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Phylogenetic diversity in cadmium : phosphorus ratio regulation by marine phytoplankton

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Abstract

We examined the effect of irradiance and growth rate on cadmium : phosphorus ratio in five species of phytoplankton representing four different phylogenetic groups to determine the relative importance of growth rate, irradiance, and taxonomic differences on Cd : P ratios. Irradiance and growth rate are responsible for >2 orders of magnitude variation in the Cd : P of phytoplankton. Diatoms exhibit an increase; cyanobacteria, a decrease; and prasinophyte and dinoflagellate species, no functional response in Cd : P with increasing growth rate because of changes in irradiance. These results are consistent with a metabolic demand for Cd in diatoms and a metabolic sensitivity to Cd by cyanobacteria.

In the ocean, Cd exhibits a nutrient-type vertical profile that is highly correlated with P (Boyle et al. 1976). This correlation, and the incorporation of Cd^{2+} and Ca^{2+} into the structural lattice of foraminiferal tests in relative proportion to their ambient concentrations in seawater forms the basis for using Cd : Ca in foraminiferal calcite as a paleoproxy for phosphate concentrations in surface (Elderfield and Rickaby 2000) and deep waters (Boyle 1988). The fidelity of the foraminiferal Cd : Ca paleoproxy for phosphate is dependent on current knowledge of the relationship between dissolved Cd and phosphate in the modern ocean and the assumption that this relationship has been static over time. It has been hypothesized that the biogeochemical cycles of Cd and P are linked though their uptake by phytoplankton in surface waters, export into the deep sea as phytoplankton aggregates and zooplankton fecal pellets, and remineralization at depth (Boyle et al. 1976; Saager and Baar 1993; Loscher et al. 1997). If this is the case, then a mechanistic understanding of the uptake and accumulation of Cd and P into phytoplankton biomass is essential for understanding the biogeochemical cycling of Cd and P in the ocean and the interpretation of foraminiferal Cd : Ca as a paleoproxy for phosphate (Cullen et al. 2003; Cullen 2006).

Laboratory and field studies over the last decade have stimulated an evolution in our understanding of the controls on Cd and P biogeochemical cycling in seawater. Particulate Cd : P in phytoplankton has been shown to be a function of a suite of environmental factors, including the concentration of free Cd and phosphate (Kudo et al. 1996; Payne and Price 1999), CO_2 (Cullen et al. 1999), iron (Sunda and Huntsman 2000; Frew et al. 2001; Cullen et al. 2003), and the trace metals Mn and Zn (Sunda and Huntsman 1998*a*; Sunda and Huntsman 2000; Cullen and Sherrell 2005). Two recent iron addition experiments in high nutrient, low chlorophyll regions provide apparently inconsistent responses in the Cd : P of phytoplankton. In a mesoscale experiment south of the Polar Front (61°S, 140°E), Frew et al. (2001) found that iron additions resulted in an increase in particulate Cd : P and a decrease in seawater Cd : PO_4 as estimated from the difference in Cd : P from filtered and unfiltered samples. In contrast, when using bottle experiments, Cullen et al. (2003) found

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that Fe additions to an Fe-limited region of the Southern Ocean led to a decrease in Cd : P in phytoplankton biomass. Phenotypic regulation of the uptake of Cd and P by phytoplankton in response to environmental conditions that differ between these experiments could account for the apparent inconsistency.

Irradiance is one of the ultimate driving factors regulating phytoplankton growth in the oceans; much of the standing stock of oceanic phytoplankton is limited by light. There is relatively little work on the effect of irradiance on Cd : P incorporation into phytoplankton biomass. Sunda and Huntsman (2004) documented that Zn : P in phytoplankton decreases with increasing irradiance and growth rate similar to the observed behavior of Fe : P and Mn : P (Sunda and Huntsman 1997; 1998*b*; 2004), suggesting that many micronutrient : P ratios may decrease with increasing irradiance because of the growth-dilution effect. Finkel et al. (2006) found that many species became highly enriched in several trace elements, Fe, Mn, Zn, Cu, Co, and Mo relative to cellular P, under extremely low irradiances (<30 μmol quanta m^{-2} s^{-1}). Observations that environmental factors alter elemental ratios in plankton biomass and that the addition of Fe in high nutrient low chlorophyll (HNLC) regions can result in a decrease in particulate Cd : P suggests that particulate Cd : P and the Cd : Ca paleoproxy for phosphate may be largely regulated by phytoplankton growth rates (Cullen et al. 2003; Cullen 2006). In the coastal diatom *Thalassiosira pseudonana* increases in irradiance and temperature have been shown to cause corresponding increases in the uptake of Cd and Zn (Miao and Wang 2004). If growth irradiance significantly affects the accumulation of Cd : P in phytoplankton biomass, then it may be necessary to further revise our interpretation of the Cd : Ca paleoproxy for seawater phosphate.

Here we investigate the effect of irradiance and growth rate on Cd : P in five species of phytoplankton, representing four different phylogenetic groups. This will allow us to determine the relative importance of growth rate, irradiance, and taxonomic differences on Cd : P ratios.

