Vol. 138: 245–254, 1996 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published July 25

Cadmium toxicity and zinc limitation in centric diatoms of the genus *Thalassiosira*

Philippe D. Tortell*, Neil M. Price

Department of Biology, McGill University, 1205 Ave. Dr. Penfield, Montréal, Québec, Canada H3A 1B1

ABSTRACT: Cadmium toxicity and Zn limitation were examined in centric diatoms to test the hypothesis that resistance to high concentrations of toxic metals was related to the assimilation of essential ones. Nine species of the genus *Thalassiosira* were cultured in artificial seawater media under Zn deficiency and in trace-metal-replete media with Cd additions ranging from 10 nM to 10 µM. Cadmium sensitivity, measured as $[Cd^{2+}]$ required to inhibit growth by 50% (pCd^{50}), varied significantly among species ($p < 0.001$). Clones isolated from offshore oligotrophic environments were significantly more resistant to Cd toxicity and Zn deficiency than those indigenous to coastal regions ($p < 0.01$). Differences in Cd resistance could not be explained by cellular exclusion since all species accumulated remarkably similar amounts of Cd (Cd quota ~ 30 µmol Cd:mol C) at the highest concentrations tested. pCd^{50}s were positively correlated to QCd^{50}s (Cd quotas required to inhibit growth by 50%). Tolerance of the diatoms to intra- and extracellular Cd was positively correlated to their growth rates under Zn-limiting conditions so that species most resistant to Cd toxicity were those least affected by Zn limitation. Cadmium concentrations that were not toxic under trace-metal-saturating conditions inhibited growth rate of Zn-limited *T. weissflogii* cultures by approximately 70%, but had no effect on Fe-limited cultures. The results thus suggest that Cd toxicity is mediated in part through the impairment of Zn assimilation in diatoms.

KEY WORDS: Cadmium · Zinc · Deficiency · Toxicity · Phytoplankton · Trace metals · Diatoms

INTRODUCTION

Total dissolved concentrations of trace metals decrease by 3 to 4 orders of magnitude between coastal and oceanic waters, from micro- to nano- and picomolar levels. This chemical gradient exists for both essential and non-essential metals and has apparently imposed significant constraints on the biota. In some offshore habitats, for example, concentrations of Fe are so low that they limit phytoplankton productivity and favour growth of small-sized autotrophs (Price et al. 1991, 1994, Martin et al. 1994). Unbalanced supply of metals relative to cellular demands in upwelling environments can also contribute to metal deficiency through competitive interactions (Sunda et al. 1981). Phytoplankton have undoubtedly adapted to counteract these stresses and to minimize their impact by processes that have been shaped by natural selection during the course of their evolution.

One of the most discriminating features of oceanic and coastal phytoplankton concerns their ability to grow under metal-deficient conditions. All species that have been isolated from offshore waters have considerably lower trace metal requirements than closely related neritic species (Ryther & Kramer 1961, Brand et al. 1983, Sunda et al. 1991, M.T. Maldonado & N. M. Price unpubl.). As a result, oceanic isolates can maintain near maximal rates of growth in metal-poor medium while coastal isolates are barely able to reproduce. These dramatically different responses persist even after decades of growth of the isolates in highly metal-enriched media. Low metal concentrations thus appear to have influenced the evolution of phytoplankton in the open ocean and are presently affecting their ecology.

The impact of high concentrations of metals on the coastal phytoplankton community has been more diffi-

*Present address: Department of Ecology and Evolutionary Biology, Princeton University, New Jersey 08544-1003, USA. E-mail: ptortell@phoenix.princeton.edu

cult to detect. Production of metal-detoxifying phytochelatins by nearshore assemblages implies that phytoplankton are experiencing some sort of metal stress (Ahner et al. 1994). Metal-tolerant strains of phytoplankton have also been isolated from habitats impacted by point source pollution (Jensen et al. 1974), but these are exceptional cases. Pervasive low-level enrichment of potentially toxic metals more commonly occurs from rivers and sediments, contributing to higher concentrations inshore (Martin & Whitfield 1983). Brand et al. (1986) recognized the existence of this oceanic gradient, and hypothesized that coastal species would have developed greater resistance to toxic metals (because they have been constantly exposed to them) than oceanic species living in comparatively pristine environments. The results of their experiments, however, did not support the predicted habitat-related pattern in Cd sensitivity of phytoplankton.

Given our present understanding of metal interactions in phytoplankton (Sunda 1988/89, Price & Morel 1994), we have reexamined the issue of habitat-related patterns of toxicity resistance. Cadmium, one of the most toxic elements (Davies 1978), is highly concentrated in coastal waters relative to the open sea (Bruland 1980, 1992, Bruland & Franks 1983). It is known to substitute for Zn and promote growth of Zn-limited phytoplankton (Price & Morel 1990) and to interfere with Mn and Fe assimilation at very high concentrations (Hart et al. 1979, Foster & Morel 1982, Harrison & Morel 1983). If Cd exerts its toxic effects by interfering with the utilization of essential metals, then those species that are most tolerant of essential metal deficiency or that require very small amounts of these elements for growth may be more immune to its effects.

In the present study, we tested the hypothesis that oceanic phytoplankton with low metal requirements would be more resistant to toxic metals than coastal species by examining the relationship between Cd toxicity and Zn deficiency. Nine species of *Thalassiosira* from a variety of ocean habitats were grown in media containing a range of Cd ion concentrations, and intracellular Cd quotas and growth rates were measured. Experiments were also conducted to elucidate the importance of Cd and essential metal antagonisms in one of the test species.

MATERIAL AND METHODS

Study organisms. All algal clones, centric diatoms of the genus *Thalassiosira*, were obtained from the Center for Culture of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine, USA. Clones were isolated from a variety of marine habitats, including eutrophic estuarine waters, oligotrophic oceanic gyres, and coastal and offshore upwelling areas, and ranged in volume from 40 fl (1 fl = 10^{-15} l) (*T. pseudonana*, clone 3H) to 877 fl (*T. decipiens*, clone 983) (Table 1). Cultures were maintained at 20°C under continuous saturating light (150 µE m^{-2} s^{-1}). Although sterile techniques were used throughout the experiments, not all cultures were axenic. Coulter Counter measurements (see 'Experimental measurements'), however, revealed that bacterial biovolume was small relative to that of phytoplankton and that most bacteria were removed during filtration of samples prior to analysis.

