Vol. 231: 179–186, 2002 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published April 22

Relative importance of dissolved versus trophic bioaccumulation of copper in marine copepods

Sung Il Chang, John R. Reinfelder*

Department of Environmental Sciences, Rutgers University, 14 College Farm Road, New Brunswick, New Jersey 08901, USA

ABSTRACT: In order to evaluate the relative contribution of water and food to the accumulation of copper in herbivorous marine zooplankton, the accumulation and loss rates of Cu in coastal copepods (*Acartia* sp. and *Temora* sp.) were measured and applied to a kinetic bioaccumulation model. Experiments were performed to measure stable Cu uptake from the dissolved phase in field-collected copepods acclimated for 2 to 3 d to a low Cu diet. The copepods were exposed to a range of free-Cu ion concentrations ($[Cu^{2+}] = 10^{-14.8}$ M, $10^{-12.8}$ M, $10^{-9.8}$ M), but a significant Cu accumulation rate was only observed in copepods exposed to $10^{-9.8}$ M Cu^{2+}. Based on the Cu uptake rate measured at $10^{-9.8}$ M Cu^{2+}, a free ion-specific uptake rate constant of dissolved Cu of 1.1×10^4 l g^{-1} d^{-1} was estimated. Efflux rate constants of Cu in copepods estimated from depuration experiments were 0.056 ± 0.016 and 0.076 ± 0.012 d^{-1} in laboratory-fed and unacclimated copepods, respectively. Application of these Cu uptake and efflux parameters, and those for Cu trophic transfer from previous studies, to a kinetic bioaccumulation model shows that food is the dominant source of Cu (>75% of total accumulation) at free-Cu ion concentrations ranging from $10^{-14.8}$ to $10^{-11.8}$ M. At free-Cu concentrations ≥$10^{-11.8}$ M, dissolved uptake becomes a significant portion (>20%) of total Cu accumulation accounting for almost 60% of the total Cu in copepods at $10^{-9.8}$ M Cu^{2+}. These results suggest that herbivorous marine zooplankton accumulate Cu mainly by trophic transfer, but that dissolved uptake could be important in contaminated waters.

KEY WORDS: Copper · Bioaccumulation · Dissolved uptake · Kinetic model · Zooplankton · Copepods

INTRODUCTION

The bioavailability and trophic transfer of metals in aquatic food chains has received considerable attention in recent years (Wang et al. 1996, Fisher et al. 2000, Lee et al. 2000) in an effort to better understand the geochemical cycling of metals and the nutritional and toxicological effects of metals in aquatic organisms. A quantitative analysis of the pathways of metal bioaccumulation is also important to the development of water quality criteria, since levels based only on dissolved metal concentrations in natural waters and sediments may not be appropriate to assess the full extent and impact of metal contamination in aquatic environments. For many aquatic invertebrates, metal accumulation from ingested food is the main route of overall bioaccumulation (Luoma et al. 1992, Fisher & Reinfelder 1995, Wang et al. 1996, Munger & Hare 1997). The bioavailability of metals associated with food particles can therefore be important to the assessment of the potential impacts of metal contamination in such organisms.

The relative importance of dissolved versus trophic accumulation of metals in herbivorous zooplankton depends on the chemical properties of each metal and the physiology and activity of the zooplankton. These geochemical and biological properties can be used to quantify the 2 routes of accumulation separately in a bioenergetic-based kinetic model. Essential information includes accumulation rates of ingested metals,

*Corresponding author. E-mail: reinfelder@envsci.rutgers.edu

uptake rates for the dissolved phase, and depuration rates of metals in consumer animals (Landrum et al. 1992, Thomann et al. 1995, Wang & Fisher 1997). Kinetic bioaccumulation models have been applied to the bioavailability and trophic transfer of a variety of essential (Co, Se, and Zn) and non-essential (Ag, Am, Cd, and Hg) trace metals in copepods (Wang & Fisher 1998, Fisher et al. 2000), mussels (Wang et al. 1996, Wang & Fisher 1997), and other marine and freshwater animals. These efforts have been supported by substantial data on the bioaccumulation of such metals in marine phytoplankton and aquatic herbivore organisms (Fisher & Reinfelder 1995). However, despite its potential toxicity and elevated concentrations in impacted coastal waters (Moffett et al. 1997), Cu has been little studied with regard to its bioaccumulation by herbivorous zooplankton or other marine consumers (Chang & Reinfelder 2000). There is an indication that Cu concentrations in some polluted estuarine waters are high enough to affect the survival and production of marine copepods (Sunda et al. 1987), but a quantitative study of the pathways of Cu accumulation in such marine herbivores has not been done. The paucity of data may be due to the lack of a readily available, long-lived Cu radioisotope or a stable isotope of low mass abundance.