Materials and methods

Phytoplankton species investigated in this study include one nitrogen-fixing Cyanophyceae, *Cyanothece* sp. (WH8904, provided by Dr. J. Waterbury), a Prasinophyceae, *Pycnococcus provasolii* (CCMP 1203), a dinoflagellate (Dinophyceae), *Amphidinium carterae* (CCMP 1314), and two diatoms (Bacillariophyceae): *Thalassiosira weissflogii* (CCMP 1336) and *Chaetoceros calcitrans* (CCMP 1315). The algae were grown at 19 ± 1°C by using an on/off 12 h : 12 h light dark cycle at five irradiances: 15, 30, 100, 250, and 500 μmol quanta m^{-2} s^{-1}. A minimum of three replicate bottles for each species under each of the experimental irradiances were maintained in exponential growth through a minimum of six generations before harvesting. For the case of 250 μmol quanta m^{-2} s^{-1} treatment, some species had more than three replicate bottles: *C.* sp., *P. provasolii*, and *T. weissflogii* had 7, 6, and 6 replicate bottles, respectively. Outliers were removed as described in Finkel et al. (2006), resulting in two replicates measurements for Cd : P for *P. provasolii* at 100 μmol quanta m^{-2} s^{-1} and *C.* sp at 30 μmol quanta m^{-2} s^{-1}. For *C. calcitrans*, Cd : P is only available for three irradiances, 15, 30, and 100 μmol quanta m^{-2} s^{-1}, because this species did not grow at 250 and 500 μmol quanta m^{-2} s^{-1} under our growth conditions, whereas, for *A. carterae*, one bottle became contaminated at the 15, 30, 100, and 500 μmol quanta m^{-2} s^{-1} treatments, leaving two replicate bottles for these light treatments, and a growth rate for *Cyanothece* sp. at 250 μmol quanta m^{-2} s^{-1} was not recorded. The number of cells per unit volume of media and the equivalent spherical diameter (ESD) was determined with a calibrated Multisizer Coulter particle counter, cell counts were used to calculate growth rates.

Medium preparation, algal culturing and sampling, and elemental analysis were prepared according to rigorous acid-cleaning procedures. Cells were cultured in the medium Aquil, and prepared and sterilized following established protocols. Synthetic ocean water was enriched with sterile and metal-free 150 μmol L^{-1} $NaNO_3$, 10 μmol L^{-1} Na_2HPO_4, and 40 μmol L^{-1} Na_2SiO_3, plus 0.1 μmol L^{-1} vitamin B_{12}, 0.1 μmol L^{-1} biotin, 20 μmol L^{-1} thiamin, and 100 nmol L^{-1} Na_2MoO_4. In the presence of 100 μmol L^{-1} ethylenediamine tetraacetic acid (EDTA), total trace metal concentrations were as follows: $Mn_T = 120$ nmol L^{-1}, $Zn_T = 80$ nmol L^{-1}, $Cu_T = 20$ nmol L^{-1}, $Co_T = 50$ nmol L^{-1} and $Cd_T = 15$ nmol L^{-1}, calculated to yield inorganic metal ion concentrations of $Mn' = 10$ nmol L^{-1}, $Zn' = 20$ pmol L^{-1}, $Cu' = 0.2$ pmol L^{-1}, $Co' = 20$ pmol L^{-1} and $Cd' = 20$ pmol L^{-1}, further details are provided in Ho et al. (2003). Low Cd concentrations in the culture media were chosen to reflect typical concentrations in surface waters. Cadmium and phosphorus were assayed with sector field high resolution inductively coupled plasma mass spectrometry (HR-ICPMS, *Element*, ThermoFinnigan). Two isotopes, Cd 110/111, were measured to confirm low interference, methodological details are provided in Ho et al. (2003) and Quigg et al. (2003). Cd : P ratios were log transformed ($\log_{10}$ [Cd : P]) to homogenize the variance. Observations from 250 μmol quanta m^{-2} s^{-1} treatment has been previously published in Falkowski et al. (2004), Ho et al. (2003), and Quigg et al. (2003), and are reported here for comparison.

A two-way analysis of variance (ANOVA) was used to quantify and evaluate the statistical significance of phylogeny, the phenotypic response to irradiance, and the interaction between phylogeny and phenotype as factors predicting variation in log (Cd : P). Because of experimental and analytical difficulties, the design was not completely balanced (orthogonal), as a result, the sums of squares for each of the main effects depends on the order in which they are included in the model. For our data, this amounted to only a small ($<5\%$) variation; analyses reported are an average of the two possible models.

Results

Phylogenetic differences in Cd : P—Ignoring the effect of irradiance, there are significant taxonomic differences in log (Cd : P) (two-way ANOVA, $p << 0.01$). The di-

Table 1. Geometric mean Cd : P (mmol : mol), equivalent spherical diameter (ESD in μm), and growth rate (day^{-1}) of phytoplankton as a function of growth irradiance (I, μmol quanta m^{-2} s^{-1}), note data for the 250 μmol quanta m^{-2} s^{-1} treatment provided in Table 2.

Taxa	I	μ (d^{-1})	SE (%)	ESD (μm)	SE (%)	Cd : P (mmol : mol)	SE (%)
T. weissflogii	15	0.13	1.80	12.43	0.44	0.005	3.30
	30	0.16	0.20	12.43	0.44	0.004	1.45
	100	0.39	11.70	13.74	1.79	0.018	1.95
	500	0.50	0.40	12.52	6.96	0.040	1.48
P. provasolii	15	0.26	4.00	2.24	1.18	0.028	3.58
	30	0.36	0.60	2.23	1.24	0.013	0.23
	100	0.56	0.60	2.27	1.26	0.030	4.03
	500	0.80	16.30	2.25	0.75	0.028	1.24
Cyanothece sp.	15	0.25	10.90	2.39	0.74	0.031	2.56
	30	0.39	6.10	2.25	1.91	0.026	2.17
	100	0.48	2.00	2.27	2.03	0.012	3.78
	500	0.74	0.90	2.65	1.56	0.003	1.54
A. carterae	15	0.14	1.20	9.26	0.57	0.255	0.31
	30	0.27	2.20	8.97	2.51	0.274	0.23
	100	0.46	3.60	9.35	0.68	0.226	2.59
	500	0.59	1.60	9.25	0.83	0.058	0.45
C. calcitrans	15	0.21	7.30	3.01	0.64	0.001	2.41
	30	0.30	0.80	3.33	0.67	0.002	1.75
	100	0.59	1.30	3.46	0.42	0.021	5.62

