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NOTE

Replacement of zinc by cadmium in marine phytoplankton

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ABSTRACT: The concentration of cadmium varies like that of a nutrient in the open ocean. Detailed studies of the marine diatom *Thalassiosira weissflogii* have shown that cadmium can act as an algal nutrient under conditions of zinc limitation. We show here that cadmium can also enhance the growth of a variety of species, including a chlorophyte and some prymnesiophytes, when they are zinc limited. The replacement of zinc by cadmium occurs at environmentally relevant inorganic cadmium and zinc concentrations. Very low concentrations of inorganic cadmium that are beneficial under conditions of moderate zinc-limitation become toxic in cultures severely limited by zinc. The role of cadmium as an algal nutrient is thus observable in a narrow, species-specific range of inorganic zinc and cadmium concentrations.

KEY WORDS: Cadmium/zinc replacement · Trace metal limitation · Trace metal toxicity · Micronutrients · Phytoplankton

The distribution of Cd within the ocean strongly suggests that it is used as a nutrient by marine phytoplankton. Like the major nutrients nitrate and phosphate, Cd is depleted from surface waters, increases with depth, and reaches a fairly constant concentration in deep waters (Boyle et al. 1976, Bruland & Franks 1983). This distribution is *prima facie* evidence of biological control over the distribution of Cd in the marine environment due both to remineralization (Lee & Fisher 1993) and scavenging in surface waters.

Until recently, however, Cd had no known role as a nutrient and rather was considered one of the most toxic trace metals. A few years ago, Price & Morel (1990) demonstrated that under conditions of Zn limitation, Cd restored the growth rate of the marine diatom *Thalassiosira weissflogii* to near maximal levels. In further work we have shown (Lee et al. 1995) that Cd enhances the growth of Zn-limited cultures of *T. weissflogii* at inorganic Cd (Cd') and Zn (Zn') levels which are close to those that have been measured in the surface water of the open ocean (~10 pM Zn', ~1 pM Cd'; Bruland 1989, 1992) although Cd cannot completely replace Zn. Over a wide range of external Cd' and Zn' concentrations, Cd uptake kinetics are regulated and the intracellular Cd quotas are maintained at relatively constant levels. The same low level of inorganic Cd that enhances the growth rate of Zn-limited cells restores the activity of carbonic anhydrase (Lee et al. 1995), which is thought to be the key enzyme limiting growth of *T. weissflogii* at low Zn' (Morel et al. 1994). Cadmium also coelutes with some of the isoforms of carbonic anhydrase detected by a post-electrophoresis enzyme assay indicating that Cd substitution in carbonic anhydrase is likely partly responsible for the nutritional role of Cd.

If the nutrient role of Cd demonstrated for *Thalassiosira weissflogii* is the explanation for the nutrient-like distribution in the oceans, many other phytoplankton should be able to replace Zn with Cd. We have thus extended our detailed work with *T. weissflogii* to determine whether Cd can replace Zn in other species of marine phytoplankton: a smaller coastal diatom and an oceanic diatom, 2 coastal and 1 oceanic prymnesiophyte, 2 chlorophytes, and a dinoflagellate.

Methods. Cultures of the 9 species of marine phytoplankton listed in Table 1 (obtained from the Center for the Culture of Marine Phytoplankton, Bigelow Laboratory, Maine, USA) were grown in synthetic ocean water prepared according to the recipe for Aquil (Price et al. 1988/89) with the following modifications: inorganic Co was omitted from the recipe since Co, like Cd, can substitute for Zn (Price & Morel 1990); Zn varied (inorganic Zn, Zn', was reduced to 3.2 and 0.2 pM instead of the normal Aquil level of 16 pM); and Cd was added at a level of 4.6 pM inorganic Cd (Cd').