Culture media. Basal culture medium, consisting of synthetic ocean water (SOW) and major nutrient solutions, was prepared, chelexed, and sterilized as described by Price et al. (1988/89). Sterile medium was transferred into acid-washed, autoclaved polycarbonate bottles to which filter-sterilized (0.2 µm Acrodisc filters) trace-metal-stock solutions and *f*/2 vitamins were subsequently added.

Table 1. *Thalassiosira* spp. Clonal designations, collection sites, and habitat classification. Cell volumes are those of control cultures grown in metal-replete medium lacking added Cd

Species	Habitat	Collection site	Cell volume (fl)
Thalassiosira oceanica clone 13-1	Oceanic	Sargasso Sea 33° 11' N, 65° 15' W	103
Thalassiosira oceanica clone 1003	Oceanic	Sargasso Sea 36° 11' N, 69° 35' W	102
Thalassiosira parthenaia clone Thal 9	Oceanic	Equatorial Pacific 0° 02' N, 139° 59' W	116
Thalassiosira pseudonana clone 1014	Oceanic	North Pacific Gyre 28° N, 155° W	52
Thalassiosira subtilis clone 50ait	Oceanic	Equatorial Pacific 0° 02' N, 139° 59' W	840
Thalassiosira decipiens clone 983	Coastal	Magdalena Bay, Baja California 25° N, 112° W	877
Thalassiosira pseudonana clone 3H	Coastal	Moriches Bay, Forge River, Long Island, NY 39° 10' N, 72° 45' W	40
Thalassiosira rotula clone 1016	Coastal	Peru Upwelling 10° S, 78° W	75
Thalassiosira weissflogii clone Actin	Coastal	Gardiner's Island, Long Island, NY 41° 05' N, 72° 06' W	660

Concentrations of trace metals in the media for Cd toxicity experiments were the same as those used by Brand et al. (1986) with 100 µM nitrilotriacetic acid (NTA) as a chelating agent, yielding the following free ion concentrations (–log free metal ion concentration = pMetal): pFe 19.29, pMn 7.53, pCu 12.25, pZn 9.04, pCo 10.05, pSe 10.57, pMo 7.00. Various amounts of $CdCl_2$ were added to achieve a range in final concentration from 10^{-8} to 10^{-5} M corresponding to pCd values of: 9.85, 9.37, 8.85, 8.37, 7.85, 7.37, 6.85. Calculation of free metal ion concentrations, performed using the chemical equilibrium program MINEQL (Westall et al. 1976), showed that the addition of Cd did not appreciably affect the speciation of other metals in the media. Because Cd is not as ubiquitous a contaminant as Fe, Zn, and Cu, a background level of <1 nM Cd (an order of magnitude less than our lowest experimental addition) was assumed in control (no added Cd) media. For Zn-deficient medium, concentrations of Fe, Mn, Cu, Co, Se, and Mo were those given by Price et al. (1988/89) using 100 µM EDTA, with Zn^{2+} concentration set at $10^{-12.5}$ M (pZn = 12.5, total [Zn] = 6.26 nM). Preparation and manipulation of all trace metal solutions were performed in a laminar flow hood using sterile and trace metal clean techniques (Price et al. 1988/89). All media were allowed to equilibrate chemically for at least 12 h before use.

Determination of growth rates. Cultures were maintained in acid-washed 28 ml polycarbonate tubes using the semi-continuous batch culture method described by Brand et al. (1981). Culture tubes were sterilized by microwaving (Keller et al. 1988). *In vivo* chlorophyll *a* fluorescence of the cultures was measured at least daily with a model 10-AU Turner Designs fluorometer, and growth rates were determined from the slopes of least-squares regressions of logarithmically transformed fluorescence data plotted against time. Acclimation was assumed to be complete when cells had completed a minimum of 10 doublings in the experimental medium and the coefficient of variation of growth rates in successive transfers was less than 10%. Relative growth rates were calculated by dividing the measured growth rates (μ) by the maximum growth rates (μ_{max}) determined in metal-replete control media, lacking added Cd. Species' relative growth rates in Zn-deficient medium (pZn 12.5) were used as a measure of their ability to tolerate Zn limitation.

Experimental measurements. Acclimated cultures were inoculated in triplicate, and growth of all replicates was monitored. Inoculations were made in a sterile, trace-metal clean room equipped with a positively pressurized, filtered (High Efficiency Particulate Air filter, 0.3 µm) air system. Elemental C was measured in mid-exponential phase cultures grown in the absence of added Cd, by collecting 25 ml onto pre-combusted Whatman GF/C filters (4 h at 425°C). Samples were analyzed with a Carlo Erba elemental analyzer. Cell volumes and densities were also determined in these cultures using a Coulter Counter (model Z_{256}) that was calibrated with 5 µm diameter latex beads. Volume-normalized carbon cell quotas (QC^{vol}) were calculated for each species from C $cell^{-1}$ (pmol $cell^{-1}$) and cell volume (fl $cell^{-1}$) measurements. These quotas were used to calculate C $cell^{-1}$ in the Cd-treated cells from cell volume measurements in mid-exponential phase cultures. Preliminary experiments showed that despite significant changes in cell volume under Cd stress, QC^{vol} in *Thalassiosira weissflogii* was unaffected (C. D. Payne & N. M. Price unpubl.). The same was assumed to be true for all species.