noflagellate *A. carterae* has significantly higher log (Cd : P) than the diatom *T. weissflogii*, and the green alga *Pycnococcus provasolii* has significantly higher log (Cd : P) than the small diatom *C. calcitrans* and the nitrogen-fixing cyanobacterium *Cyanothece* sp. (Tables 1 and 2). The differences in log (Cd : P) between species is generally larger if each irradiance is considered separately, but the relative ranking of Cd : P for the different species changes with irradiance. A extended list of Cd : P from a range of species grown at 250 μmol quanta m^{-2} s^{-1} from Falkowski et al. (2004), Ho et al. (2003), and Quigg et al. (2003) indicate there may be weak systematic cell size and taxonomic differences in Cd : P (Table 3).

Effect of growth irradiance and interaction with phylogeny on Cd : P—The growth rate of most of the species increased with irradiance up to 250 μmol quanta m^{-2} s^{-1} and generally began to saturate at 500 μmol quanta m^{-2} s^{-1}, except for *C. calcitrans*, which would not grow at irradiances above 100 μmol quanta m^{-2} s^{-1} (Fig. 1). Ignoring species-specific differences in log (Cd : P), there are small but significant differences in log (Cd : P) between the five different growth irradiances. The combination of phylogeny, phenotypic response to irradiance, and their interaction accounts for a large portion (94%) of the total variation in log (Cd : P) (Table 2). There are clearly significant taxonomic differences in the effect of irradiance on log (Cd : P); there is an increase in Cd : P in the diatoms and a decrease in Cd : P for *Cyanothece* sp. with increasing irradiance (Fig. 2).

Cd : P is a species-specific response to changes in $\mu : \mu_{max}$—Log (Cd : P) is a species-specific function of relative growth rate ($\mu : \mu_{max}$, dimensionless) as regulated by irradiance. Log (Cd : P) increases with relative growth rate for the diatoms, *Chaetoceros calcitrans* and *T. weissflogii*, decreases for *Cyanothece* sp., and has no clear functional relationship in *P. provasolii* and *A. carterae* (Fig. 3). The change in Cd : P with $\mu : \mu_{max}$ results in a change in the relative differences in Cd : P between species with growth irradiance, so that under low growth irradiance, the diatom species have higher Cd : P than diazotroph *Cyanothece* sp., while under growth saturating irradiance *Cyanothece* sp. has higher Cd : P than the diatom species.

Steady state net influx of Cd relative to P—There are three different functional responses in the steady state influx of Cd relative to PO_4 ($\mu \times$ Cd : P, mol Cd [mol P h]$^{-1}$). The diatoms exhibit an increase while *Cyanothece* sp. has a decrease in the log of the steady state influx of Cd relative to P with increasing irradiance. The dinoflagellate and prasinophyte species exhibit no clear trend in the log of the steady state influx of Cd relative to P in response to

Table 2. Two-way analysis of variance of log (Cd : P) as a function of phylogeny and phenotypic response to growth irradiance.

	df	SS	% SS	MS	*F*	*p*-Value
Phylogenetic effect (P)	4	23.39	62	5.85	265.4	<0.00001
Irradiance (I)	4	1.48	4	0.37	16.8	<0.00001
P $\times$ I	14	11.99	32	0.86	38.9	<0.00001
Residuals	50	1.10	3	0.02		
Total	73	37.96	100			

Table 3. Particulate Cd : P (mmol : mol) in a range of phytoplankton taxa under nutrient and light saturated (250 μmol quanta m^{-2} s^{-1}) conditions and particulate organic matter from field observations. Data from Quigg et al. (2003), Ho et al. (2003), Falkowski et al. (2004), and current study.

Taxonomic groups	Volume (μm^3)	μ (day^{-1})	Cd : P (mmol : mol)	SE (%)
Cyanobacteria			0.007	53.1
Cyanothece sp.	8.5		0.002	0.9
Anabeana flos-aquae			0.005	0.8
Trichodesmium sp. (IMS101)			0.005	3.0
Synechococcus sp.			0.016	2.8
Diatoms			0.101	40.6
Diatoms <1500 μm^3			0.042	158.4
Nitzschia brevirostris	119.0	0.67	0.019	1.7
T. weissflogii	930.0	0.98	0.066	0.3
Diatoms >1500 μm^3			0.160	6.8
Thalassiosira excentrica	6627.0	0.27	0.152	0.5
Ditylum brightwellii	6995.0	0.43	0.168	1.2
Dinoflagellates			0.257	70.1
A. carterae	514.0	0.52	0.730	0.6
Prorocentrum minimum	833.0	0.52	0.115	0.3
Thoracosphaera heimii	1353.0	0.33	0.097	0.4
Gymnodinium chlorophorum	3410.0	0.20	0.092	2.2
Green algae			0.178	68.4
Greens <20 μm^3			0.018	76.7
Pynococcus provasolii	10.0	0.64	0.028	0.2
Nannochloris atomus	14.0	0.69	0.008	1.4
Greens >200 μm^3			0.284	68.4
Dunaliella tertiolecta	227.0	0.72	0.102	0.9
Tetraselmis sp.	300.0	0.27	0.150	0.7
Pyramimonas parkeae	587.0	0.69	0.600	1.6
Coccolithophorids			0.335	10.6
Emiliania huxleyi	142.0	0.64	0.360	2.7
Gephyrocapsa oceanica	142.0	0.79	0.310	3.5
Particulate matter				
Coastal upwelling (Martin and Knauer 1973)			0.068	
Southern ocean (Cullen et al. 2003)			0.36–1.3	
Oligotrophic open ocean (see Ho et al. 2003)			0.51–0.54	

irradiance or growth rate (Fig. 4). This indicates that growth rate is not solely responsible for changes in Cd : P in the biomass of the diatoms and the cyanobacterium *Cyanothece*.