Our objectives were to measure Cu influx and efflux rates in marine copepods and to determine the relative importance of dissolved and trophic pathways to overall Cu bioaccumulation. With the use of trace metal clean culture techniques and trace-metal-buffered seawater, we attempted to measure gross uptake and loss rates of stable Cu in copepods. Another objective was to relate Cu accumulation in copepods to the concentration of free Cu^{2+}, which is proportional to that of total inorganic Cu and represents the bioavailable pool. Copper accumulation and toxicity in marine phytoplankton (Sunda & Guillard 1976, Anderson & Morel 1978, Sunda & Huntsman 1983) and invertebrates (Zamuda & Sunda 1982, Sanders et al. 1983, Sunda et al. 1987) has been related to the free-Cu ion concentration, and mechanistic relationships between Cu accumulation and free or kinetically labile Cu pools have been inferred. It is not known which chemical species of Cu are taken up by copepods, but since deleterious effects were observed at free-Cu concentrations of 10^{-10} to 10^{-11} M (Sunda et al. 1987, Sharp & Stearns 1997), we measured Cu accumulation over the range of $10^{-14.79}$ to $10^{-9.79}$ M.

MATERIALS AND METHODS

Diatom cultures. Axenic cultures of the marine diatom *Thalassiosira weissflogii* (Actin) were maintained in trace-metal-buffered artificial seawater media (Aquil, Price et al. 1988/89) under continuous (24 h) illumination (200 µmol quanta m^{-2} s^{-1} PAR) at 18°C. In these culture media, which include macronutrients (N, P, and Si), vitamins, and trace metals, the concentrations of free and inorganic trace metal pools are maintained constant by excess concentrations of the chelating agent EDTA (ethylenediaminetetraacetic acid). Free-Cu ion concentrations were controlled by adding a 1:1 (mol:mol) Cu:EDTA solution (Sunda & Huntsman 1995, Chang & Reinfelder 2000). Speciation calculations of trace-metal concentrations in the media were made using the MINEQL speciation program (Westall et al. 1976).

Copepods. Copepods were collected with a plankton net (63 µm Nylon mesh) from Cheesequake Creek near Raritan Bay, New Jersey, USA. Immediately following collection, adult copepods were separated from nauplii by sieving with a 350 µm Nylon netting and transferred to Cheesequake Creek water (S = 24) that had been passed through 20 µm Nylon mesh. Microscopic examination showed that the mixed assemblage contained ~90% *Acartia* sp. and ~10% *Temora* sp. (by number). Thus while the results obtained here are not strictly species-specific, they are most representative of Cu interactions with *Acartia* sp. Copepods were acclimated in diluted, trace-metal-free synthetic ocean water (SOW, S = 25) at 18°C for 2 or 3 d prior to Cu uptake experiments, during which time they were fed the diatom *Thalassiosira weissflogii* grown at pCu 14.8 (pCu = $-\log[Cu^{2+}]$).

Copper uptake in copepods from the dissolved phase. Experiments were performed to examine time-dependent copper uptake from the dissolved phase into marine copepods over a range of free-Cu ion concentrations (pCu = 14.79 to 9.79). The EDTA-buffered Cu uptake media were prepared exactly as those for the diatom cultures, including 0.2 µm filtration, except that the SOW was diluted to S = 25 to minimize the possible effects of salinity change on metal uptake and copepod physiology. The same concentrations of macro-nutrients (N, P, and Si), vitamins, EDTA, and other micro-nutrient trace elements (Zn, Fe, Mo, and Se) as used in the diatom culture media were added to the copepod uptake media. After adding a 1:1 (mol:mol) Cu:EDTA solution, the uptake media were equilibrated for at least 24 h at 18°C prior to the experiments. The trace metal speciation calculations for the diluted Aquil media are presented in Table 1. Copepods in the acclimation media were collected on a 350 µm Nylon netting and transferred to uptake media in a plastic beaker. This media was then divided into small plastic bottles, each of which held 50 to 60 individual copepods in 40 ml Cu uptake media. These bottles were maintained at 18°C in the dark for 8 to 24 h.