Intracellular Cd concentrations were measured by growing cells in media containing various amounts of carrier-free Cd^{109} (Amersham, UK, 11.7 mCi mol^{-1}). Carrier-free additions permitted high specific activities to be obtained with negligible changes in the total Cd concentration in test media. Cultures were inoculated at low densities (approximately 100 cells ml^{-1}) and allowed to complete at least 8 divisions before harvesting to ensure uniform labelling of cellular material. Cells of mid-exponential phase cultures were collected by filtering 25 ml onto 1 or 3 µm Poretics polycarbonate membrane filters. Before running dry, the cells were rinsed with 10 ml of 1 mM diethyltriaminepentaacetic acid (DTPA) dissolved in SOW to remove any Cd loosely adsorbed to the cell surface (Lee & Morel 1995). This solution was allowed to stand in the filtration apparatus for 10 min before being drained to near dryness and then rinsed with 10 ml of SOW. Samples were preserved with Lugol's solution for subsequent cell counts by microscopy in a Palmer-Maloney settling chamber. Replicate counts of at least 250 cells were made for each sample. The radioactivity on the filters was measured by liquid scintillation counting on an LKB scintillation counter with Ecolyte fluor (ICN). All data were corrected for filter adsorption of Cd^{109} by filtering an equivalent volume of labelled test media and subtracting the activity retained on the filters. Cadmium cell quotas (Q_{Cd}, amol $cell^{-1}$) were normalized to cellular C to eliminate interspecific variability in cell volumes and $Q_C{}^{vol}$.

Intracellular Cd concentrations and cell volumes were not measured for 3 of the species: *Thalassiosira parthenaia*, *T. rotula*, and *T. decipiens*. The latter 2 were only grown in the 4 highest experimental Cd concentrations.

Data analysis. Two indices of metal toxicity similar to those used in previous studies (Gavis et al. 1981, Fisher et al. 1984, Brand et al. 1986) were calculated from the growth data to compare the Cd sensitivity of the species. A least-squares, linear regression equation

describing the declining portion of each growth curve was used to estimate the greatest Cd^{2+} concentration allowing maximum growth rate (threshold Cd concentration) and the Cd concentration reducing relative growth rate to 50% (pCd^{50}). All treatments in which growth rates were significantly less than the control (i.e. $\mu/\mu_{max} < 1$), including the highest [Cd] treatment allowing maximal growth, were used in the regression.

RESULTS

Cadmium toxicity

Relative growth rates of all phytoplankton clones exhibited the same general response to increasing cadmium ion concentrations (Fig. 1). On average, growth rates were unaffected by pCd greater than 8.5 ($pCd = -\log[Cd^{2+}]$), but declined linearly at higher Cd^{2+} concentrations. Cadmium thresholds and pCd^{50}s were significantly correlated ($r^2 = 0.63$, $p < 0.01$), and both indices varied significantly among species (ANOVA, $p < 0.001$).

At the highest concentration of Cd tested (pCd 6.85), relative growth rates ranged from zero (i.e. clones did not grow in at least 3 attempts to inoculate cultures in the experimental medium: *Thalassiosira weissflogii*, *T. rotula*) to 50% (*T. oceanica*, clone 1003). Threshold Cd concentrations ranged 7-fold from pCd 8.66 (*T. weissflogii*) to pCd 7.80 (*T. oceanica*, clone 1003), while pCd^{50} values varied by approximately an order of magnitude (7.86, *T. rotula*; 6.76, *T. oceanica*, clone 1003).

The pCd^{50}s of all isolates except *Thalassiosira subtilis*, *T. parthenaia*, and *T. pseudonana* clone 1014 were significantly different (Student-Newman-Keuls test of multiple comparisons, $p < 0.01$) (Fig. 2). As in other studies of metal toxicity (e.g. Gavis et al. 1981, Brand et al. 1986) the Cd sensitivities of clones isolated from trace-metal-rich coastal waters were compared to those of species indigenous to metal-deficient offshore regions. A distinct habitat-related trend was apparent in pCd^{50} (Fig. 2). Clones isolated from oligotrophic offshore environments [mean pCd^{50} (all data) = 7.09 ± 0.19] were more resistant to high external Cd concentrations than coastal and estuarine species [mean pCd^{50} (all data) = 7.64 ± 0.24; *t*-test, $p < 0.01$]. Cadmium thresholds also differed significantly between habitat groups although the difference was less pronounced than for pCd^{50} (oceanic = 8.05 ± 0.18, coastal = 8.37 ± 0.26; *t*-test, $p = 0.05$).

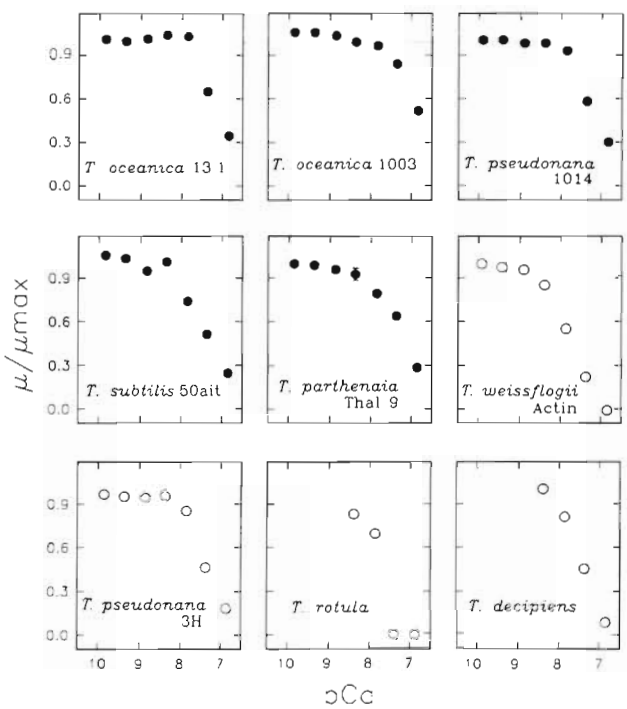

Fig. 1. *Thalassiosira* spp. Relative growth rates (μ/μ_{max}) of coastal (○) and oceanic (●) species of *Thalassiosira* as a function of free Cd ion concentration in seawater medium ($pCd = -\log[Cd^{2+}]$). Each relative growth rate is the mean of at least 6 replicates. Standard error of the mean was usually smaller than the width of the symbol

