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Responses of Zn assimilation by coastal plankton to macronutrients

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Abstract

We examined the Zn uptake in marine diatoms and its transfer to marine copepods under different nutrient-replete and -deplete conditions. Zn uptake, quantified by measurements of both total cellular and intracellular Zn accumulation in two coastal diatoms (*Thalassiosira pseudonana* and *Thalassiosira weissflogii*), was greatly dependent on the ambient nitrogen conditions. Semicontinuous culture experiments demonstrated that diatom cells accumulated much less Zn with decreasing ambient nitrate concentration. The calculated Zn uptake rate decreased by 1.8 times in *T. pseudonana* and 1.5 times in *T. weissflogii* with a decrease in ambient N concentration from 176 μM to 17.6 μM. N-starved cells also accumulated much less Zn compared with N-enriched cells. The uptake rate decreased by 2.5–2.6 times when both diatoms were starved of N for 2 d. The intracellular partitioning of Zn in the diatom *T. pseudonana* was positively related to the ambient N level within the short-term exposure period (5 h). P starvation, however, resulted in an increase in Zn accumulation in the diatoms, whereas Si starvation did not significantly affect the rate of Zn uptake in diatoms. The trophic transfer was quantified by measurement of Zn assimilation efficiency in two copepods (*Calanus sinicus* and *Acartia spinicauda*) feeding on diatoms with a different N quota. Assimilation generally increased with an increase in the N quota of the diatoms. In one experiment (*C. sinicus* feeding on diatom *T. weissflogii*), the influence of different N status in diatoms on Zn assimilation by copepods was statistically significant. In contrast, the elimination rate (physiological turnover rate) was independent of the N quota of the cells. Nitrogen enrichment may lead to an increase in Zn uptake and transfer in marine plankton. Our study therefore suggests that there is considerable interaction between macronutrients and Zn uptake by plankton in aquatic systems.

Many trace metals are biologically required by aquatic organisms but can be toxic at elevated concentrations. Trace metal uptake in marine phytoplankton has been extensively investigated over the past decades because of the significance of phytoplankton in ecosystem dynamics (Sunda 1994; Sunda and Huntsman 1998). Various geochemical and biological processes are known to affect metal uptake in these organisms (Campbell 1995; Sunda and Huntsman 1998, 2000). It is now well established that metal speciation critically affects trace metal accumulation in marine phytoplankton. In contrast to the extensive studies on the influences of metal geochemical factors on metal uptake, relatively few studies have considered the influence of environmental factors on metal accumulation. Recent studies have shown that the ambient pCO_2 level can greatly affect Zn and Cd uptake in marine phytoplankton (Morel et al. 1994; Cullen et al. 1999). The influence of the pCO_2 level on Zn uptake is primarily due to the involvement of Zn as a cofactor in carbonic anhydrase that converts inorganic biocarbonate into CO_2 for photosynthesis. Similarly, Cd is involved in carbonic anhydrase, and its uptake is influenced by the ambient pCO_2 level (Cullen et al. 1999). Recently, we have shown that the nutritional conditions of the algal cells can influence metal uptake in marine phytoplankton (Wang and Dei 2001*a*). Among the different species of phytoplankton examined, Cd uptake was most affected by the N concentration, whereas anionic metals such as Cr(VI) and Se(IV) were related to the ambient phosphate and silicate concentrations, but not the addition of nitrate (Wang and Dei 2001*b*).

In many estuarine and coastal waters, a disproportionate input of macronutrients has resulted in considerable change in the composition and productivity of the phytoplankton community (Sanders et al. 1987; Vitousek et al. 1997; Smith et al. 1999). The consequences of nutrient enrichment include hypoxia/anoxia and the increasing occurrence of harmful algal blooms that have been documented in many coastal and estuarine waters (Paerl 1988), including Hong Kong. Whether nutrient enrichment has resulted in changes in the cycling of toxic metals in the marine ecosystem remains essentially unknown. In many coastal waters, nutrient enrichment and metal pollution may have occurred simultaneously because of sewage and atmospheric deposition, and there is considerable interest in the interaction between trace metals and macronutrients in aquatic systems (Breitburg et al. 1999; Cloern 2001).

The influences of macronutrients on the uptake of metals by marine phytoplankton and their subsequent transfer along marine food webs are relatively less well studied. In most previous studies on metal–phytoplankton interaction, metal accumulation in algal cells was determined in a chemically defined medium without addition of macronutrients or at a fixed concentration. The transfer of metals from phytoplankton to zooplankton potentially can be influenced by the physiological conditions of the algal cells, but this remains essentially unknown. Such information is important for the prediction of the fate of metals in a planktonic system affected by enrichment from various nutrients. Furthermore,

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toxicity testing using algal cells (such as the diatom *Skeletonema costatum*) can be affected if metal uptake, and thus potential toxicity, is dependent on the ambient nutrient condition. In freshwater systems, considerable evidence indicates that the stoichiometry of carbon, nitrogen, and phosphorus of algal cells can affect the cycling and transfer of these major nutrients in planktonic food chains (Sterner and Hessen 1994; Elser and Urabe 1999).

In this study, we examined the influence of macronutrients on Zn uptake in marine phytoplankton and zooplankton. Zn is an essential metal to aquatic organisms but can be toxic at high concentrations. There is now a substantial concern about the setting of water quality criteria for this metal, primarily because it can be regulated by many aquatic animals (Chapman et al. 1996). We measured Zn uptake in two diatom cells manipulated under different nutrient conditions, including nutrient starvation and long-term nutrient acclimation (i.e., in a semicontinuous culture). In a previous study, we demonstrated that Zn uptake in phytoplankton was affected by short-term acclimation to different N levels (Wang and Dei 2001*a*). No previous study has examined the Zn uptake in N-starved cells and in cells maintained in semicontinuous culture. The Zn uptake in this study was quantified by measurements of both total Zn and intracellular Zn accumulation in the diatoms. We further determined the trophic transfer of Zn, quantified by measurement of Zn assimilation efficiency, to marine copepods feeding on diatoms of different N status.

Materials and methods

Phytoplankton and copepods—Two species of the coastal diatom *Thalassiosira pseudonana* (clone 3H) and *Thalassiosira weissflogii* (CCMP 1048) were obtained from the Provasoli-Guillard Phytoplankton Collection Center, Maine, and were maintained in axenic conditions in an f/2 medium (Guillard and Ryther 1962) at 18°C and under light illumination of 100 μmol photons m^{-2} s^{-1} with a 14 : 10 h light : dark (LD) cycle. The seawater used in all experiments was collected 10 km off East Hong Kong. The calanoid copepods (*Calanus sinicus* and *Acartia spinicauda*) were collected by net tows (250 and 500-μm mesh sizes) from Port Shelter, Clear Water Bay, Hong Kong. Copepods were fed with the diatoms during the 1-d acclimation period in the laboratory.