Table 1. Total metal concentrations required to achieve specified free metal concentrations (pM = –log[M^{z+}]) in diluted salt (S = 25) Aquil media. Metal speciation calculated with MINEQL (Westall 1976)

Metal	Total concentration (M)	pM
Cu	3.05×10^{-9}	14.79
	3.05×10^{-7}	12.79
	3.05×10^{-4}	9.79
Fe	8.32×10^{-6}	18.70
Mn	1.21×10^{-7}	8.42
Zn	7.97×10^{-8}	11.07

At each sampling time, copepods were separated from uptake media using a 350 µm Nylon netting and transferred to 10 ml trace-metal-free SOW in a plastic beaker. The copepods were then collected on preweighed 3 µm polycarbonate membrane filters and sequentially rinsed with 10 ml of 1 mM EDTA in trace-metal-free SOW and 10 ml of SOW to remove adsorbed Cu from the surfaces of the copepods. The copepods were also rinsed with 10 ml of 0.7 N ammonium formate to remove inorganic sea-salts. The filter with copepods was dried at 60°C overnight and weighed with a microelectronic balance (copepod dry weight = 7.51×10^{-3} mg per individual copepod).

Efflux rates of copper from laboratory-fed and unacclimated copepods. Laboratory-fed copepods were fed diatoms grown in Aquil as described above, except that the free-Cu ion concentration was $10^{-12.79}$ M. Mid-exponential growth phase cells (cell densities of $5–7 \times 10^4$ cells ml^{-1}) were collected on 3 µm polycarbonate (PC) membrane filters and rinsed sequentially with 10 ml of 1 mM EDTA in trace-metal-free SOW and 10 ml SOW to remove adsorbed Cu from the cell surfaces. Diatoms were resuspended in 500 ml of diluted (S = 25) SOW in a 500 ml PC bottle and 600 to 700 copepods were added. The feeding suspension was maintained at 18°C. After 1 d of feeding, the copepods were collected on a 350 µm Nylon netting, rinsed with SOW, and transferred to 100 ml of particle-free (0.2 µm filtered) SOW and allowed to clear their guts for 1 h. They were then transferred to 500 ml of particle-free SOW and allowed to depurate in the absence of food for 2 d at 18°C. At each sampling time, 50 to 60 individual copepods were removed from the bottle by filtering 40 ml of depuration media through a 350 µm Nylon mesh. After transferring the copepods to 30 ml of SOW, they were immediately collected on preweighed 3 µm PC membrane filters and rinsed with 10 ml of 0.7 N ammonium formate solution to remove inorganic salts. The filter with the copepods was dried at 60°C overnight and weighed with a microelectronic balance. Additional experiments were conducted to examine the efflux rates of copper from copepods that accumulated Cu from the dissolved phase and by trophic transfer in their natural environment (unacclimated copepods). For these studies, freshly collected copepods were immediately transferred to the diluted SOW and allowed to depurate Cu in the absence of food for 3 d at 18°C.

The rates with which trace metals are eliminated from aquatic invertebrates show first-order dependence on the concentration of the metal in the organism (Reinfelder et al. 1997, Wang & Fisher 1998). The efflux of Cu from copepods was therefore fit to a first-order, exponential loss model in order to calculate Cu loss rate constants:

$$Cu_t = Cu_0 e^{-kt}$$

where Cu_t is the concentration of Cu (µg g^{-1} dry wt) retained in the copepods at time t (d), Cu_0 is the initial Cu concentration in the copepods, and k is the Cu efflux rate constant (d^{-1}).

Sample digestion and Cu analysis. For sample digestion, PC filters with copepods were placed in 30 ml fused-quartz crucibles and 3 ml concentrated nitric acid (OPTIMA grade, Fisher) was added. After 2 d incubation at room temperature, filters were removed and the solution was slowly evaporated on a hot plate at 90°C. Copper in the residue was extracted with 1 ml of 1% nitric acid (OPTIMA grade), and the extract was transferred to a 1.25 ml acid-cleaned, micro-centrifuge tube. The sample was centrifuged at $12\,000 \times g$ for 3 min at room temperature to spin down any particles in the extract and copper concentrations in the supernatant were determined by graphite furnace atomic absorption spectrometry (GFAAS, Perkin-Elmer 4100 ZL) using an external calibration method. Quality assurance samples (NIST SRM 1643d, 'trace elements in water') were analyzed with each set of samples (certified value = 20.5 ± 3.8 µg l^{-1}; measured value = 20.1 ± 0.9 µg l^{-1}, n = 8) and Cu concentrations were corrected for method blanks.

