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Influence of N, P additions on the transfer of nickel from phytoplankton to copepods

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Higher nitrate or phosphate levels will facilitate the biological uptake of Ni by phytoplankton and subsequently improve its transfer to marine copepods.

Abstract

We examined the influence of macronutrient (nitrate and phosphate) additions on Ni uptake by phytoplankton (*Prorocentrum donghaiense* and *Skeletonema costatum*) and its subsequent transfer to marine copepods (*Calanus sinicus* and *Labidocera euchaeta*). Ni uptake by phytoplankton after 24 h of exposure was markedly dependent on nutrient conditions, with a higher nutrient quota facilitating Ni accumulation in the algae. Trophic transfer was quantified by measurements of the Ni assimilation efficiency in *C. sinicus* and *L. euchaeta*, feeding on the algae under different nutrient treatments. Ni assimilation efficiency generally increased with an increase of nutrient concentration in the algae. A significant positive-correlation was found between the Ni assimilation efficiencies of the copepods and the %intracellular Ni in the algal cells. However, ambient nutritional conditions had little effect on the physiological turnover rate constant of Ni by copepods. Thus, nutrient enrichment may lead to an increase in Ni uptake and transfer in marine plankton.

Keywords: Ni uptake; Assimilation efficiency; Plankton

1. Introduction

In many estuarine and coastal waters, eutrophication has become a serious environmental problem. Several studies have shown that eutrophication considerably affects phytoplankton species composition and primary production, with consequent changes in ecosystem structure and function (Sanders et al., 1987; Vitousek et al., 1997; Smith et al., 1999). Thus, nutrient enrichment might also affect the cycling of carbon, nitrogen, phosphorus, silicon and trace metals. Over the past few decades, there have been several studies of the interactions between trace metals and the marine phytoplankton (Morel et al., 1991; Hudson, 1998; Sunda and Huntsman, 1998), and it is well known that metal speciation critically affects metal accumulation by marine phytoplankton. However, few studies have tackled the influence of macronutrients on metal uptake by marine phytoplankton and it subsequent transfer within marine food webs.

Many previous studies have been devoted to metal accumulation or its uptake by aquatic organisms from the dissolved phase (e.g. Nugegoda and Rainbow, 1989; O'Brien et al., 1990; Rainbow and Black, 2002). These studies indicated that metal speciation and physicochemical changes in seawater influenced both metal bioavailability and its uptake by aquatic invertebrates. However, aquatic invertebrates

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accumulate metal either from solution or in their food, and the relative contribution from each route varies with invertebrate species and the relative bioavailability of the metal in the water and food (Wang and Fisher, 1999a). Recently, studies have proved that more than 50% of certain elements in marine organisms were obtained exclusively from the dietary phase (Wang and Fisher, 1998). This holds particularly true (>95%) for Se (Wang and Fisher, 1998, 1999a). Thus, the dietary exposure greatly contributes to trace metal accumulation by marine organisms and further studies will help us to understand the fate of metals in marine biogeochemical cycles. Recently, more and more attention has been devoted to the study of metal accumulation by phytoplankton and its subsequent transfer to marine copepods (Wang et al., 1996; Wang and Fisher, 1998; Wang and Dei, 2001a; Wang et al., 2001). These studies indicate that nutrient enrichment might lead to an increase in metal uptake by algae and its subsequent transfer to copepods (Wang and Dei, 2001a; Wang et al., 2001).

Nickel is an essential metal for aquatic organisms but it is toxic at elevated concentrations. It plays an important role in the functioning of urease, which is found in bacteria, fungi, phytoplankton and some invertebrates, and is a catalyst in the hydrolysis of urea to produce ammonia and carbamate (Smyj, 1997). Carbamate, when decomposed, yields a molecule of ammonium and carbonic acid. Therefore, it is essential to study the function and fate of nickel in the context of marine biogeochemical cycles. However, only a few studies have been aimed at this issue, and most of them focused on nickel accumulation in, or uptake from, the dissolved phase by marine biota (Campbell and Smith, 1986; Wong et al., 2000; Tam et al., 2001). There has been a particular lack of study on nickel bioaccumulation by phytoplankton and its subsequent transfer to copepods.

In this study, we first investigated the influence of macronutrient (nitrate and phosphate) additions on the bioaccumulation of Ni by two phytoplankton species (*Prorocentrum donghaiense* and *Skeletonema costatum*) and its subsequent transfer to two marine copepods (*Calanus sinicus* and *Labidocera euchaeta*) along a simplified food-chain, using a radioactive-tracer method. The experiments were designed to determine the influence of macronutrients on the assimilation efficiency (AE) and the physiological turnover rate constant (k) of Ni by copepods after feeding on algae-bound Ni.

2. Materials and methods

2.1. Phytoplankton and copepods

Two phytoplankton species, *P. donghaiense* and *S. costatum*, were obtained by separating them from natural assemblages collected in Xiamen Bay (People's Republic of China). They were maintained under axenic conditions in f/2 medium at 20 °C, under an illumination of 2000 μmol photon $m^{-2} s^{-1}$ in a 14:10 h light/dark cycle. The copepods *C. sinicus* and *L. euchaeta* were also collected from Xiamen Bay. Before the short feeding experiments commenced, the copepods were fed algae for 1 day and then starved for 1–2 h. Pristine seawater used in the experiments was obtained 20 km offshore in Xiamen Bay. All seawater used was filtered through a 0.45 μm acetic fiber membrane, cleared of trace metals by passing it through a Chelex ion exchange, and finally sterilized. The background concentrations of nitrate and phosphate in the collected seawater were <4 and <0.1 μmol L^{-1}, respectively.