Zn uptake in phytoplankton under different nutrient regimes—Radiotracer ^{65}Zn (in 0.1 N HCl, obtained from New England Nuclear) was used to trace the uptake of stable Zn in the cells. Addition of radioisotope ^{65}Zn corresponded to a Zn concentration of 32 pM. All nutrient stocks were passed through a Chelex ion exchange resin column to remove trace metals. To measure the rate of Zn uptake in the cells, we exposed the diatom cells to radiotracer and stable Zn (77 nM) under different nutrient conditions over a short-term exposure period (5 h). Stable Zn, radiotracer, and macronutrients were equilibrated overnight before the uptake experiments. All uptake experiments were conducted in 150 ml of 0.2-μm–filtered seawater held in acid-cleaned polycarbonate bottles. The handling of flasks, sampling, and tracer and stable Zn additions were performed in a trace metal clean bench (Class 100), whereas the filtration was performed outside the clean bench. The light illumination was 100 μmol photons m^{-2} s^{-1} and the temperature was 18°C.

To measure the Zn uptake in the diatoms, cells that were from the same batch and inoculated under different nutrient conditions were collected onto 3-μm polycarbonate membranes, rinsed, and resuspended in 0.2-μm–filtered seawater. After determining the cell density by a Coulter counter, the cells were added into 150 ml of filtered seawater containing radiotracer, stable Zn, and different additions of nutrients. Cell density in the medium was 5×10^5 cells ml^{-1} for *T. pseudonana*, and 10^5 cells ml^{-1} for *T. weissflogii*. Each experimental treatment was replicated twice. A control treatment containing the same experimental medium was used to monitor the precipitation of metals and the potential sorption of metals onto the polycarbonate membrane. Results indicated a negligible fraction of Zn detected in the polycarbonate membrane (<0.9% in experiments involving N and P additions and <3.1% in experiments involving Si additions, see below). At time intervals (1–2 h), a 10-ml aliquot was filtered onto a 1-μm polycarbonate membrane and was rinsed with filtered seawater, after which the radioactivity of the cells was measured. An increase in cell density during the 24-h radioactive exposure period was determined microscopically by cell count.

To measure dry weight, the cells were filtered onto a preweighed glass fiber filter, rinsed with ammonium formate, and dried at 80°C for 1 d. The protein content of the cells was measured by the bicinchroninic acid (BCA) method. The intracellular distribution of Zn in the diatom cells after 5 and 24 h of exposure was measured by the Ti washing technique (Hudson and Morel 1989; Hutchins et al. 1999). Hutchins et al. (1999) recently showed that this technique was very effective (>90%) in removing extracellular Zn.

Two experiments were conducted to examine the influence of nutritional conditions on Zn uptake in diatoms. In the first experiment, we examined the influence of nutrient (N, P, or Si) starvation on Zn uptake in the cells. Diatom cells in the exponentially growth phase from the same batch of cells were filtered and resuspended in either nutrient-depleted seawater (<0.2 μm) or in a nutrient-enriched medium (with a concentration of 882 μM nitrate, 36.2 μM phosphate, and 105.6 μM silicate). During starvation of one nutrient, other nutrients were added at the f/2 levels to ensure that the cells were only starved for the one desired nutrient. The cell density was monitored on a daily basis. There was no cell growth when the cells were starved of N for 2 d, P for 3 d, or Si for 1 d. Thus, the cells were starved of N for 2 d, P for 3 d, and Si for 1 d, respectively. Both the starved cells and the enriched cells were then collected and resuspended in either nutrient-depleted or -enriched filtered seawater for Zn uptake measurement. The nutrient concentration in the enriched filtered seawater was 176 μM of nitrate, 18.1 μM of phosphate, or 26.4 μM of silicate. We did not measure the Zn uptake at a higher Si concentration because our previous study found that a considerable fraction of Zn was coprecipitated with Si at >70 μM concentration (Wang and Dei 2001*a*).

In the second experiment, the influence of long-term acclimation to different N or P concentrations on Zn uptake in

diatoms was measured. Diatoms in the exponential growth phase from the same batch of cells were filtered and resuspended into filtered seawater containing different nitrate concentrations (17.6, 58.7, and 176 μM) or different P concentrations (0.72 and 7.2 μM) in a semicontinuous culture. Other macronutrients were added at f/10 levels. The cells were transferred to a new medium containing the same nitrate concentration when they reached the midpoint stage of the exponential growth phase (every 1–2 d). After five transfers, the cells were filtered again and resuspended in filtered seawater containing the same experimental nitrate concentration for measurement of Zn uptake. During the acclimation and uptake periods, the decline of nitrate concentration due to cellular uptake was monitored by methods described in Parsons et al. (1985). In general, we found that after 2 d acclimation, the ambient nitrate concentration declined by about 70% at the lowest nitrate treatment, whereas there was only a 10% decline of nitrate at the highest nitrate treatment. There was no significant decline of nitrate during the 5-h exposure period.

To determine whether the responses of Zn at 77 nM were representative of other Zn concentrations, we also conducted an experiment to quantify the Zn uptake at different ambient Zn concentrations (32 pM—only radioisotope addition—and additions of 30, 77, and 154 nM). The background Zn concentration in seawater used in our experiments was not experimentally quantified, and the free ion Zn concentration in the medium was not controlled by addition of chelating compounds. Our data indicated that the uptake rate constant (i.e., slope of the regression between the ratio of Zn concentration in diatoms to Zn concentration in ambient water and the time of exposure) was comparable among the three concentrations examined, suggesting that the uptake rate constant was relatively independent of the ambient Zn concentration ($<$154 nM).

Trophic transfer of Zn in marine copepods—The assimilation efficiency (AE) of Zn in marine copepods (*Calanus sinicus* and *Acartia spinicauda*) feeding on the diatoms *Thalassiosira weissflogii* and *Thalassiosira pseudonana* maintained under different nutrient conditions was determined using established methods (Wang and Fisher 1998*a*, 1999). The diatoms were first maintained in semicontinuous culture conditions (17.6, 58.7, and 176 μM) by transferring the cells when they reached the exponential growth phase. To radiolabel the diatoms, the cells were collected and resuspended in 100 ml of filtered seawater containing the f/2 levels of P, Si, and vitamins and the f/20 levels of Mn, Co, Fe, and Mo, as well as different nitrate concentrations (17.6, 58.7, and 176 μM). No stable Zn, Cu, and ethylenediaminetetraacetic acid (EDTA) were added. After the cells reached the exponential phase (within 2 d), they were filtered again and resuspended into a new medium containing the same concentrations of nitrate and other nutrients. After a further 2 d of growth in the medium (with the presence of ^{65}Zn), the cells were then filtered and resuspended in a small volume of water before being fed to the copepods. In another experiment, the diatom *T. pseudonana* was inoculated in f/2 nutrient levels for 3 d, allowing the cells to divide five times. The cells were then collected by filtration and resuspended in a P-depleted medium for 3 d in the presence of ^{65}Zn. Other major nutrients were added to the medium. The cells were collected before being fed to the copepods.

