} values of 14×10^{-12} M) in the absence of added Zn (Figs. 1, 2). This absence of an effect was not simply due to Co^{2+} concentrations being too low. In a range up to 590×10^{-12} M Co^{2+} (Table 2), no effects or slightly negative effects on growth rates were observed above 100×10^{-12} M Co^{2+} (Fig. 2). This response to the addition of Co is indicative of a toxic response at higher concentrations, similar to the slight reduction in biomass and growth rates of *D. brightwellii* (Graneli and Haraldson 1993). The negative effects on growth, however, make it clear that the Co is taken up by *C. calcitrans*. Obviously, uptake of Co is not followed by substitution and hence restoration of the activity of Zn-lim-

ited key enzymes. The absence of Co substitution for Zn in *C. calcitrans* was further demonstrated in an experiment in which Zn^{2+} and Co^{2+} concentrations were matched to 1.11×10^{-12} M (Fig. 4), with conditions ranging from high Zn^{2+} and low Co^{2+} concentrations to low Zn^{2+} and high Co^{2+} concentrations. Again, Zn^{2+} concentrations determined the growth response of *C. calcitrans*. High Zn^{2+} and low Co^{2+} concentrations resulted in normal (high) growth rates; low Zn^{2+} and high Co^{2+} concentrations resulted in low growth rates. The response as observed in *C. calcitrans* does fit the idea that some species can and some species can not fully (or partially) substitute Co for Zn. In *T. weissflogii*, Co addition to Zn-limited cultures resulted in a 60% increase in growth rate (Price and Morel 1990). Similarly, Sunda and Huntsman (1995) showed that in the diatom *T. pseudonana*, Co did substitute Zn, although increasing Co concentrations could not fully meet the Zn requirements. For the diatom *T.*

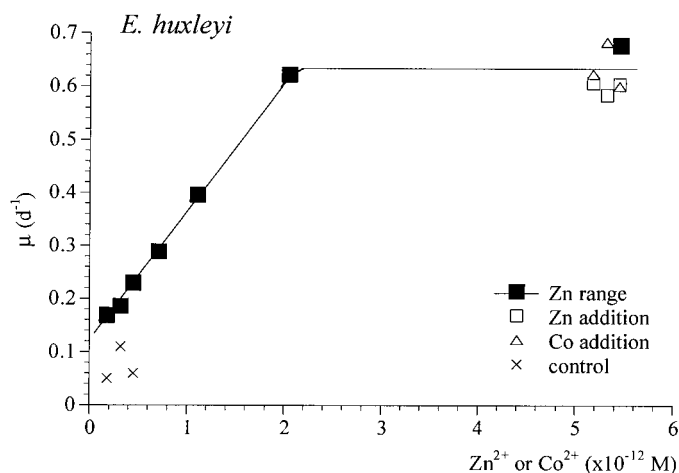


Fig. 3. Specific growth rates (d^{-1}) versus Zn^{2+} in *E. huxleyi*. Also indicated: effects of addition of 0.4×10^{-6} M Zn (5×10^{-12} M Zn^{2+}) and 0.2×10^{-6} M Co (5×10^{-12} M Co^{2+}). Crosses show growth in control incubation (neither Zn nor Co addition). See Table 3 for metal speciation details.

oceanica, Co addition under Zn limitation only resulted in a weak increase of the growth rates (Sunda and Huntsman 1995).

In contrast, under Zn^{2+} -limiting conditions, separate addition of Zn or Co resulted in increased growth rates of *E. huxleyi* comparable to those under nonlimiting conditions (Fig. 3). Obviously, *E. huxleyi* is capable of substitution of Zn by Co, resulting in alleviation of limitation. These findings corroborate the response of *E. huxleyi* as reported by Sunda and Huntsman (1995). Similarly, Graneli and Haraldson (1993) reported stimulation of biomass and growth rates in another prymnesiophyte *Chrysochromulina polylepis* upon the addition of Co. The experimental data of *E. huxleyi* also give insight into the availability of Co from vitamin B₁₂ (cyanocobalamin). A clear limitation of growth rates was observed at low Zn concentrations, in spite of the presence of 0.59×10^{-9} M vitamin B₁₂. Obviously, cobalt was not