2.2. Ni uptake in phytoplankton under different nutrient regimes

To examine the influence of nutritional status on Ni uptake in the two phytoplankton species, a long-term acclimation of algae to different N or P levels was undertaken. Algal cells from the same batch were filtered and resuspended in filtered seawater under different nutrient treatments in semicontinuous culture. The first nutritional treatment involved nitrate concentrations of 20, 80 and 320 μmol L^{-1} with a fixed phosphate concentration of 5 μmol L^{-1}, and the second treatment involved phosphate concentrations of 1 and 10 μmol L^{-1} with a fixed nitrate concentration of 20 μmol L^{-1}. Other nutrients were added to the f/2 medium in both treatments. The cells were filtered and transferred to new medium containing the same nutrient concentration every day, in order to ensure that cells were acclimated to the desired nutrient treatments. The acclimating period for *P. donghaiense* lasted for 5 days and that of *S. costatum* for 4 days. After acclimation, cells were filtered again and resuspended in filtered seawater containing the same nutrient level as in the acclimating process. Additionally, $^{63}NiCl_2$ (in 0.5 mol L^{-1} HCl, obtained from Perkin–Elmer Life and Analytical Sciences) was added into the culture medium to a final radioactivity of 0.20 μCi ml^{-1} (equal to 0.02 μg Ni ml^{-1}). All uptake experiments were conducted in acid-cleaned polycarbonate bottles containing 400 ml of filtered seawater and maintained for 24 h. Every few hours, a 10 ml aliquot was filtered through a 1 μm polycarbonate membrane and was rinsed three times with 20 ml of 1 mmol L^{-1} EDTA (pH = 7.0) to remove Ni^{2+} bound to the exterior of cells (Campbell and Smith, 1986). Thereafter, the radioactivity of the filter was measured to represent the intracellular content of Ni in the cells. In addition, at the end of the uptake experiment, another 10 ml aliquot was filtered through a 1 μm polycarbonate membrane and rinsed with 20 ml of filtered seawater three times for the measurement of radioactivity (representing the ^{63}Ni content of total cellular storage). The remaining solution was filtered and the algal cells rinsed with 100 ml of 1 mmol L^{-1} EDTA (pH = 7.0) three times before use for the pulse feeding of copepods (see below). To measure the dry weight of the algae, cells were filtered onto a preweighed glass fiber filter, rinsed with 0.5 mol L^{-1} ammonium formate, and dried at 80 °C for 1 day.

The accumulation factor of Ni by algae at the end of the uptake experiments was expressed as %intracellular Ni (which represented the percentage of intracellular Ni content to total cellular storage). Meanwhile, the DCF (the dry weight concentration factor), described as the ratio of intracellular Ni content (radioactivity kg^{-1} dry wt. cells) to total Ni content in the water sample (radioactivity L^{-1}) was used to determine the uptake of Ni by phytoplankton during the experiment.

2.3. Trophic transfer of Ni to marine copepods

The AE of Ni by the copepods (*C. sinicus* and *L. euchaeta*), feeding on the algae (*P. donghaiense* and *S. costatum*) was determined using established methods (Wang and Fisher, 1999b; Wang et al., 2001). After a long-term acclimation for the designed nutritional treatments and a subsequent uptake experiment in the presence of ^{63}Ni (as described above), the algal cells were filtered and pulse-fed to copepods in beakers filled with 300 ml of filtered seawater at 22 °C. The cell densities of *S. costatum* and *P. donghaiense* were maintained at 4×10^5 cells ml^{-1} and 6×10^4 cells ml^{-1}, respectively. Copepod densities were 0.3 inds ml^{-1} for *C. sinicus* and 0.5 inds ml^{-1} for *L. euchaeta*. Our previous studies showed that copepods rarely produced any feces within 20 min in the process of reingestion, after they were fed with algae for 1 day and then starved in 200 ml of filtered seawater for 1–2 h. After 20 min of feeding, some copepods (14–25 individuals) were immediately collected on a 300 μm mesh screen and rinsed with filtered seawater. Subsequently the copepods were radio-assayed and the radioactivity was regarded as the content of algae-bound Ni ingested by copepods during this short-term feeding. Meanwhile, the rest of the copepods were transferred and depurated in unradioactive medium with the same algal diets in 200 ml of filtered seawater. The depuration was maintained for 25–34 h, and at time

intervals of a few hours, the radioactivity retained in the copepods was determined by renewing the medium and algal diets simultaneously. The AE and the turnover rate constant (k) were calculated, respectively, as the y-intercept and the slope of the linear regression between the natural log of the percent of metal retention in copepods and the time of depuration (between 9 and 25 or 34 h) (Wang et al., 1996; Wang and Fisher, 1999b).

The radioactivity on the filter or in the copepods was counted in 10 ml of a liquid scintillation cocktail for aqueous and non-aqueous samples (obtained from Sigma), using a Packard Tricarb 4640 liquid scintillation counter with a "wide ^{63}Ni" window. The counting process was routinely conducted for a counting error <5%.

2.4. Statistical tests

The statistical differences between two or three nutrient treatments were processed by an independent-samples t-test or one-way ANOVA using SPSS 11.5 software. Significant difference was described at $P < 0.05$.