The radiolabeled diatom cells were pulse-fed to the copepods maintained in 100 ml of filtered seawater at 22°C. The cell density of the diatoms was 10^5 cells ml^{-1} for *T. pseudonana* and 10^4 cells ml^{-1} for *T. weissflogii* (corresponding to a biomass of 2.2–2.4 mg L^{-1}), and the copepod density was 0.3 copepods ml^{-1}. After 15 min of feeding on radiolabeled diatoms in the dark, copepods were removed with a mesh, and their radioactivity was counted immediately. Any feces produced during the radiolabeled feeding period was also collected by a mesh (20 μm) and radioassayed. The copepods were depurated in nonradioactive waters for 30–48 h in the presence of the same diatom diet in 120 ml of filtered seawater. At time intervals (every few hours), the radioactivity retained in the copepods was determined. Water and diatom diets were renewed each time the radioactivity in copepods was counted. The AE and elimination rate were calculated as the *y*-intercept and the slope of the linear regression between the natural log of the percentage of metals retained in copepods and the time of depuration (between 6 and 30 or 48 h) (Wang and Fisher 1998*a*, 1999).

$$A\% = A_0\% \exp(-kt) \quad (1)$$

A% is the percentage of Zn retained in copepods at time t (during the second compartment of depuration, or the physiological loss); A_0% is the initial percentage of Zn retained in the copepods, or the AE; k is the depuration rate constant; and t is the time of depuration. This calculation assumed that the first compartment of loss represented the digestive process and the second compartment of loss represented the physiological turnover of metals in copepods.

We also quantified the ingestion activity of copepods by measuring the initial and final cell density during the 15-min period of radioactive feeding. The fraction of Zn in the algal cytoplasm was measured as described in Fisher et al. (1983).

Radioactivity of ^{65}Zn was determined by a Wallac gamma detector at 1,115 keV. Counting times of the samples were 1.5–3 min, and the propagated errors were generally $<$3%.

Results

Influences of macronutrients on Zn uptake in the diatoms—In general, a linear pattern of Zn uptake against time (between 1 and 5 h) was observed during the short-term exposure period (Figs. 1–3). The *y*-intercept of such linear regression may largely reflect the surface sorption of Zn onto the diatoms (*see* Mirimanoff and Wilkinson 2000). When the diatoms *Thalassiosira pseudonana* and *Thalassiosira weissflogii* were starved of N for 2 d, their Zn accumulation was considerably lower than cells maintained in an N-enriched medium (Fig. 1). In these experiments, about 43–54% and 50–59% of ambient total Zn were removed by the diatoms *Thalassiosira pseudonana* and *Thalassiosira weissflogii*, respectively, at 5 h of exposure. In both diatom species, the Zn concentrations increased 2.1–2.2 times more in N-enriched cells than in N-starved cells after 5 h exposure (1–5 h). The uptake rates, calculated from the slope of the Zn

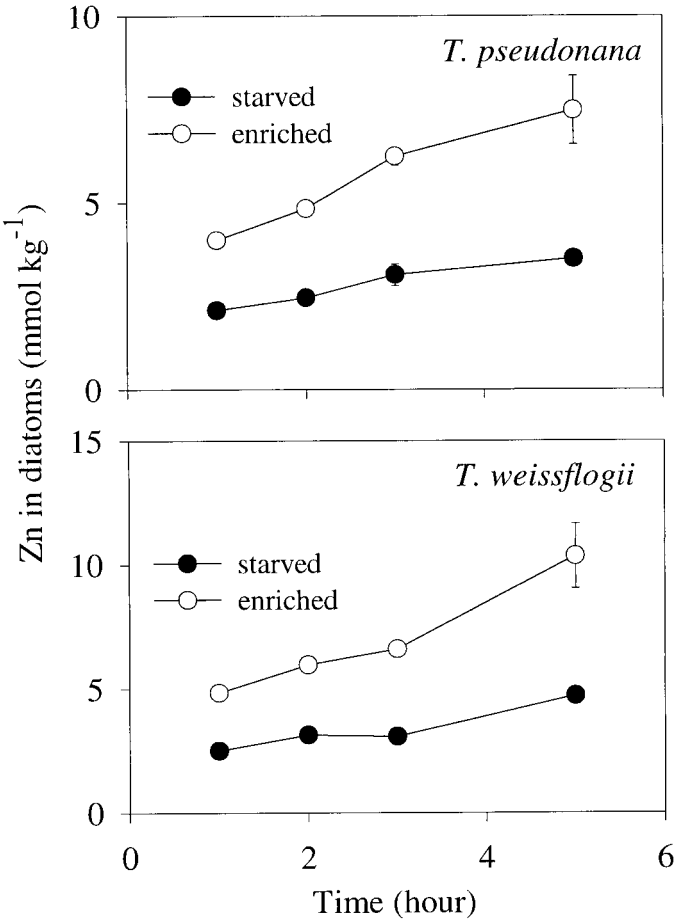

Fig. 1. Accumulation of Zn in the marine diatoms *Thalassiosira pseudonana* and *Thalassiosira weissflogii* starved or enriched with nitrate. Data are means ± semirange ($n = 2$).

concentration in diatoms against time of exposure, were 2.5–2.6 times higher in N-enriched cells than in N-starved cells (Table 1). Similarly, more Zn was found to partition into the intracellular compartment in N-enriched cells than in N-starved cells (Table 1). For example, at 5 h of exposure, 19.6% Zn in *T. pseudonana* was associated with the intracellular pool in N-starved cells, compared with 32.4% of Zn in N-enriched cells. At 24 h, 22.2% of Zn was associated with the intracellular pool in N-starved cells, compared with 37.0% of Zn in N-enriched cells.

P-starved cells had a much higher Zn accumulation than the P-enriched cells (Fig. 2). This experiment was repeated three times, and the results were consistent among the three replicate experiments. The major difference in Zn uptake between the P-starved and P-enriched cells was primarily within the first 1 h of exposure. The uptake rate, calculated from the slope of Zn concentration in cells against the time of exposure (1–5 h) was 1.9–2.4 times higher in P-starved cells than in P-enriched cells. We also examined whether the difference in P concentration in the exposure medium affected the Zn uptake in either P-starved or P-enriched cells. There was no difference in the accumulation of Zn by P-starved and P-enriched cells at different P concentrations (data not shown). In this experiment, more Zn was found in the diatom's intracellular pool in P-enriched cells than in P-starved cells. For example, 35% of Zn was associated with the intracellular pool in P-starved cells at 24 h, compared with 60% of Zn in P-enriched cells. In contrast, the uptake rate of Zn was relatively unaffected by Si starvation (Table 1), although Zn concentration in Si-enriched diatoms was somewhat higher than its concentration in Si-starved cells. Higher intracellular Zn distribution was also found for Si-enriched cells than for Si-starved cells.

When both diatom cells were maintained in a semicontinuous culture (under a 14 : 10 h LD cycle) at three different nitrate levels, the growth rate constants were 0.395 ± 0.126, 0.611 ± 0.273, and 0.852 ± 0.287 d^{-1} (mean ± SD) for *T. pseudonana* and 0.365 ± 0.154, 0.505 ± 0.183, and 0.548 ± 0.175 d^{-1} for *T. weissflogii* at 17.6 μM, 58.7 μM, and 176 μM, respectively. During the 24-h radioactive exposure period, the growth rate constants of the diatoms were 0.194 ± 0.011, 0.385 ± 0.001, and 0.515 ± 0.017 d^{-1} (mean ± semirange, $n = 2$) for *T. pseudonana* and 0.136 ± 0.021, 0.204 ± 0.012, and 0.370 ± 0.007 d^{-1} for *T. weissflogii* at

Table 1. The calculated Zn uptake rate (measured at a Zn concentration of 77 nM) and the distribution of Zn in the intracellular compartment of diatom *Thalassiosira pseudonana* and *Thalassiosira weissflogii* following 5 and 24 h exposure under different nutrient conditions. Mean ± semirange ($n = 2$). Nd, not determined.