RESULTS

Copper uptake from the dissolved phase

The measurement of stable Cu accumulation in lab-acclimated copepods (fed low-Cu diatoms) was not sensitive enough to determine dissolved Cu uptake rates at pCu = 14.79 and pCu = 12.79 (Fig. 1). Copepods exposed to pCu = 12.79 and 14.79 showed an initial lack of significant accumulation of dissolved Cu followed by an apparent and transitory increase. Since the variability of Cu concentrations in copepods at different time points was similar to that within replicates

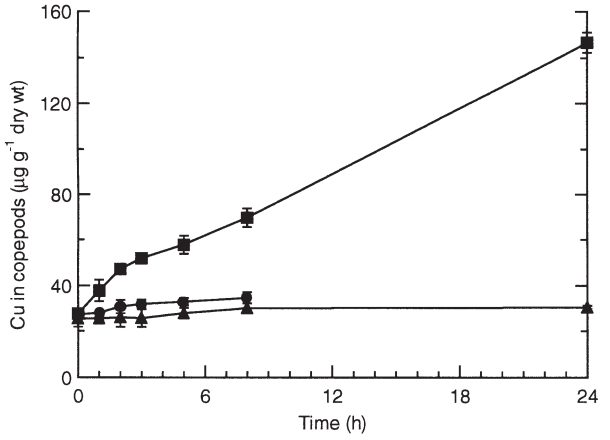

Fig. 1. Accumulation of dissolved Cu in copepods. Copepods were exposed to dissolved Cu at pCu = 14.79 (▲), 12.79 (●), 9.79 (■), where pCu = –log[Cu^{2+}]. Data points are means ± 1 SD

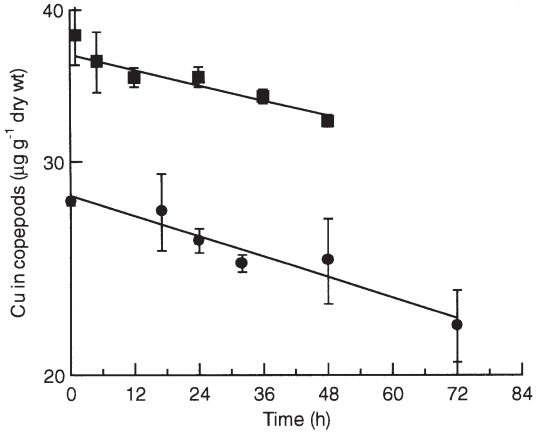

Fig. 2. Efflux of Cu from copepods fed diatoms grown with $10^{-12.79}$ M free Cu (■) and unacclimated Cheesequake Creek copepods (●). Data points are means ± 1 SD

sampled at the same time, no significant Cu accumulation rate could be determined. A significant Cu uptake rate was observed in copepods exposed to pCu = 9.79 (F = 1097, $p < 0.01$). Dissolved Cu uptake in copepods exposed to $10^{-9.79}$ M free Cu showed rapid uptake during the first 2 h followed by slower, constant accumulation thereafter (Fig. 1). Although EDTA rinsing results (Table 2) suggest that most of the accumulated Cu was internalized by the copepods, the initial rapid uptake during the first 2 h at pCu = 9.79 could be due to non-steady-state surface accumulation (apparently not removable by 1 mM EDTA). The Cu uptake rate at pCu = 9.79 was therefore calculated using data from 2 h onward. Very little mortality (= 5%) of copepods occurred during the Cu uptake experiments and was not corrected for in rate calculations. The free Cu-specific uptake rate constant of dissolved Cu in copepods was estimated using the Cu uptake rate measured at pCu = 9.79 (110 µg g^{-1} d^{-1}) and was found to have a value of 1.1×10^4 l g^{-1} d^{-1}.