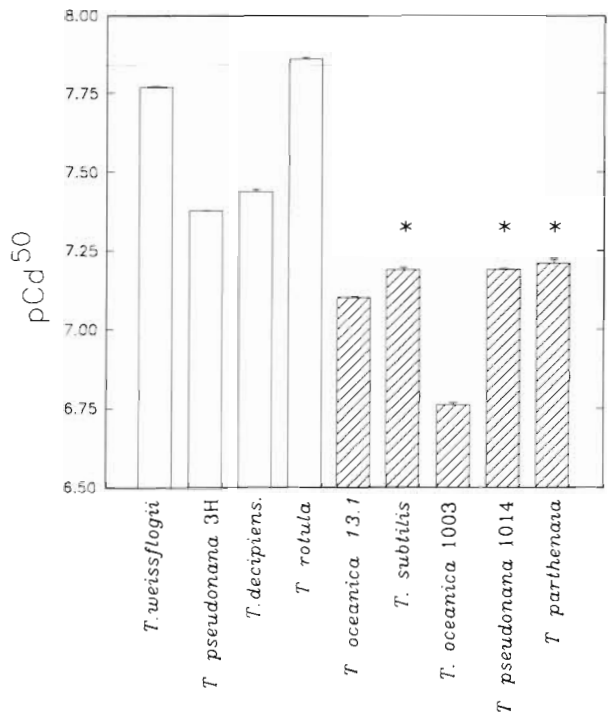

Fig. 2. The extracellular free Cd^{2+} concentration that reduced relative growth rates of *Thalassiosira* spp. by 50% (pCd^{50}). Error bars represent 1 SE and asterisks indicate pCd^{50}s that were not significantly different (ANOVA, $p > 0.05$). Average pCd^{50}s of coastal (open bars) and oceanic clones (hatched bars) were significantly different (Student-Newman-Keuls test of multiple comparisons, $p < 0.01$)

Cadmium cell quotas were measured in 2 coastal and 4 oceanic species to determine whether the greater resistance of oceanic cells could be explained by differential cellular exclusion of Cd. In *Thalassiosira oceanica* clone 13.1, *T. subtilis*, and both clones of *T. pseudonana*, Cd quotas (µmol Cd:mol C) increased in constant proportion to extracellular Cd^{2+} concentrations over a pCd range of 10 to 8. At these lower Cd^{2+} concentrations the slopes relating quota to dissolved Cd^{2+} were considerably less than 1 (0.055 to 0.33), suggesting transport system regulation as opposed to passive uptake. At higher Cd^{2+} concentrations (pCd < 8), Cd quotas for all species, except *T. weissflogii*, remained nearly constant despite increasing [Cd^{2+}], indicative of transport system saturation, feedback regulation of Cd uptake, and possibly efflux (Fig. 3). *T. weissflogii* showed very little change in cellular Cd over intermediate pCd values followed by a 10-fold increase from pCd 8.5 to 7.5. At the lowest Cd^{2+} concentration tested (pCd 9.85), Cd quotas ranged from 0.05 (*T. oceanica*, clone 1003) to 5 µmol Cd:mol C (*T. subtilis* clone 50ait) and at the highest Cd^{2+} concentration tested (pCd 6.85), *T. subtilis* contained approximately 100 (µmol Cd:mol C) while all other species contained approximately 30 µmol Cd:mol C. Relative growth rates of the species showed inhibition over a much smaller range in internal Cd quotas than for external concentrations (Fig. 4). Intracellular Cd concentrations reducing relative growth rates to 50% (QCd^{50}) were calculated as described for pCd^{50} values. A significant positive correlation between QCd^{50} and pCd^{50} values (r^2 = 0.63, p = 0.05) indicated that oceanic clones resistant to external Cd^{2+} were also more tolerant of intracellular Cd burdens. Thus, the greater resistance of oceanic diatoms relative to coastal species was not a consequence of greater cellular Cd exclusion.

Zn limitation

Coastal and oceanic species also differed significantly in their ability to grow in culture media containing low Zn concentrations (*t*-test, p = 0.002). At a pZn of 12.5 all oceanic species, with the exception of *Thalassiosira pseudonana* clone 1014, maintained maximum growth rates, whereas coastal species were severely Zn-limited with growth rates ranging from 0 to 30% of μ_{max} (control medium; pZn = 10.88) (Table 2). *T. pseudonana* clone 1014, isolated from the oceanic North Pacific gyre, was significantly Zn-limited and grew at approximately 40% of μ_{max}.

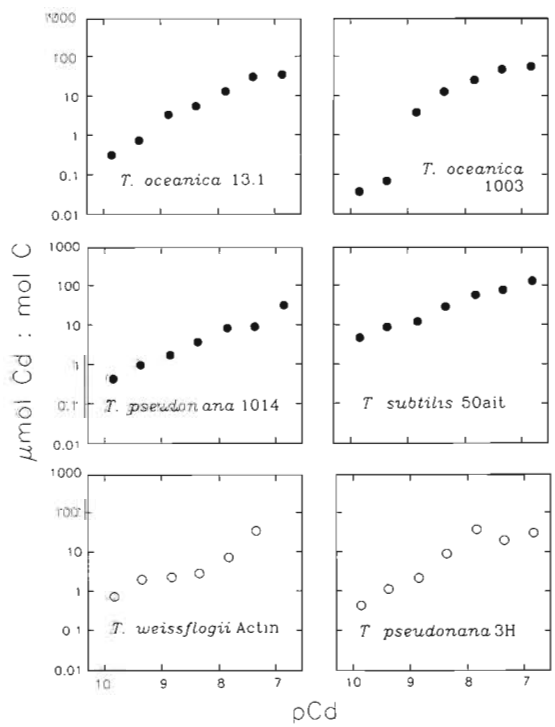

Fig. 3. *Thalassiosira* spp. Carbon-normalized Cd quotas (µmol Cd:mol C) of coastal (○) and oceanic (●) species of *Thalassiosira* as a function of free Cd^{2+} ion concentrations in seawater medium. The standard error of the mean was smaller than the width of the symbol