Diatom	Nutrient condition	Uptake rate (mmol kg^{-1} h^{-1})	% intracellular Zn at 5 h	% intracellular Zn at 24 h
T. pseudonana	N-starved	0.353 ± 0.022	19.6 ± 4.9	22.2 ± 0.6
	N-enriched	0.884 ± 0.208	32.4 ± 8.7	37.0 ± 5.6
	P-starved (Expt. 1)	6.966 ± 0.509	Nd	Nd
	P-enriched (Expt. 1)	2.862 ± 2.190	Nd	Nd
	P-starved (Expt. 2)	3.311 ± 0.255*	29.0 ± 1.6	34.6 ± 0.9*
	P-enriched (Expt. 2)	1.705 ± 0.174*	33.3 ± 2.5	60.1 ± 3.0*
	P-starved (Expt. 3)	2.449 ± 0.234*	Nd	Nd
	P-enriched (Expt. 3)	1.168 ± 0.080*	Nd	Nd
	Si-starved	1.384 ± 0.096	17.3 ± 3.8	36.1 ± 4.4
	Si-enriched	1.696 ± 0.248	31.7 ± 2.0	44.6 ± 2.3
T. weissflogii	N-starved	0.533 ± 0.067	17.2 ± 0.9*	Nd
	N-enriched	1.370 ± 0.277	22.7 ± 0.6*	Nd

* Statistically significant difference between two treatments at $P < 0.05$.

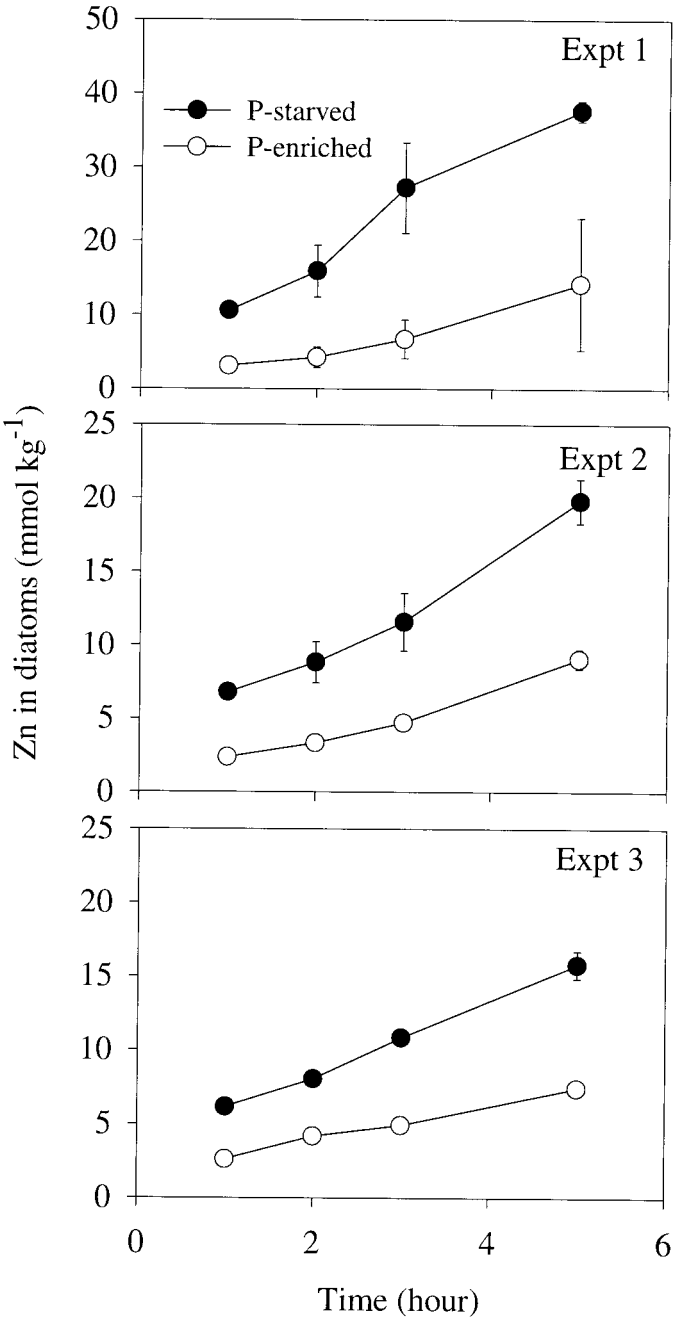

Fig. 2. Accumulation of Zn in the marine diatom *Thalassiosira pseudonana* starved or enriched with P. Three replications of the experiment are presented. Data are means ± semirange (n = 2).

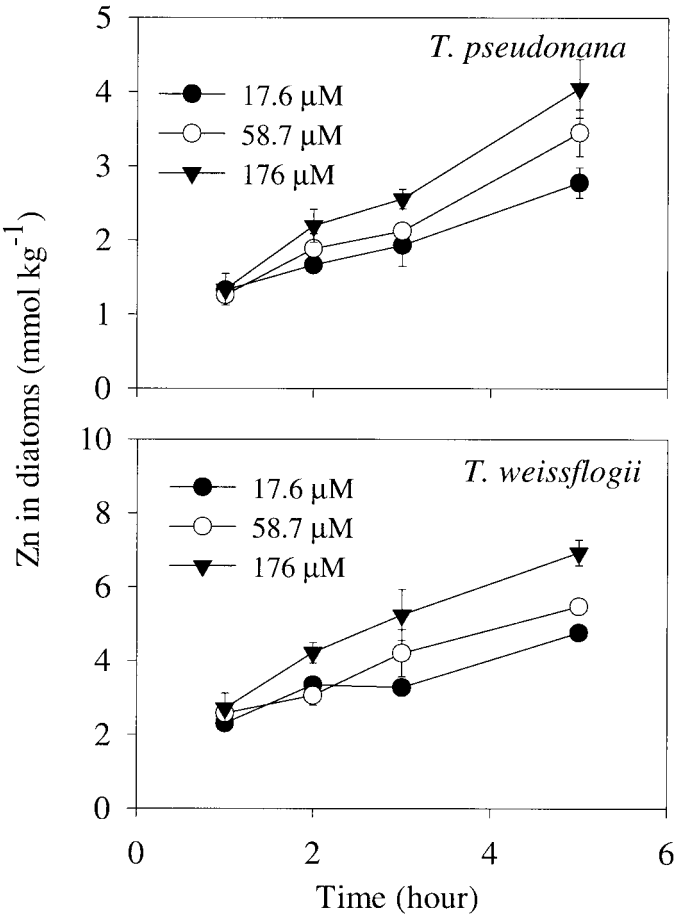

Fig. 3. Accumulation of Zn in the marine diatoms *Thalassiosira pseudonana* and *Thalassiosira weissflogii* inoculated in a semicontinuous culture. Diatom cells were maintained at 17.6, 58.7, or 176 μM of nitrate. Data are means ± semirange (n = 2).