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Table 1 List of species of marine phytoplankton in this study. Division, clone designation, place of origin of the sample from which each species was isolated, size, and optimum growth rates in doublings d^{-1} are provided

Class	Species	CCMP number	Clone	Cell vol [1] (μm^3)	Place of origin[2]	Max. growth rate (doub. d^{-1})
Bacillariophyceae	*Thalassiosira pseudonana*	1015	3H(UW)	71	Long Island, NY, USA	2.6[d]
	Thalassiosira oceanica	1005	13-1	75	Sargasso Sea	1.8[b]
	Thalassiosira weissflogii	1336	Actin	800	Long Island, NY, USA	2.6[c]
Prymnesiophyceae	*Emiliana huxleyi*	373	BT6	50	Sargasso Sea	1.0[b]
	Pavlova lutheri	1325	Mono	75	Finland	2.2[c]
	Pleurochrysis carterae	645	CoccoII	630	Woods Hole, MA, USA	2.7[c]
Chlorophyceae	*Dunaliella tertiolecta*	1320	Dun	350	Coastal (origin unknown)	1.9[c]
	Tetraselmis maculata	897	TTM	425	Departure Bay, WA, USA	1.6[c]
Dinophyceae	*Heterocapsa pygmaea*	1322		600	Galveston, TX, USA	0.8[c]

[1]Cell volumes are from Ahner et al. (1995) except for *T. pseudonana*, which was measured by J. R. Reinfelder (pers. comm.)

[2]Places of origin were obtained from the Provasoli-Guillard Center for the Cultures of Marine Phytoplankton, Bigelow Laboratory, ME, USA, except for that of *T. maculata*, which was given by G. Wickfors (pers. comm.)

[d]Sunda & Huntsman (1992); [b]Maximum growth rates from this study; [c]Ahner et al. (1995)

Inorganic trace metal concentrations, M', were calculated from total concentrations using the computer program MINEQL (Westall et al. 1976). Details of media preparation are described in Lee et al. (1995). Maximal growth rates under Zn- and Co-sufficient conditions are also given in Table 1

This metal-defined medium was inoculated from stock cultures maintained in autoclaved, metal-sufficient Aquil medium. Cultures were acclimated in 1 transfer of metal-defined medium with the desired Cd' and Zn' levels prior to all experiments. Cultures were grown in 30 ml, acid-washed, polycarbonate tubes. Cultures were incubated at 21°C under a constant photon flux density of 90 to 100 $\mu E\ m^{-2}\ s^{-1}$. Growth was monitored by *in vivo* fluorescence and growth rates were determined during exponential phase growth as per the method of Brand et al. (1981). Replicates were performed by subsequent transfer into fresh medium if Cd increased growth.

Results. We were able to lower Zn' enough to limit growth for almost all of the species of algae we studied. The growth rates of *Thalassiosira pseudonana*, *T. weissflogii*, *Pavlova lutheri*, *Pleurochrysis carterae*, *Tetraselmis maculata* and *Heterocapsa pygmaea* were less than maximal at a Zn' level of 3.2 pM (Fig. 1A). *Emiliana huxleyi*, although not limited at 3.2 pM Zn', was limited by Zn at 0.16 pM Zn' (Fig. 1B). *Thalassiosira oceanica* and *Dunaliella tertiolecta*, however, were able to grow at near-maximal rates (99 and 82% respectively of maximum) even at 0.16 pM Zn'.