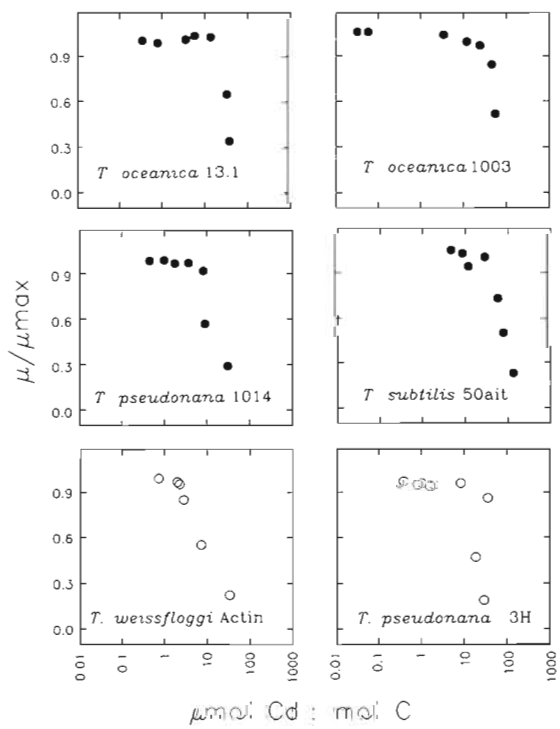

Fig. 4. *Thalassiosira* spp. Relative growth rates (μ/μ_{max}) of coastal (○) and oceanic (●) species of *Thalassiosira* as a function of intracellular cadmium quotas (µmol Cd:mol C). Standard error of the mean was smaller than the width of the symbol

Table 2. *Thalassiosira* spp. Relative (μ/μ_{max}) and maximum (μ_{max}) growth rates in Zn-deficient culture medium (pZn 12.5). Rates are means of at least 6 replicates ± 1 SD

Species	Habitat	μ_{max} (doublings d^{-1})	μ/μ_{max} (pZn 12.5)
Thalassiosira oceanica clone 13-1	Oceanic	1.75 ± 0.15	1.0 ± 0.088
Thalassiosira oceanica clone 1003	Oceanic	2.11 ± 0.04	1.0 ± 0.027
Thalassiosira parthenaia clone Thal 9	Oceanic	2.11 ± 0.08	1.0 ± 0.065
Thalassiosira pseudonana clone 1014	Oceanic	2.60 ± 0.13	0.42 ± 0.016
Thalassiosira subtilis clone 50ait	Oceanic	1.39 ± 0.08	1.0 ± 0.029
Thalassiosira decipiens clone 983	Coastal	1.53 ± 0.15	0.11 ± 0.017
Thalassiosira pseudonana clone 3H	Coastal	2.72 ± 0.15	0.33 ± 0.056
Thalassiosira rotula clone 1016	Coastal	1.66 ± 0.12	0
Thalassiosira weissflogii clone Actin	Coastal	2.07 ± 0.11	0.35 ± 0.026

Cd/Zn interactions

The results of Cd toxicity and Zn limitation experiments were combined to investigate Cd/Zn interactions. Species' growth rates under Zn-limiting conditions showed a significant positive correlation with their sensitivity to extracellular Cd ($r^2 = 0.62$, $p = 0.012$) (Fig. 5) and intracellular Cd ($r^2 = 0.76$, $p = 0.014$). Oceanic isolates that were tolerant of Zn deficiency were more resistant to Cd than coastal species whose reproduction was severely limited both by high [Cd] and low [Zn]. Thus, Cd tolerance of the phytoplankton was related to their ability to withstand Zn deficiency, consistent with a Cd/Zn antagonism.

To provide experimental evidence for a Cd/Zn antagonism, cultures of *Thalassiosira weissflogii* were grown under Zn and Fe deficiency (pZn 12.0, pFe 20.5, 100 µM EDTA, all other metal activities as in Price et al. 1988/89) in the absence of added Cd at relative growth rates of approximately 60% of μ_{max} (Fig. 6). A parallel set of Fe- and Zn-limited cultures were enriched with a Cd concentration (5.67 µM) that was not toxic to this species in metal-replete medium. Cd additions reduced the growth rates of the Zn-limited cultures by 70% (from 1.2 to 0.38 doubling d^{-1}), but had no measurable effect on the reproduction of Fe-limited cultures. Cadmium toxicity was thus specifically enhanced under Zn-deficient conditions.

DISCUSSION

The principal finding of this study concerns the different growth responses of marine diatoms to high

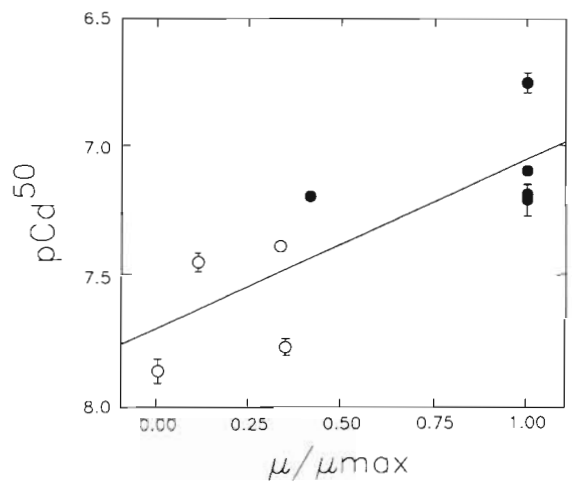

Fig. 5. Free Cd^{2+} ion concentrations reducing relative growth rates of coastal (○) and oceanic (●) isolates of *Thalassiosira* spp. to 50% (pCd^{50}) as a function of their relative growth rates (μ/μ_{max}) in zinc-deficient medium (pZn 12.5). Error bars represent 1 SE. The line through the data points was fit by a least-squares procedure ($r^2 = 0.62$, $p = 0.012$)