3. Results

3.1. Influence of macronutrient additions on Ni uptake in phytoplankton

Intracellular Ni uptake by two phytoplankton species maintained at various nutrient concentrations is shown in Figs. 1 and 2. Because a radioisotope was used to trace the uptake of Ni by algae, we regarded the DCF of Ni by phytoplankton at the beginning of uptake as zero (Figs. 1 and 2). Higher N concentrations resulted in a higher uptake rate of Ni by both algae after 24 h of exposure (Fig. 1). Thus, the intracellular Ni concentration in both *S. costatum* and *P. donghaiense* significantly increased (about three times) with an increase of the N concentration from 20 to 320 μmol L^{-1} after 24 h of exposure ($P < 0.01$). The intracellular Ni concentration also increased about twice in the 10 μmol L^{-1} P-enriched diatom *S. costatum*, in contrast to the 1 μmol L^{-1} treatment after exposure for 24 h, as it did in the dinoflagellate *P. donghaiense* under 10 μmol L^{-1} P-enrichment ($P < 0.05$) versus the 1 μmol L^{-1} treatment (Fig. 2). However, in the 5 μmol L^{-1} fixed phosphate concentration, Ni accumulation in *S. costatum* was lower than at either 1 or 10 μmol L^{-1}, whereas in *P. donghaiense*, Ni accumulation is higher at the 5 μmol L^{-1} concentration than either the 1 or 10 μmol L^{-1} concentrations (Figs. 1 and 2).

Our results also showed that the dinoflagellate *P. donghaiense* exhibited a different pattern of Ni uptake from the diatom *S. costatum*. A linear pattern of Ni uptake (particularly between 0 and 12 h) by *S. costatum* under various nutrient concentrations was observed during the 24 h exposure, whereas Ni uptake in *P. donghaiense* reached an equilibrium during the first 3 h of exposure and was relatively constant during the remaining exposure time (Figs. 1 and 2).

3.2. Assimilation of Ni in copepods

In general, the algal cells maintained at higher N concentrations but a fixed P concentration, or at higher P concentrations with a fixed N concentration tended to have a higher Ni distribution in the intracellular pool of the algal medium (Table 1).

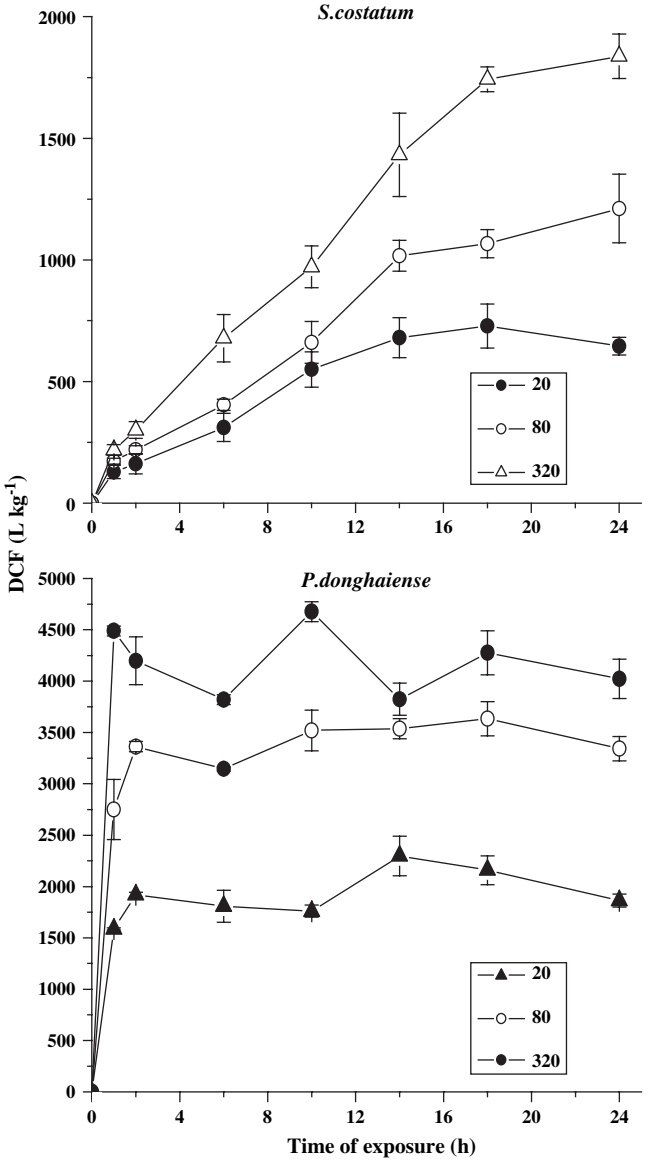

Fig. 1. Intracellular accumulation of Ni by two algal species (*Skeletonema costatum* and *Prorocentrum donghaiense*) maintained at different nitrate concentrations (20, 80 and 320 μmol L^{-1}) with a fixed phosphate concentration (5 μmol L^{-1}). Data are means ± SD ($n = 3$).