17.6 μM, 58.7 μM, and 176 μM, respectively. The growth rate constants measured during the radioactive uptake period were lower than those measured during the semicontinuous culture because no nutrient other than N (at different concentrations) was added into the medium. There was a notable difference in Zn uptake among different treatments (Fig. 3). The Zn concentrations in the cells, for example, were 1.3–1.5 times higher in the 176-μM treatment than in the 17.6-μM treatment in both diatoms. In these experiments, about 21–40% and 39–54% of ambient total Zn was removed by the diatom *T. pseudonana* and *T. weissflogii*, respectively, at 5 h of exposure. The uptake rate of Zn was calculated from the slope of the linear regression between Zn accumulation in diatoms against the time of exposure (1–5 h). An increase in nitrate concentrations resulted in a significant increase in Zn uptake in *T. weissflogii* ($P < 0.01$, one-way ANOVA), but not in the diatom *T. pseudonana* ($P = 0.065$, one-way ANOVA). Uptake in *T. pseudonana* was 1.8 times higher at 176 μM than at 17.6 μM. In *T. weissflogii*, the uptake rate was 1.5 times higher at 176 μM than at 17.6 μM. By the end of 5 and 24 h of exposure, the fraction of Zn in the intracellular pool generally increased with increasing N concentration in the diatom *T. pseudonana*, whereas its subcellular distribution was not greatly affected by ambient N concentration in diatom *T. weissflogii* (Table 2).

When the diatom cells were maintained in a semicontinuous culture (under a 14 : 10 h LD cycle) at two different P levels, the growth rate constants were 0.835 ± 0.100 and 0.782 ± 0.185 d^{-1} (mean ± SD) for *T. pseudonana* and 0.352 ± 0.170 and 0.457 ± 0.178 d^{-1} for *T. weissflogii* at 0.72 μM and 7.2 μM, respectively. During the 24-h radioactive exposure period, the growth rate constants of the diatoms were 0.316 ± 0.005 and 0.328 ± 0.010 d^{-1} (mean ±

Table 2. The calculated Zn uptake rate (measured at a Zn concentration of 77 nM) and the distribution of Zn in the intracellular compartment of diatom *Thalassiosira pseudonana* and *Thalassiosira weissflogii* following 5 and 24 h exposure under different nitrate and phosphate conditions in semicontinuous culture. Mean ± semirange ($n = 2$).

Diatom	N or P concentration (μM)	Uptake rate (mmol kg^{-1} h^{-1})	% intracellular Zn at 5 h	% intracellular Zn at 24 h
N experiment				
T. pseudonana	17.6	0.360 ± 0.050	21.2 ± 2.2	40.8 ± 1.1
	58.7	0.534 ± 0.065	29.2 ± 3.2	51.4 ± 3.3
	176	0.658 ± 0.044	35.8 ± 2.0	47.1 ± 1.3
T. weissflogii	17.6	0.569 ± 0.010**	23.4 ± 2.5	32.5 ± 3.7
	58.7	0.750 ± 0.031**	24.2 ± 3.0	36.4 ± 1.1
	176	1.028 ± 0.003**	19.6 ± 0.7	35.6 ± 3.3
P experiment				
T. pseudonana	0.72	1.111 ± 0.309	48.3 ± 2.4	60.1 ± 1.6
	7.2	1.458 ± 0.055	35.4 ± 6.0	53.8 ± 4.6
T. weissflogii	0.72	1.023 ± 0.128	36.3 ± 1.2	48.4 ± 3.3
	7.2	0.985 ± 0.095	35.1 ± 3.5	46.0 ± 2.1

** Statistically significant difference between two treatments at $P < 0.01$.

semirange, $n = 2$) for *T. pseudonana* and 0.161 ± 0.013 and 0.204 ± 0.019 d^{-1} for *T. weissflogii* at 0.72 μM and 7.2 μM, respectively. The growth rate constants measured during the radioactive uptake period were lower than those measured during the semicontinuous culture because no N was added into the medium. There was no major difference in the calculated Zn uptake rate between the two P levels for both species of diatoms (Table 2).

Trophic transfer of Zn in copepods—In general, the diatoms maintained at a lower N concentration tended to have a lower Zn distribution in algal cytoplasm, although such a trend was not pronounced in some cases (Table 3). In these experiments, the diatoms were exposed to different N concentrations for several divisions, and the responses of subcellular distribution to nutrients may have been different from those responses determined within a short-term exposure period (see above). Diatoms also contained a higher protein content with increasing N concentrations, but the protein concentrations measured in experiments involved with copepod *A. spinicauda* were lower than the protein concentrations measured in diatoms involving copepod *C. sinicus* (Table 3). Depuration of ingested Zn in marine copepods (*C. sinicus* and *A. spinicauda*) feeding on *T. pseudonana* and *T. weissflogii* inoculated under different N levels is shown in Figs. 4, 5. Depuration was characterized by a rapid egestion of unassimilated Zn within the first 3 h and then a slower depuration between 6 and 30 or 48 h of depuration. More Zn was egested within the first 3 h in animals feeding on diatoms with a lower N quota. In a previous study, Wang and Fisher (1998*a*) defined the first phase of depuration as the digestion and assimilation of ingested Zn and the second, slower phase of depuration as the physiological turnover (e.g., elimination). The Zn AE was thus calculated as the *y*-intercept of the slower depuration compartment and increased with an increase in the N quota of diatom cells (Table 3).

Statistical analysis indicated that N-status of the diatoms significantly affected the Zn AEs in copepod *C. sinicus* feeding on the diatom *T. weissflogii* ($P < 0.05$, one-way ANOVA), whereas the influences of N-status did not significantly affect the Zn AEs in copepod *C. sinicus* feeding on the diatom *T. pseudonana* and in copepod *A. spinicauda* feeding on both diatoms. However, the AEs in *A. spinicauda* feeding on *T. pseudonana* increased from 28 to 49% with an increase in N concentration from 17.6 to 176 μM. The Zn AE from P-starved cells was comparable to the Zn AE measured at the highest nitrate concentration (176 μM). The elimination rate constant was relatively independent of the N status of the diatom cells, and essentially remained comparable among different N treatments between the two copepod species and between the two diatom diets. The ingestion activities of copepods were also quantified during the short 15-min feeding period and were found to be comparable among different N treatments.

There was no significant correlation between the Zn AE and Zn distribution in diatom cytoplasm when all experiments were considered in this study (Fig. 6). However, a significant correlation was found when all experiments but one involving *C. sinicus* feeding on diatom *T. pseudonana* were considered together. For *C. sinicus* feeding on diatom *T. pseudonana* inoculated at different N concentrations and under P starvation, the measured AE appeared to increase with an increase in Zn distribution in diatom cytoplasm.