Discussion

Field observations of Cd : P in plankton indicate that environmental conditions, Cd (Martin and Broenkow 1975), Zn, Mn, Fe (Sunda and Huntsman 2000; Cullen and Sherrell 2005; Cullen 2006), and CO_2 concentrations (Cullen et al. 1999), control Cd : P in phytoplankton biomass. Laboratory experiments confirm that these environmental variables can cause shifts in Cd : P in phytoplankton biomass through several not necessarily independent physiological responses: (1) a change in the relative steady-state uptake of Cd relative to phosphate in response to changes in the concentration and/or ratio of phosphorus and free Cd concentrations (Kudo et al. 1996; Sunda and Huntsman 1998*a*; Payne and Price 1999); (2) a biodilution of cellular Cd relative to the macronutrients phosphorus or carbon in response to an increase in growth rate, without a corresponding increase in uptake (Kudo et al. 1996; Sunda and Huntsman 1998*a*; Payne and Price 1999); (3) nonspecific transport of Mn, Zn, Co, Fe, and Cd can result in variable uptake and incorporation of Cd relative to P, depending on the free concentrations of these competing elements (Foster and Morel 1982; Sunda and Huntsman 1998*a*; Sunda and Huntsman 2000); (4) a change in cellular demand for Cd (or Zn and Co) in carbonic anhydrase of diatoms, the only taxonomic group with a demonstrated use for Cd, in response to the availability of inorganic carbon (Cullen et al. 1999 ; Lane et al. 2005) or demand for competing elements (Cullen 2006), and (5) nutrient availability may trigger a change in P or Cd storage and thus alter the Cd : P ratio in total cellular biomass (Walsh and Hunter 1992).

It is notable that although taxonomic differences in particulate Cd : P have been documented in laboratory studies (Payne and Price 1999), there are few unequivocal field observations of a relationship between surface particulate Cd : P and the taxonomic structure of the phytoplankton community. Experimental work on single species under optimal growth conditions (saturating

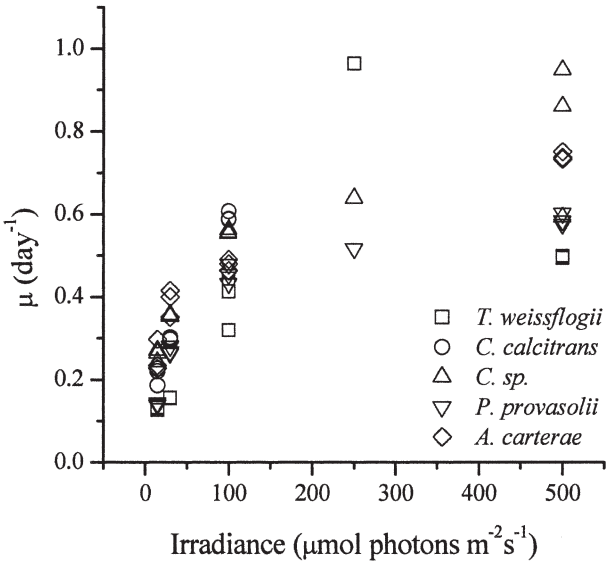

Fig. 1. Growth irradiance relationships for the diatoms *Chaeotoceros calcitrans* and *T. weissflogii*, cyanobacterium *Cyanothece* sp., dinoflagellate *A. carterae*, and prasinophyte *P. provasolii*.

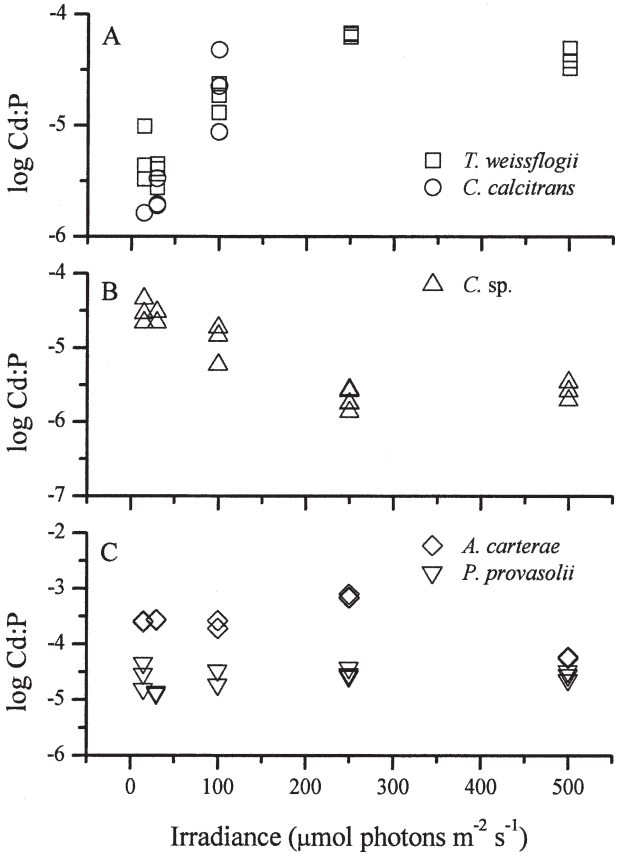

Fig. 2. Species specific responses of log Cd : P (mol : mol) to growth irradiance (μmol quanta m^{-2} s^{-1}): for: (A) the diatoms: *Chaeotoceros calcitrans* and *T. weissflogii*, (B) the cyanobacterium *Cyanothece* sp., and (C) the dinoflagellate *A. carterae* and prasinophyte *P. provasolii*.