However, in the case of *S. costatum*, fed upon by either copepod, there was an anomaly between the Ni accumulation results for the (fixed) 5 μmol L^{-1} P and 20 μmol L^{-1} N treatment and the 1 and 10 μmol L^{-1} P and (fixed) 20 μmol L^{-1} N treatment in that the former results were lower than both of the latter, despite the common N concentration. Statistical analysis indicated that the nutrient status of the phytoplankton significantly influenced the assimilation of Ni in the copepods *C. sinicus* and *L. euchaeta* (Table 1; Figs. 3 and 4). The Ni AEs by *C. sinicus*, feeding on *S. costatum* and *P. donghainese*, increased from 16.6 to 41.2% and from 9.7 to 39.5% with an increase of N concentration from 20 to 320 μmol L^{-1}, respectively, ($P < 0.01$) when the P concentration was fixed. The assimilation of Ni by *L. euchaeta* feeding on *S. costatum* also increased significantly with increasing levels of nitrate

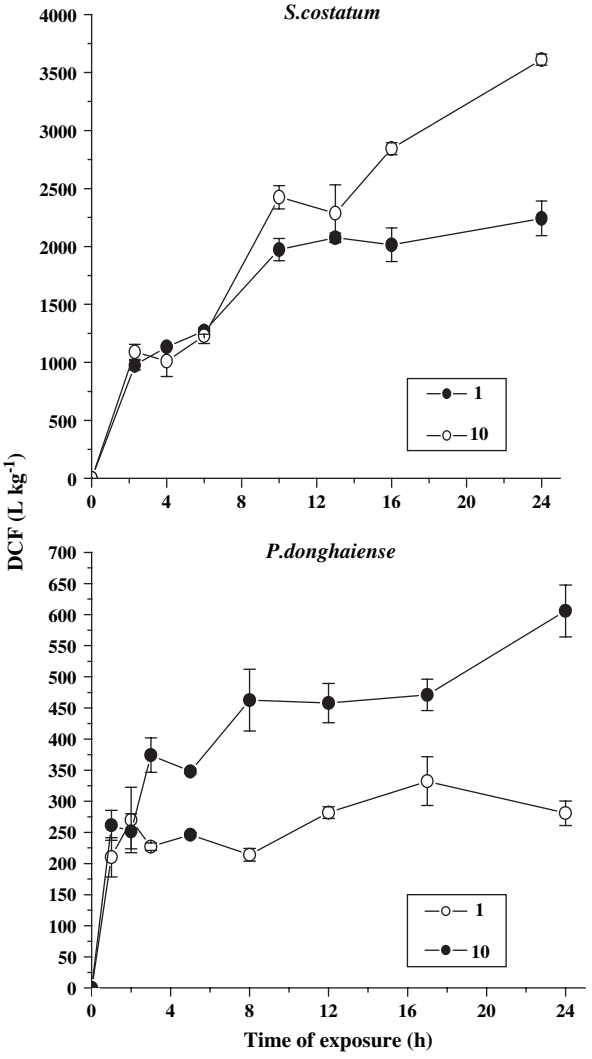

Fig. 2. Intracellular accumulation of Ni by two algal species (*Skeletonema costatum* and *Prorocentrum donghaiense*) maintained at different phosphate concentrations (1 and 10 μmol L^{-1}) with a fixed nitrate concentration (20 μmol L^{-1}). Data are means ± SD ($n = 3$).

($P < 0.05$), but fixed levels of phosphate. The Ni AEs by copepods feeding on *S. costatum* grown in 10 μmol L^{-1} phosphate were significantly higher than those in 1 μmol L^{-1} phosphate ($P < 0.05$). However, the same anomaly referred to above appeared when the 5 μmol L^{-1} P concentration results were compared with the 1 and 10 μmol L^{-1} results, i.e. the 5 μmol L^{-1} AE results were lower than both the 1 and 10 μmol L^{-1} results for *S. costatum*.

There was a significant positive-correlation between the Ni AEs of the copepods and the Ni content in the intracellular pool (Fig. 5; $P < 0.01$). A higher intracellular uptake of Ni by phytoplankton generally led to a higher assimilation of Ni by the copepods, though no statistical significance was found (Fig. 6; $P > 0.05$).

3.3. Depuration by marine copepods

Depuration of Ni from copepods following ingestion of algae cultured under different nutrient levels is shown in Figs. 3 and 4. There was a dramatic reduction of Ni retention in the copepods within 8–9 h of depuration, although subsequently a minor amount of Ni was scavenged by the copepods. The calculated physiological turnover rate constant (k) of Ni in copepods was independent of different N or P concentrations (Table 1; $P > 0.05$). The physiological turnover rate constant of Ni by *C. sinicus* fed on *S. costatum* fluctuated only from 0.390 to 0.495 with an increase of N concentration from 20 to 320 μmol L^{-1} at a fixed P concentration of 5 μmol L^{-1}, and the physiological turnover rate constant in *L. euchaeta* feeding on *S. costatum* changed only between 0.375 and 0.468, with an increase of the N concentration from 20 to 320 μmol L^{-1} ($P > 0.05$) at a fixed (5 μmol L^{-1}) P concentration.

The physiological turnover rate constant for Ni (k) varied more with copepod species than with nutrient concentrations (Table 2; $P < 0.01$). The physiological turnover rate constant of Ni for *C. sinicus* was generally higher (0.390–0.678) than that for *L. euchaeta* (0.194–0.468), regardless of phytoplankton species, although this trend was not pronounced in some cases (Table 1). Particularly, the physiological turnover

Table 1
The assimilation efficiency (AE) and the physiological turnover rate constant (k) of Ni in the copepods *Calanus sinicus* and *Labidocera euchaeta* feeding on the algae *Prorocentrum donghaiense* (Pd) and *Skeletonema costatum* (Sc) maintained under different nutrient treatments (N, Nitrate; P, Phosphate)