Discussion

Macronutrient influences on Zn uptake in diatoms—Our study using nutrient starvation and semicontinuous cultures demonstrated that the Zn uptake in marine diatoms was dependent on the ambient nitrate concentrations. In a recent study, we also found that the Zn uptake in several species of phytoplankton increased significantly with increasing N concentration when the cells were acclimated to different nitrate concentrations for 2 d (Wang and Dei 2001*a*). In that

Table 3. Physiological conditions of two diatom species (*Thalassiosira pseudonana* and *Thalassiosira weissflogii*) inoculated under different N concentrations and the assimilation efficiency (AE) and the elimination rate constant (k) of Zn in the marine copepod *Calanus sinicus* and *Acartia spinicauda*. Data are means ± semirange or SD (n = 2–3). Nd, not determined.

	T. weissflogii			*T. pseudonana*			
	16.7 μM N	58.7 μM N	167 μM N	16.7 μM N	58.7 μM N	167 μM N	P-starved
C. sinicus							
Protein content (pg cell^{-1})	40.3 ± 0.4	41.2 ± 0.2	60.7 ± 1.2	3.40 ± 0.05	3.76 ± 0.06	4.95 ± 0.09	3.72 ± 0.24
% Zn in diatom cytoplasm	52.2 ± 2.6	52.8 ± 0.5	58.7 ± 0.2	18.1 ± 0.1	23.5 ± 0.8	37.7 ± 1.5	33.5 ± 0.1
Zn AE (%)	43.7 ± 6.4	55.7 ± 1.8	69.6 ± 0.8	56.1 ± 1.8	65.2 ± 4.2	68.6 ± 3.2	66.9 ± 1.9
Zn k (d^{-1})	0.304 ± 0.037	0.296 ± 0.008	0.292 ± 0.020	0.205 ± 0.022	0.219 ± 0.019	0.240 ± 0.022	0.238 ± 0.070
Ingestion rate (cells copepod^{-1} h^{-1})	28,000 ± 5670	29,908 ± 1083	30,735 ± 8996	Nd	Nd	Nd	Nd
A. spinicauda							
Protein content (pg cell^{-1})	16.1 ± 1.7	29.1 ± 0	45.9 ± 0.9	1.33 ± 0.01	2.52 ± 0.00	2.89 ± 0.29	Nd
% Zn in diatom cytoplasm	38.2 ± 5.0	26.2 ± 0.6	45.0 ± 12.2	22.4 ± 4.0	34.6 ± 1.8	27.1 ± 0.2	Nd
Zn AE (%)	46.2 ± 5.3	49.1 ± 6.6	52.1 ± 5.4	27.7 ± 6.1	42.4 ± 4.5	48.8 ± 16.4	Nd
Zn k (d^{-1})	0.374 ± 0.045	0.359 ± 0.011	0.330 ± 0.051	0.376 ± 0.018	0.364 ± 0.020	0.343 ± 0.049	Nd

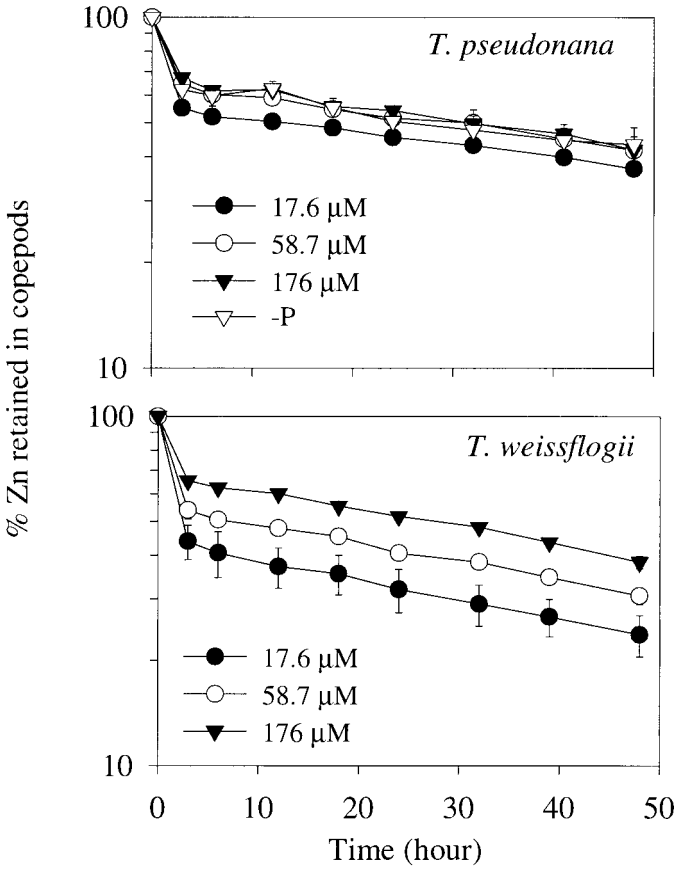

Fig. 4. The percentage of Zn retained in marine copepods (*Calanus sinicus*) following a pulse feeding on two marine diatoms (*Thalassiosira weissflogii* and *Thalassiosira pseudonana*) maintained at different nitrate concentrations in a semicontinuous culture or in a P-depleted medium. Data are means ± SD (n = 3).

study, Zn uptake in phytoplankton was quantified by the measurements of concentration factor (the ratio of Zn concentration in the cells to Zn concentration in the water) because no stable Zn was added into the medium. Our present study using a semicontinuous culture further indicated that Zn uptake was related to the ambient N concentration. In a semicontinuous culture, the ambient N concentration was maintained as constant as possible by transferring the cells to the new medium when they reached the midpoint of exponential growth phase (within 1–2 d). It is assumed that the cells had acclimated to a specific nitrate level after several transfers.

In our experiments, we did not control the speciation of Zn using the buffer solution, as has been done in many previous studies on Zn uptake in marine phytoplankton (e.g., Sunda and Huntsman 1998, 2000). The speciation of Zn in the exposed medium thus remained unclear. Further studies are needed to examine Zn responses to macronutrient additions using buffer solutions. However, because different treatments were carried out under identical conditions (except different experimental nutrient concentrations), difference in Zn uptake was probably not related to the medium chemistry but may be related to the different physiological conditions of the cells.