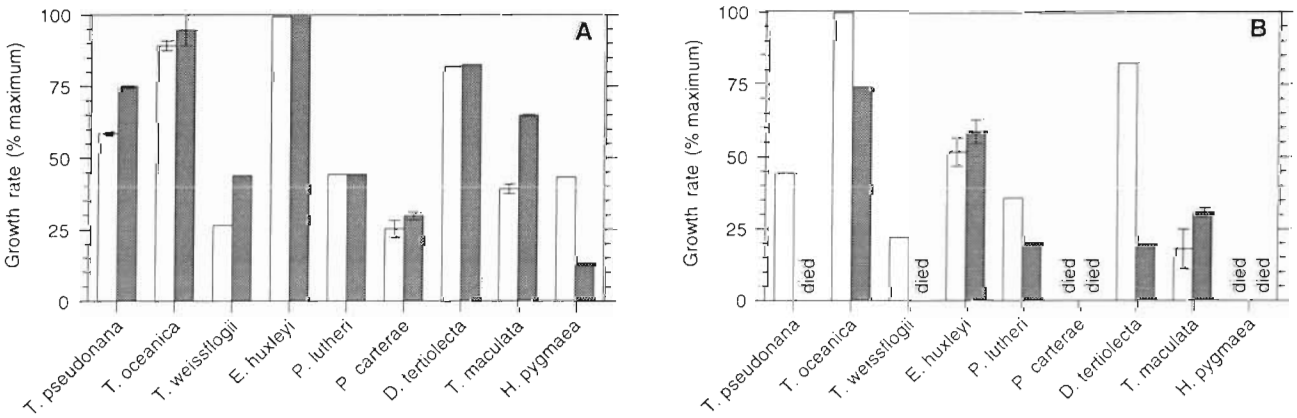

Fig. 1 Growth rates of different species of marine phytoplankton at Zn' concentrations that are (A) mildly limiting(3.2 pM Zn') and (B) severely limiting (0.16 pM Zn') to *Thalassiosira weissflogii*. Filled bars represent cultures supplemented with 4.6 pM Cd' and open bars represent no added Cd. Error bars span the range of duplicates. Cultures were preconditioned at a given Cd' and Zn' for 1 transfer. Growth rates were determined by monitoring *in vivo* fluorescence during exponential phase growth in subsequent transfers and normalized to maximum rates (see Table 1)