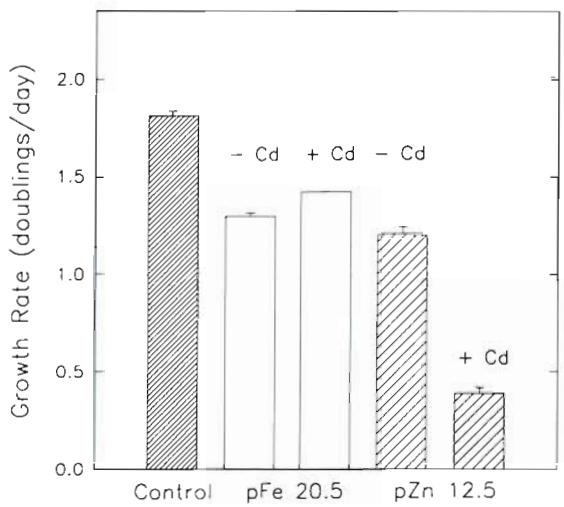

Fig. 6. *Thalassiosira weissflogii*. Relative growth rates of Fe-deficient (pFe 20.5, open bars), Zn-deficient (pZn 12.0, coarsely hatched bars), and control (Fe, Zn-saturated, finely hatched bar) cultures of *T. weissflogii* clone Actin with and without Cd additions (5.67 µM). Error bars represent 1 SE

Cd. Threshold concentrations and pCd^{50}s varied considerably among the species, but were significantly lower on average for isolates derived from oceanic habitats. These species were thus more resistant to Cd toxicity than coastal ones which were growth-rate-limited by much lower Cd^{2+} concentrations (higher pCd^{50}s).

Cadmium quotas

Cadmium quota measurements (Fig. 3) established that the interspecific variability in resistance was not due to Cd exclusion by some species. QCd^{50}s and pCd^{50}s were positively correlated to one another (r^2 = 0.63, $p = 0.05$), demonstrating that clones more resistant to high extracellular Cd were also more tolerant of high intracellular Cd. The maximum quotas in all diatoms were similar and extraordinarily high (about 30 µmol Cd:mol C or 3 to 50 mmol Cd l^{-1} cell volume). Slopes of the curves relating internal Cd quotas of the phytoplankton to external concentrations were also similar to one another and to those observed for other algal taxa (Ahner et al. 1995, Payne & Price unpubl.). This correspondence among species implies that they possess similar mechanisms for regulating cellular Cd concentrations. Increased Cd resistance of oceanic diatoms compared to coastal isolates was apparently a result of their greater tolerance of high cellular burdens.

One possible explanation for the greater tolerance may be differences between the cellular distribution and function of Cd among the isolates. Relative partitioning of Cd between high and low molecular weight biochemicals of the cytosol depends on the absolute amount of Cd and the presence of other metals (Price & Morel 1990). Systematic variations in the relative concentrations of these pools and hence in the proportions of reactive and non-reactive Cd might easily account for the habitat pattern observed. The available evidence, however, does not strongly support this explanation.

Detoxification of Cd occurs through the biosynthesis of small, metal-complexing phytochelatins (Gekeler et al. 1988), but oceanic and neritic species produce similar amounts of these peptides in response to Cd stress (Ahner et al. 1995). A greater capacity for Cd detoxification in oceanic species thus seems unlikely. Cadmium may also play a beneficial role for phytoplankton by substituting for Zn in certain cellular functions (Price & Morel 1990). Because oceanic species have evolved in habitats where essential metals are scarce, they might be hypothesized to be more adept in making such substitutions. If so, then Cd would be less deleterious to their growth compared to coastal species. Although neither of these explanations can be entirely ruled out, as discussed below, the habitat related pattern in Cd tolerance most likely reflects the greater ability of oceanic clones to tolerate essential metal deficiency.

Deficiency and toxicity of trace metals are not necessarily independent phenomena. Low concentrations of essential metals, for example, can amplify the deleterious effects of toxicants, as in the case of Cd and Zn (Lee et al. 1995). Cellular receptor ligands of phytoplankton exhibit similar coordination properties and so are unable to completely discriminate among cationic trace metals (Sunda 1988/89). Indeed, competitive inhibition of uptake is reported for a number of trace metals such as Cu and Mn (Sunda & Huntsman 1983), Cu and Zn (Rueter & Morel 1981), and Cu and Fe (Murphy et al. 1984). These antagonistic and substitutive reactions may be detrimental to cellular physiology, because they displace essential metals from their characteristic biochemical coordination sites. Such interference with transport and or assimilation may create toxic effects that ultimately result from a deficiency of an essential metal. Species that endure metal deficiency would thus be predicted to be more resistant to the high concentrations of Cd.

Cadmium/zinc interactions

Support for such an essential and toxic metal interaction was provided by examining the relationship between Cd-stressed and Zn-deficient growth rates. In low Zn medium, oceanic species maintained maximal rates of growth as expected (Sunda & Huntsman 1992), but coastal species were severely rate-limited. Both pCd^{50}s and QCd^{50}s were positively correlated to growth rates in low Zn so that phytoplankton most resistant to Cd toxicity were those least affected by Zn limitation. Oceanic species with low metal requirements thus appear to be less sensitive to elevated Cd concentrations than coastal species.

This correlative relationship by itself cannot be interpreted mechanistically as resulting from a specific Cd/Zn antagonism. Phytoplankton that grow well in medium containing low Zn are also able to tolerate low concentrations of other essential metals. Relative growth rates of Fe- and Zn-limited phytoplankton are indeed positively correlated (r^2 = 0.75, $p < 0.05$; data adapted from Brand et al. 1983). Any one of these elements could be equally important in explaining the species variability in Cd toxicity.

More direct evidence of the specific nature of the Cd-essential metal interaction was provided by the metal-limitation/threshold experiment. This experiment was designed to discriminate between Cd and Fe

or Cd and Zn antagonisms by measuring growth of one of the diatom species with various combinations of these metals. Because potential competitive interactions between toxic and essential metals are exacerbated when the essential ones are growth-rate-limiting (e.g. Cu/Zn, Rueter & Morel 1981; Cd/Zn, Lee et al. 1995), the concentrations of Fe and Zn were lowered in the media.

One of the first studies of metal antagonisms documented a Cd and Mn transport competition in *Chlorella pyrenoidosa* (Hart et al. 1979). Such an antagonism cannot be entirely ruled out in the present study, but for some of the most Cd-intolerant diatoms we were unable to induce Mn limitation even when Mn was completely omitted from the media. The lack of effect of low Mn in *Thalassiosira weissflogii* was not a result of Mn contamination of our base media, because we were easily able to limit *T. pseudonana* of Mn using the same medium (N. M. Zador & N M. Price unpubl.). At least in the test species, *T. weissflogii*, Cd growth toxicity was unlikely to be caused by an antagonism with Mn.