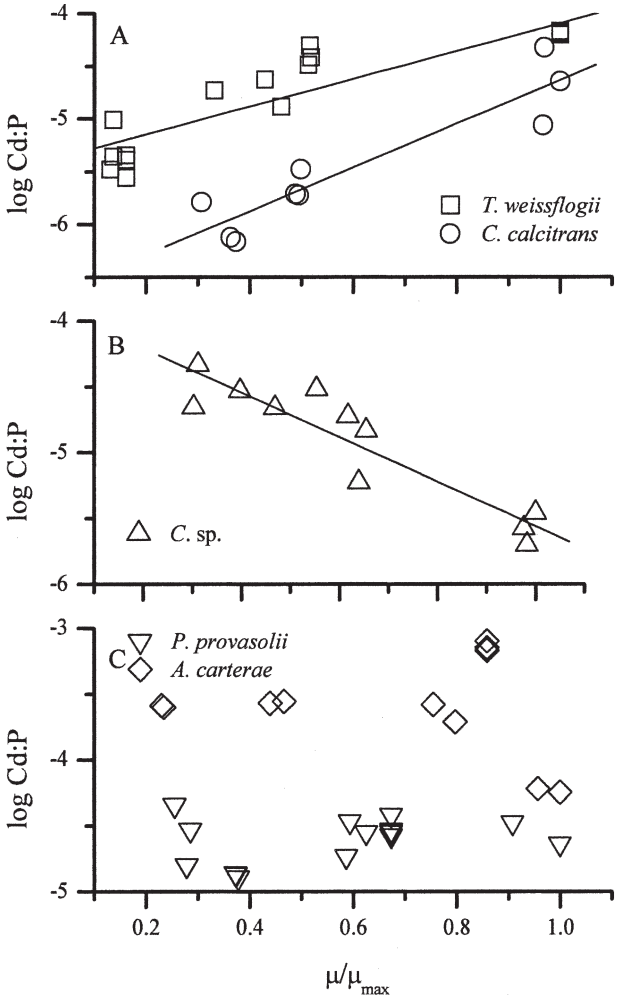

Fig. 3. Species specific responses of log Cd : P (mol : mol) as a function of relative growth rate ($\mu : \mu_{max}$) for the diatoms: (A) *Chaeotoceros calcitrans* and *T. weissflogii*, (B) cyanobacterium *Cyanothece* sp., and (C) dinoflagellate *A. carterae* and prasinophyte *P. provasolii*.

irradiance and nutrient concentrations) suggest that Cd : P is low in the cyanobacteria, and especially low in diazotrophs (Table 3). Diatoms, dinoflagellates, and green algae exhibit a wide range of values in Cd : P, but larger species of diatoms and green algae tend to have higher Cd : P than smaller species from the same taxonomic group. The prasinophyte *Pyramimonas parkeae* and the coccolithophorid species display the highest Cd : P (Table 3). Taxonomic differences in the regulation of Cd : P in response to environmental conditions, such as irradiance, and other factors not yet identified, may obscure the identification of both taxonomic and environmental regulation of Cd : P from field observations.

Cullen et al. (2003) propose a variety of reasons for the discrepancies between two Fe-enrichment experiments on particulate Cd : P, including methodology: Frew et al. (2001) monitored dissolved Cd and PO_4 in filtered and nonfiltered seawater from inside and outside an Fe-enriched mesoscale patch, whereas Cullen et al. (2003)

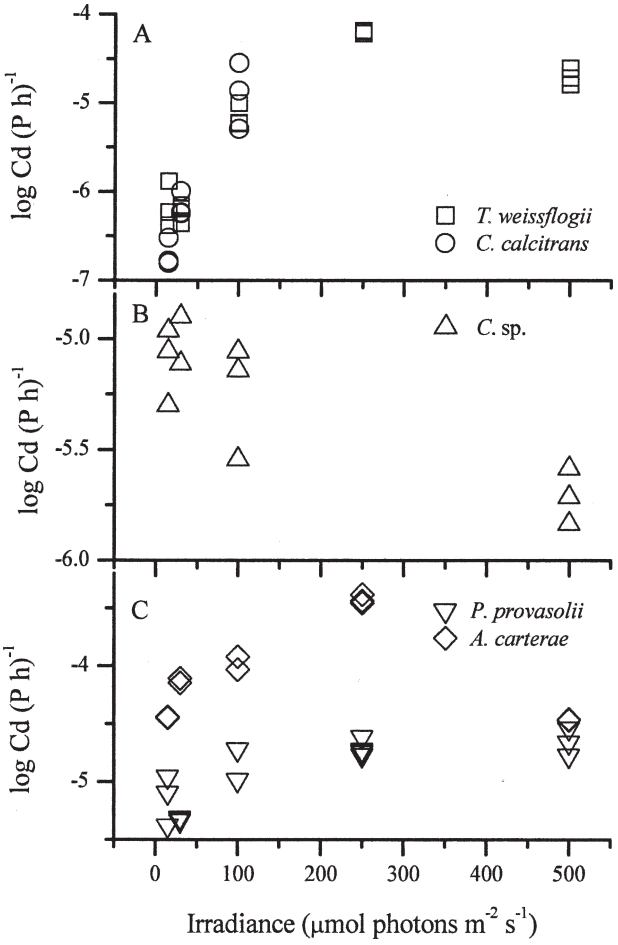

Fig. 4. Species specific responses in the log net steady state uptake of Cd : P (mol Cd mol P^{-1} h^{-1}) as a function of growth irradiance (μmol quanta m^{-2} s^{-1}) for the diatoms: (A) *Chaeotoceros calcitrans* and *T. weissflogii*, (B) cyanobacterium *Cyanothece* sp., and (C) dinoflagellate *A. carterae* and prasinophyte *P. provasolii*.