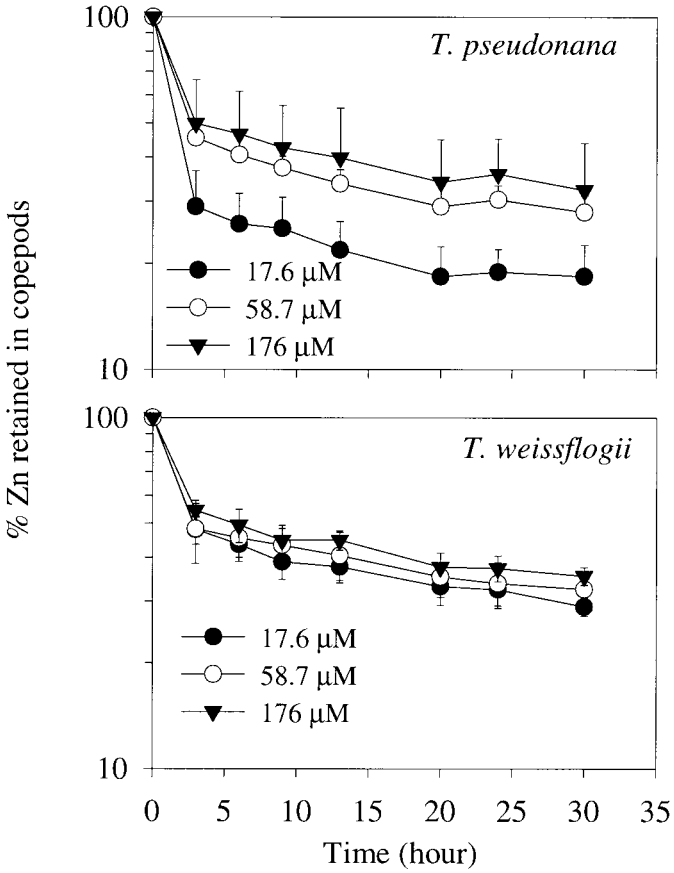

Fig. 5. The percentage of Zn retained in marine copepods (*Acartia spinicauda*) following a pulse feeding on two marine diatoms (*Thalassiosira weissflogii* and *Thalassiosira pseudonana*) maintained at different nitrate concentrations in a semicontinuous culture. Data are means ± SD ($n = 3$).

Nitrogen starvation also appeared to significantly affect Zn uptake in the diatoms. Harrison et al. (1990) found that after 2 d of nitrate starvation, the protein fraction decreased, the carbohydrate fraction increased, and the lipid fraction remained unchanged in diatom *T. pseudonana.* Similarly, we have recently found that N starvation can considerably reduce Cd uptake in the cells (Wang et al. unpubl. data). For example, the Cd uptake rate in *T. pseudonana* decreased by about four times when the cells were starved of N for 2 d. These experimental results therefore strongly indicated that the physiological conditions affected by different nitrogen conditions critically influenced Zn uptake in phytoplankton. The mechanisms underlying the influence remain to be studied but may be related to the change in protein ligands available for Zn binding for internalization (Mirimanoff and Wilkinson 2000). Consistent with this hypothesis, the intracellular Zn uptake was found to significantly relate to the N conditions in the medium. More Zn was partitioned into the intracellular compartment with increasing N levels in the medium. In addition, the higher metabolism (e.g., carbon metabolism) brought about by a higher N concentration may result in a higher demand for Zn that may be involved in carbonic anhydrase that converts biocarbonate to CO_2 for photosynthesis. The higher growth rate of the cells may have resulted in a higher demand for Zn. Rijstenbil et al. (1998) have demonstrated that diatom cells (*T. pseudonana*) inoculated in N-enriched water had a much higher level of glutathione than cells that were maintained without N enrichment. They further showed that the cellular Zn level was significantly correlated with the glutathione pool as a result of Zn involvement in glutathione synthesis. Consequently, the cellular Zn concentration increased with N over a 14-d batch culture (Rijstenbil et al. 1998).

Enhanced Zn uptake under P starvation was rather unexpected in our study, although the results were consistent among three replicate experiments. In contrast, Zn uptake was not affected by different P levels when the cells were grown under conditions without P limitation. Few studies have examined the physiology and biochemistry of diatoms under P starvation conditions. La Roche et al. (1993) detected a high molecular weight membrane-associated protein induced by P limitation in the chlorophyte *Dunaliella tertiolecta.* Graziano et al. (1996) further indicated that the high molecular weight stress protein associated with the internal cell membrane was produced under P-limited conditions and increased in abundance with increasing P limitation. However, the stress protein was not produced under N or Fe limitation. It is unknown whether the induced stress protein may have been involved in Zn uptake and transport. It was noticeable that the influence of P starvation occurred primarily during initial uptake (within the first 1 h), during which Zn sorption presumably dominated the uptake (Mirimanoff and Wilkinson 2000). Furthermore, our data also indicated that most Zn was associated with the extracellular pool in P-starved cells. In contrast to N and P starvation, we did not find any influence of Si starvation on the rate of Zn uptake.

Thus, macronutrients, in addition to aqueous metal chemistry, should be considered when studying Zn uptake in marine phytoplankton. In many coastal waters, nitrogen enrichment may have considerably increased the biological uptake of Zn in phytoplankton. Conversely, in regions where nitrate concentration is relatively low (i.e., oceanic), the biological uptake of Zn may be limited, which could lead to Zn limitation in the cells because of their biological need for it. Field verification of this hypothesis is needed. In addition, whether the Zn concentration in the cells was dependent on the ambient N concentration remains to be studied further. According to a kinetic model, Zn concentration in cells can be predicted from the Zn uptake rate divided by the growth rate constant of the cells (Sunda and Huntsman 1998). Because both Zn uptake rate and cell growth rate are dependent on the N conditions, whether Zn concentration in the cells will change with ambient N concentration is determined by the degree to which Zn uptake rate and growth rate are affected by the N concentration. Breitburg et al. (1999) recently showed a strong interaction between nutrients (added as N and P) and trace elements (added together as As, Cu, Cd, Zn, and Ni) on phytoplankton communities in mesocosm experiments. Trace metal addition had little or no effect in the absence of added nutrients but greatly decreased the responses of phytoplankton (e.g., becoming toxic) with the addition of nutrients. One of the likely explanations for such an interaction is the enhanced uptake of metals by phyto-