A Cd concentration that had no effect on metal-replete cultures became clearly toxic to Zn-limited *Thalassiosira weissflogii*, reducing its growth rate to 30% of μ_{max}. This enhanced toxicity did not result from a general stress imposed on cells by essential metal deficiency because cells that were Fe-limited to the same extent were completely unaffected by the same Cd additions. Thus, Cd levels that were not toxic under Zn-saturating conditions became severely inhibitory when Zn concentrations were low, providing strong evidence of a specific Cd/Zn interaction.

Although the growth rate antagonism between Cd/Zn was only experimentally demonstrated under Zn-limiting conditions, it is a plausible explanation for the pattern of Cd tolerance occurring in Zn-replete medium (Fig. 2). Harrison & Morel (1983) previously showed that a Cd and Fe antagonistic interaction in Fe-deficient *Thalassiosira weissflogii* also existed when Fe levels were 100-fold higher, so such extrapolation is not without foundation. Establishing which Cd-essential metal antagonism occurs in metal-replete medium will ultimately provide an unambigous test of the Cd/Zn hypothesis.

Habitat-related patterns in Cd toxicity

The habitat-dependent pattern in Cd toxicity that is evident from our results has not been reported before. In a comprehensive study that examined 20 phytoplankton species from 17 genera and 4 classes Brand et al. (1986) observed significant phylogenetic differences in Cd tolerance, but no significant trends between coastal and oceanic isolates. Closer inspection of their data (Table 6, p 245), however, revealed that within every taxonomic class, oceanic phytoplankton had lower pCd^{50}s (i.e. were more resistant) than neritic isolates. The lack of significant habitat-related patterns in Cd sensitivity (Brand et al. 1986) may have resulted from the qualitative, categorical habitat classification scheme that is in general use.

Phytoplankton are classified as either 'coastal' or 'oceanic' based on the geographic distance of their collection sites from the continental boundary (Ryther & Kramer 1961). Although terrestrial influences are known to differentiate coastal waters from the open sea, a dichotomous classification scheme does not reflect the spatial variability within coastal and oceanic zones or the continuity with which biological and chemical characteristics vary between these habitats. This classification fails to account for a number of important hydrographic phenomena, such as oceanic upwelling and island effects, that can result in the emergence of persistent, local sub-habitats. To circumvent these difficulties, it is necessary to replace discrete habitat categories with a continuous, quantitative variable. Given that trace metal concentrations decrease by several orders of magnitude along coastal to oceanic transects (Bruland 1980, Bruland & Franks 1983) and that the ability of phytoplankton to tolerate essential metal-limiting conditions is an evolutionary stable trait closely reflecting the availability of these micronutrients in their natural habitats (Brand et al. 1983, Sunda & Huntsman 1992, Sunda et al. 1991), we propose that species' relative growth rates under essential trace metal deficiency can be used as such a variable.

'Coastal' and 'oceanic' phytoplankton can be distinguished by their relative growth rates under low zinc concentrations (pZn 12.5), although considerable variation exists within each group (Table 2). At low Zn^{2+} concentrations, coastal species grew very slowly whereas all but one oceanic species maintained maximum growth rates. This exceptional oceanic species, *Thalassiosira pseudonana*, exemplifies the limitations of the traditional habitat classification scheme. The clone (1014) was isolated from the 'oceanic', central north Pacific gyre, an oligotrophic region characterized by low concentrations of nutrients compared to typical coastal waters. Its relative growth rate at pZn 12.5 was 42% of μ_{max}, the lowest among species in the 'oceanic' group, but highest among species in the 'coastal' group.

To further validate the use of the quantitative habitat classification scheme, Cd toxicity data of Brand et al. (1986) were reanalyzed, replacing discrete habitat classifications with the relative growth rates of the same species under Zn-limiting conditions (Brand et

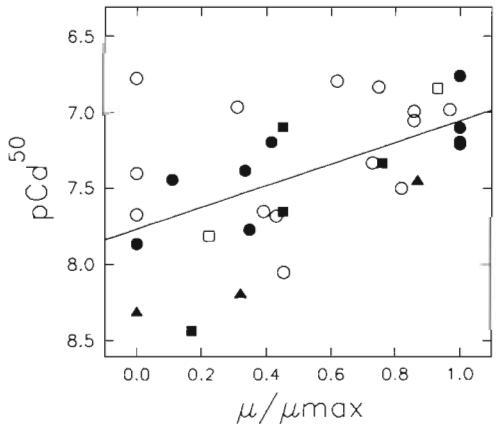

Fig. 7. Cadmium sensitivity (pCd^{50}) of a number of phytoplankton taxa as a function of their relative growth rates in Zn-deficient culture medium. The data are taken from this study, Brand et al. (1983, 1986), and Payne & Price (unpubl.). (●) *Thalassiosira* spp., this study; (○) other diatoms; (▲) dinoflagellates; (■) coccolithophorids; (□) green algae (r^2 = 0.30, n = 32, p = 0.001)

al. 1983). Data from a variety of other phytoplankton were also included in the analysis (Payne & Price unpubl.). A significant correlation (p = 0.001) was observed between Cd tolerance and Zn-limited growth rates (Fig. 7), suggesting that indeed a 'habitat'-related pattern exists among a diversity of phytoplankton taxa and that the species least affected by essential metal limitation, such as Zn, are those most resistant to Cd toxicity. As is apparent from the scatter of the plot, other ecological and physiological factors are also important in determining Cd resistance among diverse phytoplankton taxa.

Acknowledgements. We thank Chris Payne for the Cd sensitivity data and Maria Maldonado for comments. This work was supported by the Natural Sciences and Engineering Research Council of Canada and by a McGill University Faculty of Graduate Studies and Research Equipment Grant.