measured particulate Cd : P in particulate matter from shipboard incubation experiments; potential differences in grazing and recycling rates; environmental conditions, such as the light field and mixing rates; and the larger proportional drawdown of initial dissolved Cd and PO_4 to 67% and 95% in Cullen et al. (2003) versus 57% and 17% in Frew et al. (2001). Fe-enrichment resulted in increases in community growth rate and diatom biomass in both studies, but further differences in the taxonomic composition of the initial and climax phytoplankton communities (proportion of large vs. small diatoms, cyanobacteria vs. coccolithophorids, etc.) and differences in the environmental conditions (Gall et al. 2001; Cullen et al. 2003) suggest that the unique physiological responses of the different phytoplankton taxa to specific environmental conditions (such as irradiance) may have contributed to the contradicting response of particulate Cd : P to Fe-enrichment observed by Frew et al. (2001) and Cullen et al. (2003).

The unique patterns of regulation of Cd : P by the different phylogenetic groups of phytoplankton in response to growth irradiance are consistent with a facultative biological use of Cd by diatoms because of the increasing Cd : P with the rate of carbon fixation, a metabolic sensitivity to Cd by cyanobacteria because of the active net decrease in the rate of incorporation of Cd relative to P with increasing adenosine triphosphate (ATP) availability, and no clear functional response by dinoflagellate and prasinophyte species that results in a fairly stable particulate Cd : P regardless of growth rate and irradiance (Figs. 2 and 3). A potential physiological basis for these phylogenetic differences in Cd : P regulation in response to irradiance may be taxonomic differences in elemental transport systems and their specificity for Cd versus other elements in response to environmental conditions. Alternatively, based on prokaryotic influx and efflux systems it has been hypothesized that it may be more energetically costly to restrict and select entry of different ions than induce efflux (Silver 1996). Efflux of Cd^{2+} would require a direct input of ATP, or symport with an ion maintained out of equilibrium across the plasmalemma by an ATP-dependent process. The decrease in Cd : P in *Cyanothece* sp. biomass with increasing growth rate but under the same external Cd and PO_4 concentrations is consistent with the increase in energy available for the efflux of this potentially toxic metal. The phylogenetic differences in Cd : P incorporation are also consistent with the observed growth rate responses of species from these phylogenetic groups in response to a range of Cd concentrations (Brand et al. 1986; Payne and Price 1999), and field observations of lower Cd : P in smaller versus larger species from the same taxonomic group (Cullen and Sherrell 2005). Saito et al. (2003) postulated that the relative sensitivity to Cd of the prokaryotic cyanobacteria versus the various eukaryotic groups of phytoplankton is consistent with their origination and initial radiation in the sulfidic ocean of the Proterozoic where the availability of Cd would have been relatively low (Saito et al. 2003). Expanding on this idea, we hypothesize that the fundamental and striking differences in the regulation of Cd : P in the cyanobacteria versus the eukaryotic taxa, especially the diatoms, may reflect the active selection of different biochemical regulatory networks in response to the changing availability of elements over Earth's history (Ho et al. 2003; Quigg et al. 2003; Falkowski et al. 2004).

Sunda and Huntsman (1997, 1998*a*, 2004) have proposed that the trace element (E) to macronutrient (C or P) ratios are a balance of net steady state uptake and growth rate (μ):

$$E : P = \rho/\mu \quad (1)$$

where ρ is a Michaelis-Menton function of nutrient availability resulting in increased cellular quota and increased growth rate (μ), which eventually results in a decline in E : P. There is an inherent assumption that the elements that make up a large proportion of cellular biomass, such as C (or P), have cellular concentrations that are linearly related to growth rate. As a result this growth biodilution model predicts that an increase in growth rate, because of irradiance, will result in a decrease in cellular E : C or E : P, regardless of the element or phylogenetic

group. Cullen et al. (2003) proposed that the biodilution hypothesis provided a parsimonious explanation of the observed decrease in particulate Cd : P in response to Fe enrichment in the HNLC region off California. This suggests that the ratio of Cd : P in seawater relative to particulate organic material ($\alpha_{Cd:P}$) should be negatively correlated with the growth rate of the phytoplankton community and as a result the assumption of a constant or regional $\alpha_{Cd:P}$ is unlikely to provide accurate reconstructions of seawater phosphate concentrations from the Cd : Ca in foraminiferal calcite (Cullen et al. 2003).