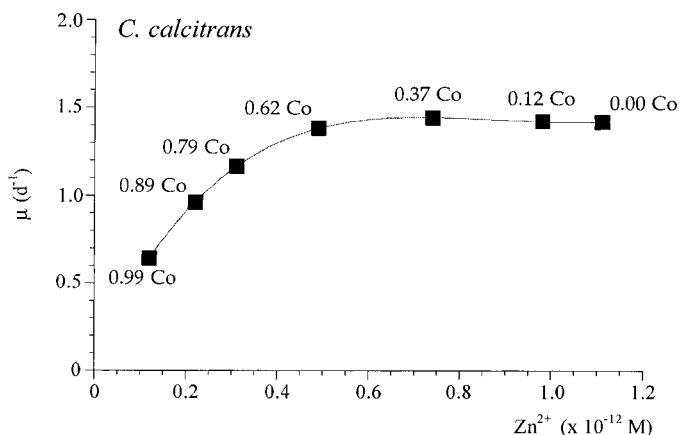


Fig. 4. Specific growth rates (d^{-1}) versus Zn^{2+} and Co^{2+} in *C. calcitrans*. Zn^{2+} concentrations are given on the x-axis and Co^{2+} concentrations ($\times 10^{-12}$ M) in the plot. The Zn and Co concentrations were chosen such that the sum of Zn^{2+} and Co^{2+} was 1.11×10^{-12} M. See Table 2 for metal speciation details.

available from the vitamin B₁₂, or the available concentrations were too low to sustain the growth rates observed under nonlimiting conditions (see Swift and Taylor 1974).

Phytoplankton species differ in their ability to substitute Zn with Co (Price and Morel 1990; Sunda and Huntsman 1995), and at least one species is unable to replace Zn with Co (this study). Metabolic substitution of Zn by Co appears not to be a general phenomenon, but it is dependent on the species considered. Different responses of species/genera will affect their competitive abilities within the algal community, leading to spatial and temporal differences in species-specific primary production. Diatoms are deemed to be superior competitors for resources, as long as the silicate concentrations are $>2 \mu M$ (Egge and Aksnes 1992). Under these conditions, they are key players in the oceanic carbon cycle (Smetacek 1999). This superiority is not, however, without limits. In addition to the need for silicate, the competitive capabilities of diatoms may be limited by an inabil-

Table 3. Speciation calculations for experiments with *E. huxleyi* (see also Table 1 for conditions). Background Fe_{diss} was 8.1×10^{-9} M, Zn_{diss} was 14.0×10^{-9} M, Co_{diss} was 0.06×10^{-9} M, and pH in the medium at the start of the experiment was 8.14. EDTA concentration was fixed at $1,000 \times 10^{-6}$ M. All concentrations are given in M, with multiplication factor ($\times 10^{-6} = \mu M$, $\times 10^{-9} = nM$, $\times 10^{-12} = pM$).

	Fe added M $\times 10^{-9}$	Fe ³⁺ M $\times 10^{-21}$	Zn added M $\times 10^{-9}$	Zn ²⁺ M $\times 10^{-12}$	Co added M $\times 10^{-9}$	Co ²⁺ * M $\times 10^{-12}$	Zn ²⁺ +Co ²⁺ M $\times 10^{-12}$
Zn							
1	1,000	1.2	0	0.18	0	2.5	0.18
2	1,000	1.2	10	0.32	0	2.5	0.32
3	1,000	1.2	20	0.45	0	2.5	0.45
4	1,000	1.2	40	0.71	0	2.5	0.71
5	1,000	1.2	70	1.11	0	2.5	1.11
6	1,000	1.2	400	5.46	0	2.5	5.46
Zn or Co addition†							
Control	1,000	1.2	0	0.18	0	0.0025	0.18
Zn	1,000	1.2	380–400	5.46	0	0.0015	5.46
Co	1,000	1.2	0	0.18	200	5.27	5.45

* For Zn, Co^{2+} uses 10×10^{-15} , whereas for Zn or Co addition, Co^{2+} uses $M \times 10^{-12}$.

† Additions of Zn (final concentration 400×10^{-9} M) or Co (final concentration 200×10^{-9} M) to subsamples taken on day 8 from the original cultures (0 to 20×10^{-9} M Zn added).

ity to substitute Co for Zn. This may be another factor that prevents diatoms from becoming dominant in all regions of the world's oceans.

The concentrations of Zn and Co in near-surface waters are low enough to limit phytoplankton growth (Martin and Fitzwater 1988; Bruland 1989). It can therefore be hypothesized that changes in the absolute concentrations of Zn and Co as well as the Zn:Co ratio in the surface oceans will have an effect on phytoplankton productivity (Sunda and Huntsman 1992, 1995; this study) and species composition (Morel et al. 1994; Sunda and Huntsman 1995; this study). Extending the theoretical concepts of resource competition (Tilman et al. 1982), it can be deduced that not only is the ability to reduce the availability of a limiting factor a competitive advantage, but also the ability to substitute another compound for a limiting resource. The ability for trace metal substitution can, in addition to, for example, N:P or N:Si ratios (Riegman 1995), availability of trace elements (Brand et al. 1983; Bruland et al. 1991; Graneli and Haraldson 1993), nutrient pulses (Riegman et al. 1992), and silicate availability (Egge and Aksnes 1992), cause spatial and temporal differences in phytoplankton biomass and species composition.

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