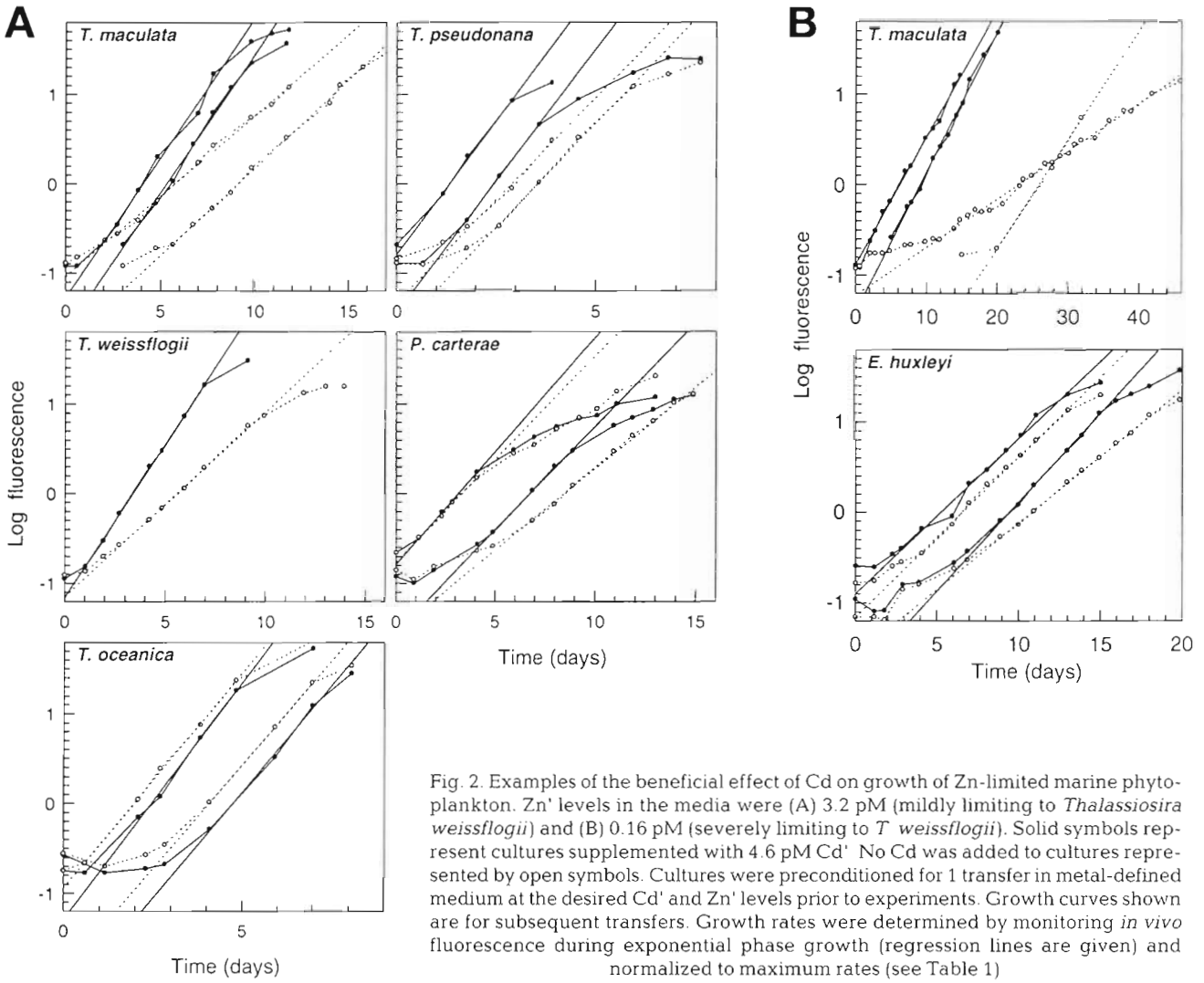

Fig. 2. Examples of the beneficial effect of Cd on growth of Zn-limited marine phytoplankton. Zn' levels in the media were (A) 3.2 pM (mildly limiting to *Thalassiosira weissflogii*) and (B) 0.16 pM (severely limiting to *T weissflogii*). Solid symbols represent cultures supplemented with 4.6 pM Cd' No Cd was added to cultures represented by open symbols. Cultures were preconditioned for 1 transfer in metal-defined medium at the desired Cd' and Zn' levels prior to experiments. Growth curves shown are for subsequent transfers. Growth rates were determined by monitoring *in vivo* fluorescence during exponential phase growth (regression lines are given) and normalized to maximum rates (see Table 1)

At 3.2 pM Zn', Cd acted as a nutrient for half of the species that were Zn limited (Figs. 1A & 2A). Adding Cd had a significant beneficial effect on growth at the 95% confidence level for *Thalassiosira weissflogii*, *Pleurochrysis carterae* and *Tetraselmis maculata* (for at least one of the replicates). Cadmium may also have had a beneficial effect on the growth of *T. pseudonana* and *T. oceanica*, but this effect is not significant at the 95% confidence level for either replicate (and *T. oceanica* was not Zn limited at 3.2 pM Zn'). Therefore a total of 3 out of the 6 species that were Zn limited at 3.2 pM Zn' were able to grow faster when supplied with Cd' at very low concentrations (Cd' = 4.6 pM). The growth curves for these species and *T. oceanica* with and without Cd are compared in Fig. 2A. At lower Zn' concentrations of only 0.16 pM, Cd benefited a smaller fraction of the species limited by Zn: only *T. maculata* and *Emiliana huxleyi* grew significantly faster at the 95% confidence level when supplemented with Cd (2 out of 7 Zn-limited species, Fig. 2B). Reproducibility of growth rates of replicates was generally good (<10% difference) except for *P. carterae* at 3.2 pM Zn' without Cd and *T. maculata* at 0.2 pM Zn' without Cd (12% difference for *P. carterae* and 39% for *T. maculata*), most likely due to Zn contamination of the second replicate during transfer.

At low concentrations of Zn' (0.16 pM), Cd was toxic to at least 5 out of the 9 species studied: *Thalassiosira oceanica*, *Dunaliella tertiolecta*, *T. pseudonana*, *T. weissflogii*, and *Pavlova lutheri* (Fig. 1B). The effects of Zn limitation and Cd toxicity could not be distinguished for *Pleurochrysis carterae* since cultures were not able to grow at all even in the absence of Cd at 0.16 pM Zn'. The same was true for *Heterocapsa pygmaea*. At the higher Zn' concentration of 3.2 pM, Cd toxicity was only observed for 1 species, *H. pygmaea*.