Efflux rate of copper from copepods fed cultured diatoms and natural foods

The depuration of Cu from both laboratory-fed (diatoms grown at pCu = 12.79) and unacclimated copepods was fairly slow, with a slightly faster release rate during the first 5 h from the cultured diatom-fed copepods (Fig. 2). By treating the copepods as a single-compartment organism, the slow phase of depuration, representing physiological loss of Cu, can be used to determine the Cu efflux rate constant. Copper loss rate constants were calculated as the linear regression slopes of natural log-transformed depuration data. We used Cu loss data for 5–48 and 0–72 h to estimate the efflux rate constants of Cu in copepods fed cultured diatoms and unacclimated copepods, respectively. Copepod mortality was 10 to 15% during the efflux experiments and was not corrected for in loss rate calculations. Significant exponential loss rates were observed in the lab-fed (0.056 d^{-1}; F = 34, $p < 0.05$) and unacclimated (0.076 d^{-1}; F = 46, $p < 0.05$) copepods and these were not significantly different from each other ($p > 0.99$) by the Tukey-Kramer test of difference of slopes (Sokal & Rohlf 1981). The maximum potential re-accumulation of Cu lost during the depuration experiments was evaluated using the dissolved Cu concentration that would have been obtained if all Cu lost from the copepods in 3 d was in the dissolved phase at the beginning of the depuration and the dissolved Cu uptake rate constant determined above. Based on bioassays with diatoms, the maximum total dissolved Cu the depuration copepods could have been exposed to corresponds to a free Cu of $<10^{-13.79}$ M ($<10^{-6}$ µg l^{-1}). Thus the maximum dissolved Cu uptake rate in the depurating copepods could have been <0.01 µg g^{-1} d^{-1}. Since this is much lower than the observed minimum Cu loss rate (1.2 µg g^{-1} d^{-1}), Cu recycling was considered unimportant in these experiments.

Table 2. Surface-adsorbed Cu (removable with 1 mM EDTA) in copepods exposed to dissolved Cu uptake media for 3 h. Values are mean ± SD (n = 3) Cu concentrations with and without EDTA rinsing

pCu	Cu in copepods (µg g^{-1} dry wt)		Percent surface-bound
	Rinsed with seawater only	Rinsed with seawater plus EDTA	
14.79	26.0 ± 0.8	25.6 ± 3.9	0
9.79	58.1 ± 1.9	51.8 ± 0.1	11

DISCUSSION

Accumulation of dissolved copper by copepods

Copepods acclimated to a diet of diatoms grown at low free Cu (pCu = 14.79) accumulated dissolved Cu at pCu = 9.79, but not at pCu = 14.79 or 12.79. Loss of Cu accumulated by the copepods prior to collection or during acclimation may have obscured dissolved Cu uptake below a $[Cu^{2+}]$ of $10^{-12.79}$ M. However, there does not appear to have been a rapid exchange of surface adsorbed Cu during the dissolved phase accumulation experiments since only 0 to 11% of Cu in the copepods was in a surface exchangeable pool (Table 2). The rapid accumulation of dissolved Cu in copepods at pCu = 9.79 was therefore mainly associated with internal tissues or strongly bound to the copepods' surfaces. At the highest exposure concentration, Cu accumulation in the copepods did not reach a steady-state in 24 h (Fig. 1), indicating an absence of Cu regulation over this time scale.

The free-Cu ion-specific rate constant of dissolved Cu accumulation in copepods measured here can be compared with those for other metals by conversion to a total metal-specific uptake rate constant with the ratio of free:total Cu in natural waters (total Cu values of the EDTA-buffered media are unrealistically high). The ratio of $[Cu^{2+}]$:Cu_T varies from 2.4×10^{-5} in the surface waters of oligotrophic open ocean surface waters (Coale & Bruland 1988) to 2.4×10^{-3} in harbor surface waters (Moffett et al. 1997). Since our copepods were collected from a tributary of Raritan Bay, New Jersey, we used the measured speciation of dissolved Cu in Raritan Bay (99.4% of total dissolved Cu is bound by organic ligands; Sunda et al. 1987) and an inorganic side reaction coefficient (α′) of 13 (Byrne et al. 1988) to estimate a $[Cu^{2+}]$:Cu_T of 4.6×10^{-4}. The calculated total Cu-specific uptake rate constant is therefore 5.1 l g^{-1} d^{-1} for these coastal waters. The uncertainty associated with this calculation notwithstanding, dissolved accumulation of Cu appears to be faster than that measured in estuarine copepods (Wang & Fisher 1998) for Cd (0.69 l g^{-1} d^{-1}), slightly slower than that for Ag (10 l g^{-1} d^{-1}) and similar to that for Zn (3.3 l g^{-1} d^{-1}).