Copepod–Phytoplankton	Nutrients	Intracellular Ni (%) at 24 h	AE (%)	k (d^{-1})
C. sinicus feeding on Sc	20 μmol L^{-1} N	27.5 ± 2.4	16.6 ± 1.0	0.390 ± 0.020
	80 μmol L^{-1} N	31.6 ± 2.9	26.3 ± 1.4*	0.495 ± 0.011
	320 μmol L^{-1} N	66.9 ± 5.0**	41.2 ± 1.4**	0.436 ± 0.035
	1 μmol L^{-1} P	55.8 ± 4.6	32.7 ± 1.2	0.478 ± 0.041
	10 μmol L^{-1} P	72.1 ± 4.0	48.4 ± 2.3*	0.442 ± 0.045
C. sinicus feeding on Pd	20 μmol L^{-1} N	13.2 ± 1.0	9.7 ± 0.9	0.556 ± 0.048
	80 μmol L^{-1} N	20.1 ± 1.8	14.6 ± 1.8	0.678 ± 0.730
	320 μmol L^{-1} N	70.1 ± 3.9***	39.5 ± 0.6**	0.490 ± 0.002
L. euchaeta feeding on Sc	20 μmol L^{-1} N	9.1 ± 0.4	13.7 ± 1.3	0.468 ± 0.067
	320 μmol L^{-1} N	29.6 ± 0.9**	40.2 ± 1.3**	0.375 ± 0.032
	1 μmol L^{-1} P	45.7 ± 2.4	17.3 ± 1.2	0.212 ± 0.019
	10 μmol L^{-1} P	68.1 ± 4.8*	28.0 ± 0.5*	0.194 ± 0.020

*Indicates $P < 0.05$, **indicates $P < 0.01$, ***indicates $P < 0.001$. Data are described as means ± semi-range or SD ($n = 2–3$).

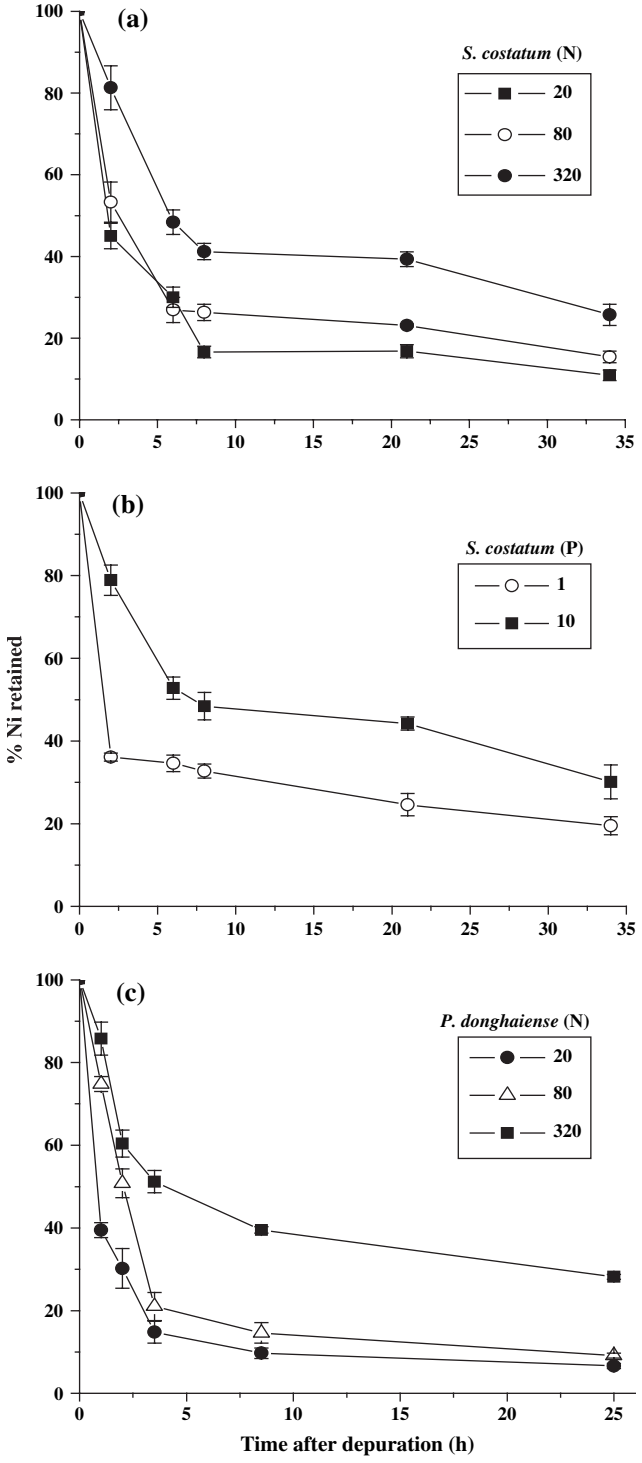

Fig. 3. The percentage of Ni retained in the marine copepod *Calanus sinicus* following a pulse feeding on the algae maintained at different nutrient concentrations. (a) *Skeletonema costatum* under different nitrate concentrations. (b) *S. costatum* under different phosphate concentrations. (c) *Prorocentrum donghaiense* under different nitrate concentrations. Data are means ± semi-range ($n = 2$).

rate constant of Ni for *C. sinicus* feeding on *S. costatum* under different P concentrations ranging between 0.442 and 0.478, increased more strikingly than in *L. euchaeta* which fluctuated between 0.194 and 0.212.