Discussion. Our previous work on the replacement of Cd by Zn in *Thalassiosira weissflogii* has shown that

limitation by Zn is a necessary condition for Cd to enhance the growth rate of cultures (Lee et al. 1995). However, from our present study there does not appear to be a straightforward pattern for predicting which species are the most prone to Zn limitation. Small size does not appear to make Zn limitation less likely: *T. pseudonana*, with a cell volume of only 71 μm^3, and *Pavlova lutheri*, with a cell volume of 75 μm^3 (see Table 1), were easily limited at 3.2 pM Zn'. Conversely, the large alga *Dunaliella tertiolecta* (350 μm^3) was able to grow as fast at 0.16 pM as at 3.2 pM Zn'. Physiological differences which allow variations in the cellular Zn requirement must overcome physical limitations imposed by diffusion which would otherwise predict that large cells are more easily nutrient limited than small ones (Hudson & Morel 1993).

Neither do oceanic algal species always appear to be less subject to Zn limitation than coastal species. The oceanic species *Emiliana huxleyi* was Zn limited at 0.16 pM Zn' while the coastal species *Dunaliella tertiolecta* grew at maximum rates. Previous work has also shown that although coastal species are usually more easily limited by low Zn' than oceanic species, that is not always the case (Brand et al. 1983). Cobalt limitation appears to play a synergistic role in Zn limitation for the oceanic coccolithophorid *E. huxleyi* since the same Zn' levels found to be limiting in this study were not limiting when Co was provided (Sunda & Huntsman 1992).

For all species studied, the effect of Cd on growth rate, positive or negative, is highly dependent on the Zn' concentrations in the medium (as well of course as the Cd' concentration; Lee et al. 1995). For each species we can thus expect that the beneficial role of Cd will only be observed over a narrow range of Cd' and Zn' concentrations. In view of this difficulty, it is remarkable that we obtained positive results with so many species. The same low levels of Zn' and Cd' which resulted in a beneficial effect of Cd on *Thalassiosira weissflogii* also lead to an enhancement of growth by Cd in several of the other species surveyed. This coincidence, which cuts across algal phyla, may be related to the fact that the Cd' and Zn' levels at which Cd replaces Zn in *T. weissflogii* are close to those which have been measured in the marine environment (Bruland 1989, 1992), although in an oligotrophic rather than coastal regime. It is of course entirely possible that other combinations of Cd' and Zn' concentrations than those used here might allow Cd/Zn replacement in the species which showed negative results.

As is the case for Cd/Zn replacement, the dependence of Cd toxicity on the inorganic Zn concentration in the medium gives a clue as to its mechanism. Either competitive inhibition of Zn and Cd uptake or biochemically ineffective replacement of Zn by Cd in proteins is the likely cause. In *Thalassiosira weissflogii*, competitive inhibition of Cd uptake by Zn does not appear important at the Zn' and Cd' levels studied here (Lee et al. 1995). More likely, then, Cd toxicity is the result of the inactivation of Zn enzymes by Cd/Zn substitution under conditions of severe Zn limitation.

Algae and higher plants produce a metal-binding polypeptide, phytochelatin, in response to exposure to Cd and other trace metals (Grill et al. 1985, Gekeler et al. 1988). This response to Cd under controlled trace metal conditions has been studied in detail by Ahner et al. (1995) for all but one of the species examined here (*Thalassiosira pseudonana*). Exposure to Cd causes a much stronger response by *T. weissflogii* in the cellular production of phytochelatin than any other trace metal (Ahner & Morel 1995). We would therefore expect that phytochelatin production would play an important role in the ability of Cd to replace Zn. It is thus of interest to note that 2 of the species that have the greatest ability to increase the levels of phytochelatin g^{-1} chl *a* as Cd' increases, *T. weissflogii* and *Tetraselmis maculata*, are also the species that show the clearest evidence of Cd/Zn replacement. The least response of phytochelatin g^{-1} chl *a* to Cd' is that of *Heterocapsa pygmaea* and this species is the most sensitive to Cd toxicity of those surveyed. The metal buffering capacity provided by phytochelatin may therefore be important for Cd/Zn replacement, but there are some species differences in this response.

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