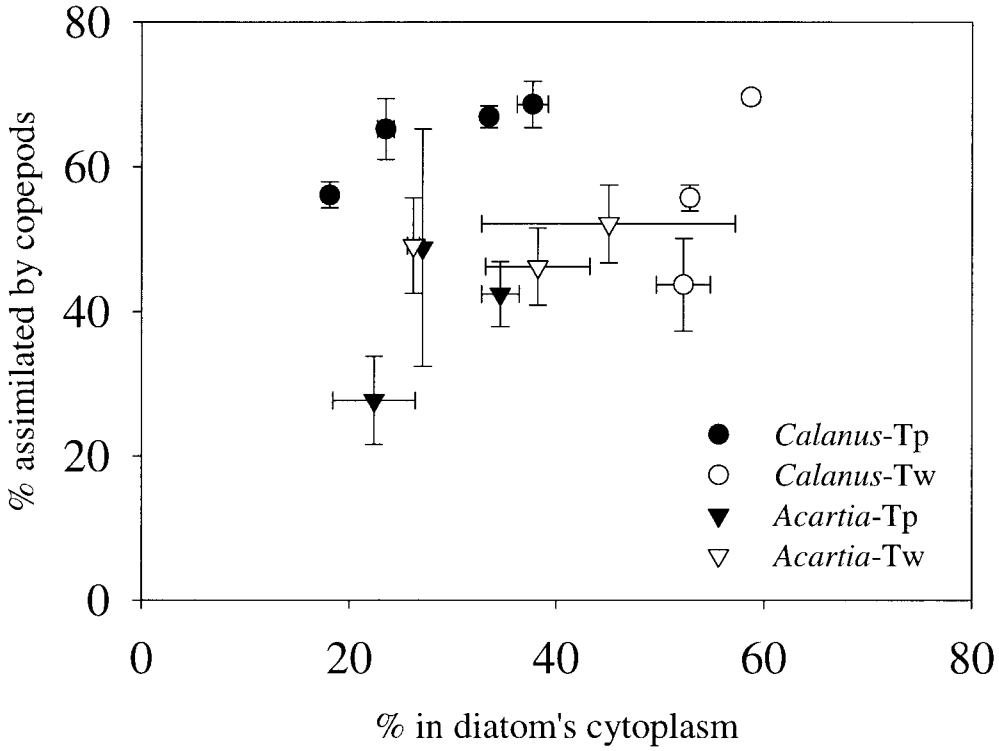

Fig. 6. Relationship between the calculated Zn assimilation efficiency in two marine copepods (*Calanus sinicus* and *Acartia spinicauda*) and Zn distribution in diatom diets (*Thalassiosira weissflogii*-Tw and *Thalassiosira pseudonana*-Tp). Data are means ± semirange or SD ($n = 2$ for Zn distribution in algal cytoplasm and $n = 3$ for Zn assimilation efficiency). Correlation between Zn assimilation (y) and its distribution in diatom cytoplasm (x) for all experiments except *C. sinicus* feeding on *T. pseudonana* was: $y = 24.8 + 0.60x$ ($r^2 = 0.501$, $P < 0.03$).

plankton because of nutrient enrichment, although metal concentration in phytoplankton cells was not determined in their mesocosm studies.

Trophic transfer of Zn in marine copepods—The increased distribution of Zn in algal cytoplasm with increasing ambient N concentration was consistent with our measurements of Zn intracellular distribution during the short-term exposure period. In these experiments, the response of Zn subcellular distribution to increasing N levels in the medium was less pronounced than the response measured within a short-term exposure period. In our study, a significant relationship between Zn AE in copepods and its distribution in diatom cytoplasm was found without considering the Zn AE in *C. sinicus* feeding on diatom *T. pseudonana*.. Reinfelder and Fisher (1991) have shown that trace element assimilation in marine copepods is directly related to their distribution in diatom cytoplasm; thus, a metal associated with the soluble cytoplasm is available for the biological uptake by copepods across the gut lining. Wang and Fisher (1996*a*), however, showed that there is no statistically significant relationship between the Zn AEs in marine mussel *Mytilus edulis* and its distribution in the cytoplasm of diverse phytoplankton diets, presumably because Zn was regulated by the mussels during the digestive period. Among the several metals studied so far (Cd, Se, Fe, Zn) (Hutchins et al. 1995; Xu et al. 2001), Zn AE in marine copepods was least correlated with its subcellular partitioning in diatoms under different environmental and physiological conditions.

Few studies have considered the influence of algal chemical compositions on metal assimilation in marine herbivores. Wang and Fisher (1996*b*) measured the AEs of Zn in marine mussels feeding on diatoms inoculated in different N levels and found that there was no significant influence of the N quota of diatoms on Zn assimilation in mussels. Thus, marine copepods were probably more responsive to difference in the N status of the cells during metal assimilation. However, the ingestion rate of copepods was relatively independent of the N status of the cells. Previous studies have indicated that the difference in food quantity and quality can considerably influence the ingestion activity of copepods (Paffenhofer and Van Sant 1985; Houde and Roman 1987).

According to a simple bioenergetic-based kinetic model (Reinfelder et al. 1998; Wang and Fisher 1998*a*), the influx of metals into marine copepods through trophic transfer can be calculated as

$$I = \mathrm{AE} \times \mathrm{IR} \times C_{\mathrm{f}}. \quad (2)$$

I is the metal influx into the copepods, AE is the assimilation efficiency of metal from ingested food sources, IR is the copepod ingestion rate, and C_{f} is the metal concentration in ingested food particles. Under steady-state conditions, the metal concentration in the copepods can be calculated by

$$C_{ss} = I/k_e. \quad (3)$$

C_{ss} is the metal concentration in copepods due to dietary uptake, and k_e is the metal efflux rate constant or the elimination rate constant.

Thus, four parameters (AE, IR, C_f, and k_e) should be considered in assessing the degree to which metal accumulation in marine zooplankton is affected by macronutrients. Of these parameters, AE, IR, and C_f can be influenced by macronutrients. The elimination rate of copepods was not affected by the N status of ingested cells; thus, the overall Zn concentration in copepods due to dietary uptake may have increased with increasing nitrogen enrichment. A recent modeling study has indicated that dietary uptake is the major pathway for the overall Zn accumulation in the marine copepod *Temora longicornis* (Wang and Fisher 1998*a*). Over 60% of Zn in copepods was predicted to derive from dietary exposure. Consequently, macronutrients can greatly affect Zn bioaccumulation in marine copepods because of their direct influence on Zn uptake and transfer in marine phytoplankton.

It is likely that a potential increase in the Zn concentration in phytoplankton under N-enriched conditions may lead to a further change in the Zn AE in copepods (i.e., a Zn concentration effect instead of a N status effect). Our recent data indicated that an increase in Zn concentration in algal cells resulted in a decrease in the Zn AE in marine copepods, leading to a potential decline in Zn influx (Xu et al. 2001). However, a Zn concentration effect was only observed at a relatively high ambient Zn concentration (154 nM).

Our measurements of the elimination rate constant of Zn were consistent with recent studies of the marine copepod *T. longicornis* (Wang and Fisher 1998*a*,*b*). Wang and Fisher (1998*a*) reported a physiological turnover rate constant of 0.25–0.29 d^{-1} in copepods pulse-fed with the diatoms *T. pseudonana* and *T. weissflogii*. When the copepods were fed with radiolabeled diatoms for a longer period (2–6 d), the physiological turnover rate of Zn was smaller and ranged between 0.05 and 0.09 d^{-1} (Wang and Fisher 1998*a*,*b*). Such a high physiological turnover rate may play a critical role in the cycling of metals in surface waters into the dissolved phase. In our study, the digestion and assimilation of dietary metals in *C. sinicus* and *A. spinicauda* were relatively rapid and were completed within 3–6 h. In *T. longicornis*, digestion and assimilation of Zn were somewhat longer (<8 h), presumably because of the lower temperature used in the experiment (15°C), which may increase the time for food to pass through the gut in copepods (Dam and Peterson 1988). The elimination rate has been shown to be a function of algal food density (Wang and Fisher 1998*b*) but was not affected by the Zn body burden in copepods (Xu et al. 2001).

Our study showed that the uptake and transfer of Zn in a marine planktonic food chain are considerably affected by ambient macronutrients. Higher uptake of Zn in marine diatoms was observed at a higher N level. N starvation may potentially lead to a reduction in Zn accumulation in diatoms. In contrast, P starvation increased the Zn uptake, whereas Si starvation did not influence Zn uptake in diatoms. The trophic transfer also was affected significantly by the nutritional conditions of food particles. The assimilation efficiency of Zn increased with an increase in the N quota of food particles. Thus, increases in Zn assimilation in copepods and possibly Zn concentration in phytoplankton diets may have increased the Zn burden in copepod bodies as a result of nitrogen enrichment. Given the increasing potential for metal uptake, metal cycling in coastal planktonic food webs may have been perturbed because of nitrogen-stimulated eutrophication. Thus, interaction between trace elements and macronutrients should be considered in ecosystem dynamic studies, as well as in ecotoxicity testing.

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