Our results and several other experimental studies have confirmed that irradiance alters the uptake rate of a range of trace elements from a variety of phytoplankton species (Strzepek and Price 2000; Miao and Wang 2004; Finkel et al. 2006). Cullen (2006) found systematic differences in the dissolved Cd : PO_4 in iron-limited high-nutrient, low chlorophyll regions of the ocean, and hypothesized that Fe-limited phytoplankton may hyper-accumulate Cd because of decreases in growth rate and/or nonspecific Cd : Fe transporters (Cullen 2006), resulting in high particulate Cd : P in these regions. If nonspecific Cd : Fe transport is an important pathway for Cd and Fe acquisition by phytoplankton then an increase in Cd : P should accompany any increase in steady-state Fe uptake with decreasing irradiance. Although cellular Fe requirements (Sunda and Huntsman 1997) and net steady state uptake of Fe (Finkel et al. 2006) do generally increase with decreasing irradiance, Cd : P (and net steady-state uptake) only increased in one (*Cyanothece* sp.) of the five species examined (Figs. 2 and 4). Based on these experimental observations, we concur that phytoplankton community growth rate may have an effect on the uptake and accumulation of Cd relative to P in phytoplankton (Sunda and Huntsman 1998*a*; Sunda and Huntsman 2000; Cullen et al. 2003), but our results indicate that both the taxonomic composition of the phytoplankton community and their specific physiological response to irradiance is as important as free Cd and phosphate and potentially more important than CO_2, Mn, Zn, or Fe (Cullen et al. 1999; Cullen et al. 2003; Cullen and Sherrell 2005), and will interact with these factors in regulating variation in Cd : P in phytoplankton. It is worth noting that the dissolved Cd : Fe ratio in our laboratory cultures is low relative to many offshore regions and that further study into the effect of the concentration and ratio of dissolved Fe and Cd under different growth irradiances for different phytoplankton species may yield further insight into the variability and biogeochemical cycling of Cd : PO_4 in the ocean.

Consideration of phylogenetic differences in Cd : P regulation by phytoplankton in response to environmental factors may also prove useful in predicting fluctuations in Cd concentrations in ecologically and economically important organisms, such as oysters and mussels (Kruzynski 2004), and improve our understanding of the high concentrations of Cd in many marine mammal species, especially those with polar distributions (Honda et al. 1987; Hansen et al. 1990). High concentrations of Cd have been found in a variety of shellfish including some oysters, mussels, and scallops that do not clearly relate to high concentrations or anthropogenic input of Cd to the sediment (Brooks and Rumsby 1965; Dietz et al. 2000; Kruzynski 2004). Shellfish off the coast of British Columbia, Canada, such as the Pacific oysters (*Crassostrea gigas*) often have concentrations of Cd consistently above limits set by the Hong Kong and European community (Kruzynski 2004). It seems plausible that differences in the Cd : P in different phytoplankton taxa under different environmental conditions in conjunction with selective feeding by species in the upper trophic levels may account for differences in the accumulation of Cd in different metazoan species (Honda et al. 1987; Bargagli et al. 1996; Dietz et al. 2000). Much more work is required as species level variation in elemental composition may not simply translate into community or ecosystem level responses as has been demonstrated for Redfield stoichiometry (Falkowski 2000). Ultimately, the importance of irradiance and taxonomic variation on particulate Cd : P must be assayed in the field, but the results of this study strongly indicate that irradiance and the taxonomic composition of the plankton should be considered in future studies of Cd : P biogeochemistry in the oceans.

References

Bargagli, R., L. Nelli, S. Ancora, and S. Focardi. 1996. Elevated cadmium accumulation in marine organisms from Terra Nova Bay (Antarctica). Polar Biol. **16:** 513–520.

Boyle, E. A. 1988. Cadmium: Chemical tracer of deepwater paleoceanography. Paleoceanography **3:** 471–489.

———, F. R. Sclater, and J. M. Edmond. 1976. On the marine geochemistry of cadmium. Nature **263:** 42–44.

Brand, L. E., W. G. Sunda, and R. L. Guillard. 1986. Reduction of marine phytoplankton reproduction rates by copper and cadmium. J. Exp. Mar. Biol. Ecol. **96:** 225–250.

Brooks, R. B., and M. G. Rumsby. 1965. The biogeochemistry of trace element uptake by some New Zealand bivalves. Limnol. Oceanogr. **10:** 521–527.

Cullen, J. T. 2006. On the nonlinear relationship between dissolved cadmium and phosphate in the modern global ocean: Could chronic iron limitation of phytoplankton cause the kink? Limnol. Oceanogr. **51:** 1361–1380.

———, and R. M. Sherrell. 2005. Effects of dissolved carbon dioxide, zinc, and manganese on the cadmium to phosphorus ratio in natural phytoplankton assemblages. Limnology and Oceanography **50:** 1193–1204.

———, Z. Chase, K. H. Coale, S. E. Fitzwater, and R. M. Sherrell. 2003. Effect of iron limitation on the cadmium to phosphorus ratio of natural phytoplankton assemblages from the Southern Ocean. Limnol. Oceanogr. **48:** 1079–1087.

———, T. W. Lane, F. Morel, and R. M. Sherrell. 1999. Modulation of cadmium uptake in phytoplankton by seawater CO_2 concentration. Nature **402:** 165–167.

Dietz, R., F. Riget, M. Cleemann, A. Aarkrog, P. Johansen, and J. C. Hansen. 2000. Comparison of contaminants from different trophic levels and ecosystems. Sci. Total Environ. **245:** 221–231.

Elderfield, H., and R. E. M. Rickaby. 2000. Oceanic Cd/P ratio and nutrient utilization in the glacial Southern Ocean. Nature **405:** 305–310.

Falkowski, P. G. 2000. Rationalizing elemental ratios in unicellular algae. J. Phycol. **36:** 3–6.

———, M. E. Katz, A. H. Knoll, A. S. Quigg, J. A. Raven, O. Schofield, and F. J. R. Taylor. 2004. The evolution of modern eukaryotic phytoplankton. Science **305:** 354–360.