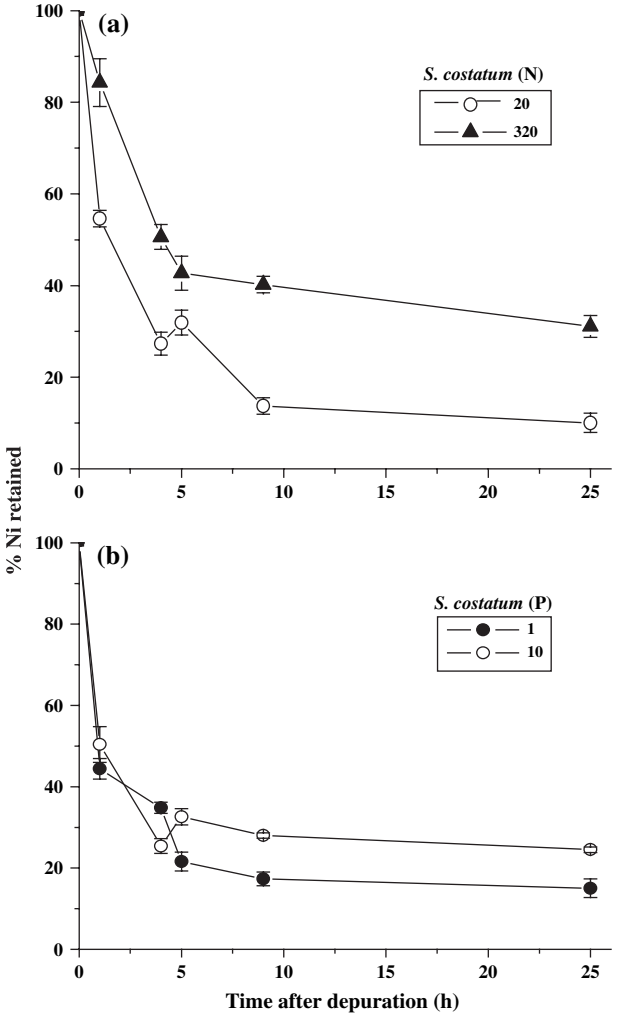

Fig. 4. The percentage of Ni retained in the marine copepod *Labidocera euchaeta* following a pulse feeding on the diatom *Skeletonema costatum* maintained at different nitrate (a) or phosphate (b) concentrations. Data are means ± semi-range ($n = 2$).

4. Discussion

4.1. Macronutrient influence on intracellular accumulation of Ni by phytoplankton

The present results showed that macronutrients (nitrate and phosphate) significantly affected the intracellular accumulation of Ni in two phytoplankton species after 24 h of exposure, with higher nutrient concentrations generally facilitating the biological uptake of Ni by algae (Figs. 1 and 2). Previous studies demonstrated that a higher concentration of nitrate would increase the uptake of Fe, Cd and Zn by phytoplankton, while various P regimes had little effect (Wang and Dei, 2001a,b,c; Wang et al., 2001). However, other studies showed that an increase in ambient phosphate concentrations enhanced the intracellular accumulation of Cd, Zn and Cr in algae significantly, with the N treatment showing little, or even an inverse, influence (Yu and Wang, 2004; Lee and Wang, 2001). A previous study on the uptake of ^{63}Ni by the diatom *Phaeodactylum tricornutum* revealed that phosphate addition

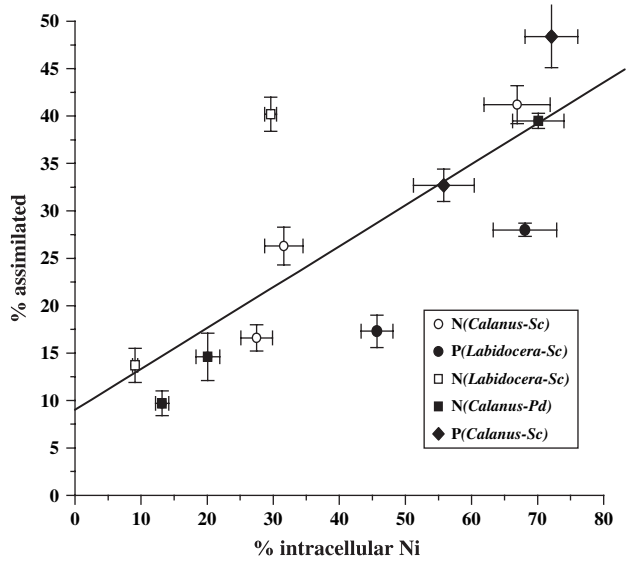

Fig. 5. Relationship between the calculated Ni assimilation efficiency in the marine copepods (*Calanus sinicus* and *Labidocera euchaeta*) and Ni intracellular distribution in the algal cells (*Prorocentrum donghaiense*, Pd and *Skeletonema costatum*, Sc). N or P represents the different nutrient treatments. Data are means ± semi-range or SD ($n = 2–3$, $R = 0.7806$, $P = 0.0027$).

markedly enhanced the nickel-binding capability of phosphate-starved cells due to phosphate being involved in the synthesis of the nickel-binding system of the cells (Skaar et al., 1974). Fuhrmann and Rothstein (1968) also demonstrated that Ni^{2+} was transported intracellularly by yeast cells, probably via a specific ion transport mechanism containing phosphate groups localized on the cell surface. Recently, Rijstenbil et al. (1998) found that N-enrichment stimulated