Efflux of copper from laboratory-fed and unacclimated copepods

The efflux of Cu from copepods fed laboratory-grown diatoms showed a slightly faster rate within the first few hours than thereafter (Fig. 2), as was observed for other trace metals in laboratory-fed, marine copepods (Wang & Fisher 1998). This initial loss, which may include Cu egestion, was not seen in the unacclimated copepods. Copper efflux rate constants for unacclimated copepods, which accumulated Cu from both the dissolved phase and food, and copepods fed cultured diatoms were not significantly different, indicating that the efflux rate of Cu is controlled primarily by copepod physiology and not by food type or route of exposure. The Cu efflux rate constants measured in this study (0.06 to 0.08 d^{-1}) are similar to that of the copepod *Calanus hyperboreus* in the Greenland Sea (0.05 d^{-1}; Ritterhoff & Zauke 1997a) and for Zn in *Temora longicornis* from the Long Island Sound (0.08 d^{-1}; Wang & Fisher 1998). They are, however, lower than those of Ag (0.3 d^{-1}), Cd (0.3 d^{-1}), and Co (0.3 d^{-1}) in *T. longicornis* (Wang & Fisher 1998) and somewhat lower than those of Pb (0.23 d^{-1}) and Zn (0.17 d^{-1}) in Greenland Sea *Calanus hyperboreus* (Ritterhoff & Zauke 1997c). Based on these limited data, it appears that the efflux kinetics of metals in marine copepods do not vary greatly across taxa or among different metals. This is similar to what has been found for metal efflux in marine bivalves (Wang et al. 1996, Reinfelder et al. 1997). However, metal elimination rates in marine copepods are an order of magnitude higher than in marine bivalves (0.01 to 0.03 d^{-1}; Wang et al. 1996, Reinfelder et al. 1997).

Our estimates of Cu efflux rate constants in copepods are at the low end of the range among the metals tested, indicating that assimilated Cu is associated with relatively slowly exchanging pools in these animals. The slow elimination of dissolved Cu from copepods may result from its strong tendency to bind protein (Bryan 1984). Copper is referred to as a 'borderline' metal that exhibits chemical reactivity with sulfur-, nitrogen-, and oxygen-bearing functional groups (Turner et al. 1981). The binding strength of Cu to sulfur-nitrogen ligands, such as cysteine or the metalloenzyme carbonic anhydrase, is greatest among the borderline metals which include Cd, Co(II), Fe(II), Ni, and Zn (Williams 1981). The slow turnover of Cu in copepods may also indicate a significant biological requirement for and/or regulation of this essential metal (see below) as has been noted for other marine invertebrates (Bryan 1984, Langston & Spence 1995). Whether or not Cu is regulated or required by marine copepods, the remineralization of Cu by copepods via physiological turnover is sufficiently slow that it is not likely to be a significant pathway for the release of Cu from suspended particles into the dissolved phase.

Kinetic model of the bioaccumulation of Cu in copepods

Kinetic bioaccumulation models are bioenergetically based models in which the concentration of a metal in

Table 3. Kinetic parameters and their values used to model Cu bioaccumulation in marine copepods

Parameter	Symbol	Value	Units
Dissolved Cu uptake rate constant (free ion specific)	k_u	1.1×10^4	l g^{-1} d^{-1}
Free Cu ion concentration	Cu^{2+}_w	10^{-7}–10^{-2}	µg l^{-1}
Copepod ingestion rate	IR	0.44[a]	d^{-1}
Cu assimilation efficiency	AE	0.4[b]	Unitless
Cu concentrations of diatom food	Cu_f	2.62–417[c]	µg g^{-1}
Cu efflux rate constant	k_e	0.08	d^{-1}
Copepod growth rate	g	0.02[d]	d^{-1}

[a]Estimated from the ingestion rates for *Acartia* sp. in the Lower Hudson River Estuary from Lonsdale et al. (1996)
[b]From Chang & Reinfelder (2000)
[c]Based on diatom-Cu-free-Cu ion concentration algorithms in Chang & Reinfelder (2000)
[d]Copepod growth rate was calculated as half of the geometric mean egg production rates for female *Acartia* sp. in the Lower Hudson River Estuary of Londsdale et al. (1996)