Finkel, Z. V., A. S. Quigg, J. A. Raven, J. R. Reinfelder, O. E. Schofield, and P. G. Falkowski. 2006. Irradiance and the elemental stoichiometry of marine phytoplankton. Limnol. Oceanogr. **51:** 2690–2701.

Foster, P. L., and F. M. M. Morel. 1982. Reversal of cadmium toxicity in a diatom: An interaction between cadmium activity and iron. Limnol. Oceanogr. **27:** 745–752.

Frew, R., A. Bowie, P. Croot, and S. Pickmere. 2001. Macronutrient and trace-metal geochemistry of an *in situ* iron-induced Southern Ocean bloom. Deep Sea Research Part II: Topical Studies in Oceanography **48:** 2467–2481.

Gall, M. P., R. Strzepek, M. Maldonado, and P. W. Boyd. 2001. Phytoplankton processes. Part 2: Rates of primary production and factors controlling algal growth during the Southern Ocean Iron RElease Experiment (SOIREE). Deep Sea Research Part II: Topical Studies in Oceanography **48:** 2571–2590.

Hansen, C. T., C. O. Nielsen, R. Dietz, and M. M. Hansen. 1990. Zinc, cadmium, mercury and selenium in Minke whales, belugas and narwhals from West Greenland. Polar Biol. **10:** 529–539.

Ho, T.-Y., A. Quigg, Z. V. Finkel, A. J. Milligan, K. Wyman, P. G. Falkowski, and F. M. M. Morel. 2003. Elemental composition of some marine phytoplankton. J. Phycol. **39:** 1145–1159.

Honda, K., Y. Yamamoto, H. Kato, and R. Tatsukawa. 1987. Heavy metal accumulations and their recent changes in southern Minke whales *Balaenoptera acutorostrata*. Arch. Environ. Contam. Toxicol. **16:** 209–216.

Kruzynski, G. M. 2004. Cadmium in oysters and scallops: The BC experience. Toxicol. Lett. **148:** 159–169.

Kudo, I., H. Kokubun, and K. Matsunaga. 1996. Chemical fractionation of phosphorus and cadmium in the marine diatom *Phaeodactylum tricornutum*. Mar. Chem. **52:** 221–231.

Lane, T. E., M. A. Saito, G. N. George, I. J. Pickering, R. C. Prince, and F. M. M. Morel. 2005. A cadmium enzyme from a marine diatom. Nature **435:** 42,

Loscher, B. M., J. van der Meer, H. J. W. De Baar, P. M. Saager, and J. T. M. de Jong. 1997. The global Cd/phosphate relationship in deep ocean waters and the need for accuracy. Mar. Chem. **59:** 87–93.

Martin, J. H., and W. W. Broenkow. 1975. Cadmium in plankton: Elevated concentrations off Baja California. Science **190:** 884–885.

———, and G. A. Knauer. 1973. The elemental composition of plankton. Geochimica et Cosmochimica Acta **37:** 1639–1653.

Miao, A.-J., and W.-X. Wang. 2004. Relationships between cell-specific growth rate and uptake rate of cadmium and zinc by a coastal diatom. Mar. Ecol. Prog. Ser. **275:** 103–113.

Payne, C. D., and N. M. Price. 1999. Effects of cadmium toxicity on growth and elemental composition of marine phytoplankton. J. Phycol. **35:** 293–302.

Quigg, A., and others. 2003. The evolutionary inheritance of elemental stoichiometry in marine phytoplankton. Nature **425:** 291–294.

Saager, P. M., and H. J. W. De Baar. 1993. Limitations to the quantitative application of Cd as a paleoceanographic tracer, based on results of a multi-box model (MENU) and statistical considerations. Global and Planetary Change **8:** 69–92.

Saito, M. A., D. M. Sigman, and F. M. M. Morel. 2003. The bioinorganic chemistry of the ancient ocean: The co-evolution of cyanobacterial metal requirements and the biogeochemical cycles at the Archean-Proterozoic boundary. Inorganica Chimica Acta **356:** 308–318.

Silver, S. 1996. Bacterial resistances to toxic metal ions—A review. Gene **179:** 9–19.

Strzepek, R. F., and N. M. Price. 2000. Influence of irradiance and temperature on the iron content of the marine diatom *Thalassiosira weissflogii* (Bacillariophyceae). Mar. Ecol. Prog. Ser. **206:** 107–117.

Sunda, W. G., and S. A. Huntsman. 1997. Interrelated influence of iron, light and cell size on marine phytoplankton growth. Nature **390:** 389–392.

———. 1998a. Control of Cd concentrations in a coastal diatom by interactions among free ionic Cd, Zn, and Mn in seawater. Environ. Sci. Technol. **32:** 2961–2968.

———. 1998b. Interactive effects of external manganese, the toxic metals copper and zinc, and light in controlling cellular manganese and growth in a coastal diatom. Limnol. Oceanogr. **43:** 1467–1475.

———. 2000. Effect of Zn, Mn, and Fe on Cd accumulation in phytoplankton: Implications for oceanic Cd cycling. Limnol. Oceanogr. **45:** 1501–1516.

———. 2004. Relationships among photoperiod, carbon fixation, growth, chlorophyll *a*, and cellular iron and zinc in a coastal diatom. Limnol. Oceanogr. **49:** 1742–1753.

Walsh, R. S., and K. A. Hunter. 1992. Influence of phosphorus storage on the uptake of cadmium by the marine algal *Macrocystis pyrifera*. Limnol. Oceanogr. **37:** 1361–1369.

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