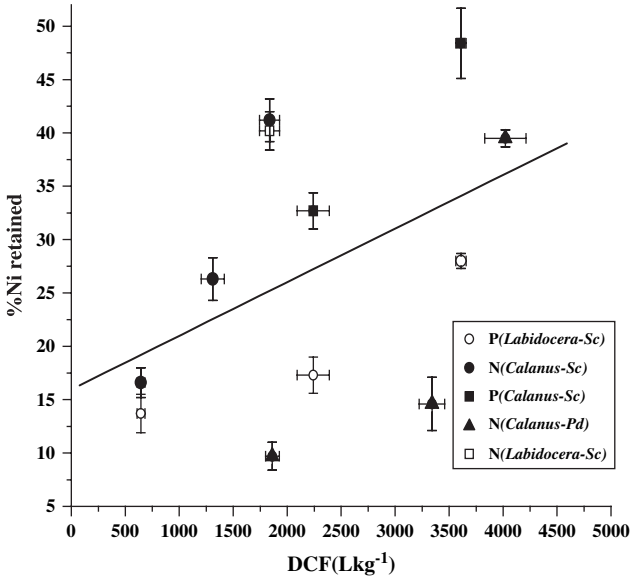

Fig. 6. Relationship between the calculated Ni assimilation efficiency in the marine copepods (*Calanus sinicus* and *Labidocera euchaeta*) and Ni DCF (after 24 h of exposure) in the algal cells (*Prorocentrum donghaiense*, Pd and *Skeletonma costatum*, Sc). N or P represents the different nutrient treatments. Data are means ± semi-range or SD ($n = 2–3$, $R = 0.4451$, $P = 0.1471$).

the production of glutathione, which might be responsible for metal detoxification, and they also found more cellular Cu, Zn and Mn in diatom cells after 14 days of batch culture under N-addition.

In our Ni uptake experiment, the growth rate of algae increased with increasing nitrate levels in the N treatment, while it was independent of P concentration in the P treatment. Previous studies also showed that growth rate increased with increasing nitrate levels (Wang and Dei, 2001a,b,c; Wang et al., 2001). Growth rate has been considered to be important in affecting metal uptake (Sunda and Huntsman, 1996, 1998), and Wang and Dei (2001b,c) demonstrated that the uptake of Cd and Zn was correlated with cell growth rate in phytoplankton. In this study, we also found an inter-relationship between growth rate and metal uptake rate in phytoplankton. However, Wang and Dei (2001b,c) also reported that there was no significant correlation between Se uptake and growth rate in the diatom. Thus, the relationship between metal uptake and growth rate is complicated, and it is difficult to conclude that the metal uptake rate is directly dependent on the cell growth rate based on present studies. A further challenge would be to quantify the exact relationship at the biochemical and cellular level, i.e. metal transport systems.

It is well known that free or dissolved metals in the environment are taken into the cells of marine organisms through membrane channels (cation transporter) (Simkiss and Taylor, 1995; Tessier et al., 1994). Also, anion transporters are also found in cell membranes, both for inorganic anions (e.g. phosphate and sulfate) and organic anions (e.g. amino acids and citrate), and hence some metals would accidentally be transported through cell membranes via anion transporters after combining with certain anions to form metal complexes (i.e. the anion ligand is assimilated as metal-anion complexes and the metal 'comes along for the ride') (Campbell et al., 2002). In fact, this piggyback transporter was observed in the penetration of metal ions into the cell membrane of algae in the form of cadmium-citrate or silver-thiosulfate complexes (Errécalde and Campbell, 2000; Fortin and Campbell, 2001). Though there is no evidence of the ''accidental'' uptake of nickel by algae in the form of Ni-nitrate or phosphate complexes, several previous studies reported that citrate could significantly facilitate the uptake of Ni by a bacterium (*Bacillus subtilis*) due to the Ni-citrate complex being transported intracellularly (Warner and Lolkema, 2002; Krom et al., 2002). So Ni might find its way into the algal cells ''accidentally'' if the nitrate or phosphate transport system could be ''fooled'' into transporting the Ni-nitrate or phosphate complexes. Therefore, this would explain why higher nutrient concentrations facilitated the biological uptake of Ni by phytoplankton. However, further studies should be undertaken to explore the exact mechanisms of the influence of macronutrients on the uptake of Ni by phytoplankton.

Our results demonstrate that two phytoplankton species show different strategies in the biological uptake of Ni. In contrast to the diatom *S. costatum*, the biological uptake of Ni by the dinoflagellate *P. donghaiense* reached an equilibrium more rapidly. As we know, metals are generally taken

Table 2
The statistical difference between the calculated physiological turnover rate constant (k) for Ni by *Calanus sinicus* and *Labidocera euchaeta* was processed using an independent-samples t-test, regardless of different N or P treatments

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
k of Ni by copepods	*C. sinicus*	16	0.49563	0.095166	0.023792
	L. euchaeta	8	0.31225	0.129287	0.045710

Independent-samples test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
k of Ni by copepods	Equal variances assumed	2.565	0.124	3.950	22	0.001	0.18337	0.046422	0.08710	0.27965
	Equal variances not assumed			3.559	10.932	0.005	0.18337	0.051531	0.06987	0.29688

An extreme difference ($P = 0.001$) is shown.

up into cells by membrane transport proteins. However, the biosorption process is also very important before metal internalization into cells. Therefore, differences in the structure and composition of the cell wall or membrane might mainly be responsible for the variant strategies of Ni uptake exhibited by the two plankton species. Further investigation is needed to study the influence of different phytoplankton species on the biological uptake of Ni.