LITERATURE CITED

Ahner BA, Kong S, Morel FMM (1995) Phytochelatin production in marine algae: I. An interspecific comparison. Limnol Oceanogr 40:649–657

Ahner BA, Price NM, Morel FMM (1994) Phytochelatin production by marine phytoplankton at low free metal ion concentrations: laboratory studies and field data from Massachusetts Bay. Proc Nat Acad Sci USA 91. 8433–8436

Brand LE, Guillard RRL, Murphy LS (1981) A method for the rapid and precise determination of acclimated phytoplankton reproductive rates. J Plankton Res 3:193–201

Brand LE, Sunda WG, Guillard RRL (1983) Limitation of marine phytoplankton reproductive rates by zinc, manganese, and iron. Limnol Oceanogr 28:1182–1198

Brand LE, Sunda WG, Guillard RRL (1986) Reduction of marine phytoplankton reproduction rates by copper and cadmium. J Exp Mar Biol Ecol 96:225–250

Bruland KW (1980) Oceanographic distributions of cadmium, zinc, nickel, and copper in the North Pacific. Earth Planet Sci Lett 47:176–198

Bruland KW (1992) Complexation of cadmium by natural organic ligands in the central North Pacific. Limnol Oceanogr 37:1008–1017

Bruland KW, Franks RP (1983) Mn, Ni, Cu, Zn, and Cd in the western north Atlantic. In: Wong CS, Boyle EA, Bruland KW, Burton JD, Goldberg ED (eds) Trace metals in the ocean. Proceedings of NATO Advanced Research Institute. Plenum Press, New York, p 395–414

Davies AG (1978) Pollution studies with marine plankton. Part II. Heavy metals. Adv Mar Biol 15:381–508

Fisher NS, Bohé M, Teyssié JL (1984) Accumulation and toxicity of Cd, Zn, Ag, and Hg in four marine phytoplankters. Mar Ecol Prog Ser 18:201–213

Foster PL, Morel FMM (1982) Reversal of cadmium toxicity in a diatom: an interaction between cadmium toxicity and iron. Limnol Oceanogr 27:745–752

Gavis J, Guillard RRL, Woodward BL (1981) Cupric ion activity and the growth of clones isolated from different marine environments. J Mar Res 39:315–333

Gekeler W, Grill E, Winnacker EL, Zenk MH (1988) Algae sequester heavy metals via synthesis of phytochelatin complexes. Arch Microbiol 150:197–202

Harrison GI, Morel FMM (1983) Antagonism between cadmium and iron in the marine diatom *Thalassiosira weissflogii*. J Phycol 19:495–507

Hart BA, Bertram PE, Scaife BD (1979) Cadmium transport by *Chlorella pyrenoidosa*. Environ Res 18:327–335

Jensen A, Rystad G, Melsom S (1974) Heavy metal tolerance of marine phytoplankton. I. The tolerance of three algal species to zinc in coastal waters. J Exp Mar Biol Ecol 15:145–157

Keller MD, Bellows WK, Guillard RRL (1988) Microwave treatment for sterilization of phytoplankton culture media. J Exp Mar Biol Ecol 117:279–283

Lee JG, Morel FMM (1995) Replacement of zinc by cadmium in marine phytoplankton. Mar Ecol Prog Ser 127:305–309

Lee JG, Roberts SB, Morel FMM (1995) Cadmium: a nutrient for the marine diatom *Thalassiosira weissflogii*. Limnol Oceanogr 40:1056–1063

Martin JH and others (1994) Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean. Nature 376: 123–129

Martin JM, Whitfield M (1983) The significance of the river input of chemical elements to the ocean. In: Wong CS, Boyle EA, Bruland KW, Burton JD, Goldberg ED (eds) Trace metals in the ocean. Proceedings of NATO Advanced Research Institute. Plenum Press, New York, p 475–508

Murphy LS, Guillard RRL, Brown JF (1984) The effects of iron and manganese on copper sensitivity in diatoms: differences in the response of closely related neritic and oceanic species. Biol Oceanogr 3:187–201

Price NM, Ahner BA, Morel FMM (1994) The equatorial Pacific Ocean: grazer controlled populations in an iron-limited ecosystem. Limnol Oceanogr 39:520–534

Price NM, Andersen LF, Morel FMM (1991) Iron and nitrogen nutrition of equatorial Pacific plankton. Deep Sea Res 38: 1361–1378

Price NM, Harrison GI, Hering JG, Hudson RJ, Nirel PMV, Palenik B, Morel FMM (1988/89) Preparation and chemistry of the artificial algal culture medium Aquil. Biol Oceanogr 6:443–461

Price NM, Morel FMM (1990) Cadmium and cobalt substitution for zinc in a marine diatom. Nature 344:658–660

Price NM, Morel FMM (1994) Trace metal nutrition and toxicity in phytoplankton. In: Rai LC, Gaur JP, Soeder CJ (eds) Algae and water pollution (Advances in limnology; Heft 42) E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, p 79–97

Rueter JG, Morel FMM (1981) The interaction between zinc deficiency and copper toxicity as it affects the silicic acid uptake mechanisms of *Thalassiosira pseudonana*. Limnol Oceanogr 26:67–73

Ryther JH, Kramer DD (1961) Relative iron requirement of some coastal and offshore plankton algae. Ecology 42: 444–446

Sunda WG (1988/89) Trace metal interactions with phytoplankton. Biol Oceanogr 6:411–442

Sunda WG, Barber RT, Huntsman, SA (1981) Phytoplankton growth in nutrient rich seawater: importance of copper-manganese cellular interactions. J Mar Res 39: 567–586

Sunda WG, Huntsman SA (1983) Effect of competitive interactions between manganese and copper on cellular manganese and growth in estuarine and oceanic species of the diatom *Thalassiosira*. Limnol Oceanogr 28:924–934

Sunda WG, Huntsman SA (1992) Mutual feedback interactions between zinc and phytoplankton in seawater. Limnol Oceanogr 37:25–41

Sunda WG, Swift DG, Huntsman SA (1991) Low iron requirement for growth in oceanic phytoplankton. Nature 351 55–57

Westall JC, Zachary JL, Morel, FMM (1976) MINEQL: a computer program for the calculation of chemical equilibrium composition of aqueous systems. Tech. Note No. 18. RM Parsons Lab for Water Resources and Environmental Engineering. MIT, Dept of Civil Engineering, Cambridge, MA

This article was submitted to the editor

Manuscript first received: January 1, 1995
Revised version accepted: March 8, 1996