4.2. Trophic transfer of Ni to copepods under different nutrient regimes

Our experiments indicated that the Ni AEs by copepods increased significantly with the increase of N or P concentrations where phytoplankton was acclimated (Figs. 3 and 4, Table 1). Previous studies demonstrated that a higher concentration of N would increase the metal content in the cytoplasm and would subsequently facilitate the assimilation of metals by copepods (Reinfelder and Fisher, 1991; Wang et al., 2001; Wang and Dei, 2001a). Furthermore, whereas previous studies reported that different P concentrations showed no relation to the assimilation of Fe (Wang and Dei, 2001a), and lower P concentration stimulated the assimilation of Zn (Wang et al., 2001) by copepods, in our study, higher copepod assimilation efficiencies were observed at a P concentration of 10 μmol L^{-1} rather than 1 μmol L^{-1}, and that was matched by the results concerning the uptake of Ni by algae under similar P treatments. In this study, there was a good relationship between intracellular DCFs of Ni in the algae and the AEs of the copepods (Fig. 6). Furthermore, a significant positive-correlation was observed between the Ni content in the intracellular pool and the subsequent copepod assimilation efficiencies (Fig. 5; $P = 0.0027$). Thus, the explanations given above might provide reasons why higher phosphate concentrations caused a higher assimilation of Ni by copepods, and this holds if following the hypothesis "copepods only assimilate the cytoplasmic pool of intracellular metal-storage (Reinfelder and Fisher, 1991; Hutchins et al., 1995; Wang and Fisher, 1999b), which is equal to one-third of the intracellular pool (Hutchins et al., 1995; Wang and Fisher, 1999b)". In our experiments, ingestion rates of algal cells by copepods were not measured. However, according to a previous study where food quantity did not appreciably affect the assimilation efficiency of metal by copepods (Wang et al., 1996), different ingestion rates might show little effect on the assimilation of Ni by copepods in the present study.

We noted in Sections 3.1 and 3.2 that the results for the fixed 5 μmol L^{-1} phosphate with 20 μmol L^{-1} nitrate treatments did not follow the trends in the 1 and 10 μmol L^{-1} phosphate experiments although both had 20 μmol L^{-1} nitrate. We have no clear explanation for this anomaly at present, although it might be caused by the different physiological status of the algae used in the experiments for nitrate and for phosphate since they were not carried out at the same time, and so the physiology of the algal cells might have been different. Another possible reason is that variations in the N:P ratio in the medium might have affected the uptake of Ni by the phytoplankton, since N:P ratio affects phytoplankton growth and physiology (Hegarty and Villareal, 1998; Vuorio et al., 2005). According to previous studies, a higher intracellular percentage of metal in algal cells leads to its subsequent higher assimilation by copepods, and so the higher %intracellular Ni in the 1 and 10 μmol L^{-1} phosphate treatments leading to higher Ni AE than in the 5 μmol L^{-1} phosphate–20 μmol L^{-1} nitrate treatment might be acceptable.

Many previous studies illustrated that the depuration process of metals by marine copepods was typically characterized by a two-compartment model (Wang et al., 1996; Wang and Fisher, 1998, 1999b; Wang and Dei, 2001a; Wang et al.,

2001), and our results further supported this model (Figs. 3 and 4). Within the first compartment, metal was egested rapidly and only the cytoplasmic content of metal could be assimilated, with the cell wall or membrane-bound metal being excreted during the defecation process (Reinfelder and Fisher, 1991; Hutchins et al., 1995). In other words, the first compartment reflected the assimilation process of metal by copepods. When depuration enters the second compartment, assimilated metal begins a process of physiological exchange between the inside and the surroundings. Therefore, the characteristics of the second compartment might be responsible for the physiological turnover rate constant of Ni by copepods being independent of the ambient nutrient status (Figs. 3 and 4, Tables 1 and 2), which seemed to be more affected by the individual physiological conditions of particular copepod species. Wang et al. (2001) also reported the same trend in their study of Zn depuration rate constant by copepods (*C. sinicus* and *Acartia spinicauda*), and showed that depuration rate constants of Zn by *C. sinicus* were generally lower than those by *A. spinicauda*, regardless of nutrient treatments or food types.

The metal ingested by copepods is divided into two pools, assimilated and egested. The egested fraction often settles to the bottom and might be recycled in the ecosystem only after being re-used by organisms, such as epi- and infauna, and bacteria, whereas the assimilated pool could easily show bioavailability to marine organisms. Therefore, the assimilated pool will be recycled and re-used by marine organisms several times before being exported from the surface waters. Many previous studies illustrated that excretion into the dissolved phase by zooplankton may be an important source for metal regeneration in surface waters (Hutchins and Bruland, 1994; Hutchins et al., 1995). In our experiments, the calculated physiological turnover rate constant of Ni fluctuated between 0.194 and 0.678 d^{-1}. This is significant because such a high physiological turnover rate constant may play an important role in Ni regeneration in surface waters by altering the portion being excreted into the dissolved phase and therefore bioavailable to phytoplankton.

5. Conclusion

Our novel results showed that environmental macronutrients (nitrate or phosphate) significantly affected the uptake of nickel by phytoplankton and also the subsequent transfer to marine copepods after ingestion. Higher N or P levels facilitated the bioaccumulation of nickel by algae, and the subsequent assimilation of Ni by copepods increased accordingly, with the physiological turnover of this metal being influenced very little by environmental nutrient conditions. Therefore, taking into account the fact that metal pollution often goes hand in hand with eutrophication in marine coastal ecosystems impacted by human activities, it is important to study the interactions between metal and macronutrients in marine ecosystems. Because of the relatively high assimilation efficiency, physiological turnover rate constant and bioavailability of Ni to marine planktonic organisms (Hutchins and Bruland, 1994), further investigation of the role and function of Ni in marine biogeochemical cycles is essential.

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