1 or more compartments of an organism is controlled by uptake and elimination. With such a model, different pathways of metal accumulation can be quantified separately and the relative importance of metal uptake from the dissolved phase and ingested food assessed (Thomann et al. 1995, Wang & Fisher 1997, 1998, Reinfelder et al. 1998). Assuming that copepods accumulate Cu in a single compartment, the steady-state concentration of Cu in copepods can be described by the following equations:

$$Cu_{ss,w} = (k_u \cdot Cu_w)/(k_{ew} + g)$$

$$Cu_{ss,f} = (AE \cdot IR \cdot Cu_f)/(k_{ef} + g)$$

$$Cu_{ss} = Cu_{ss,w} + Cu_{ss,f}$$

where $Cu_{ss,w}$ and $Cu_{ss,f}$ are the concentrations of Cu (µg g^{-1}) in copepods due to uptake from the dissolved phase and food, respectively, k_u is the uptake rate constant (l g^{-1} d^{-1}) from the dissolved phase, Cu_w and Cu_f are the dissolved (µg l^{-1}) and food (µg mg^{-1}) Cu concentrations, respectively, k_{ew} and k_{ef} are efflux rate constants (d^{-1}) following uptake from the dissolved phase and food, respectively, g is the copepod growth rate constant (d^{-1}), AE is the assimilation efficiency (%) of ingested Cu, IR is the copepod ingestion rate (d^{-1}), and Cu_{ss} is the total concentration of Cu (µg g^{-1}) in a copepod.

The kinetic parameters shown in Table 3 were used to model Cu accumulation in copepods over a range of free-Cu ion concentrations. The ingestion rate of 0.44 d^{-1} was estimated using the average ingestion rate for *Acartia* sp. in the Lower Hudson River Estuary measured by Londsdale et al. (1996), assuming a carbon content of 3.7 µg C per copepod. The AE (fraction of ingested Cu that is retained by the copepods following gut clearance) is that measured in copepods by Chang & Reinfelder (2000). The AE of Cu in copepods (like that of other metals) is proportional to the cytoplasm fraction of Cu in diatom cells and the cytoplasm, and total cellular Cu concentrations in diatoms increase linearly with the free-Cu concentration of the growth media (Chang & Reinfelder 2000). The AE of Cu in copepods is assumed here to be constant, but may increase somewhat at higher free-Cu concentrations (Chang & Reinfelder 2000).

The Cu content of food (Cu_f) is based on a diatom diet and the relationships between Cu accumulation in a marine diatom and the free-Cu concentration determined by Chang & Reinfelder (2000). In these relationships, diatom Cu was found to increase more rapidly with increasing free-Cu concentration above $10^{-11.79}$ M free Cu. We used an overall Cu efflux rate constant (k_e) to describe the loss of Cu from copepods regardless of uptake pathway with a value of 0.08 d^{-1}, the higher of the 2 measured values. Copepod growth rate was calculated as half of the geometric mean egg production rates for female *Acartia* sp. in the Lower Hudson River Estuary determined by Londsdale et al. (1996), assuming an equal number of male and female copepods (adult male copepods do not exhibit significant growth).

Over a free-Cu concentration range of 10^{-7} to 10^{-2} µg l^{-1} ($10^{-14.79}$ to $10^{-9.79}$ M), model-predicted steady-state copepod Cu concentrations varied from 5 to 2000 µg g^{-1} dry wt (Fig. 3a). Copper concentrations in copepods collected from Cheesequake Creek were 20 to 30 µg g^{-1}, which, based on the model results, corresponds to a free-Cu concentration range of 10^{-5} to 10^{-4} µg l^{-1} ($10^{-12.79}$ to $10^{-11.79}$ M). This 'bioassay' result is consistent with free-Cu concentrations determined by electrochemical methods in harbor estuaries of the northeast US (Moffett et al. 1997, Kozelka & Bruland 1998). Copepods living in such impacted waters have significantly more Cu than copepods living in cleaner waters such as the Fram Strait and Greenland Sea (4 to 8 µg g^{-1}; Ritterhoff & Zauke 1997b). However, it is noteworthy that our harbor estuary copepods have only a factor of 2 or 3 more Cu than the estimated (for decapod crustaceans with no haemocyanin) enzymatic requirement of 7 to 15 µg g^{-1} (White & Rainbow 1985, Depledge & Bjerregaard 1989).

The model results also show that Cu accumulation from food is the main pathway in waters with moderate

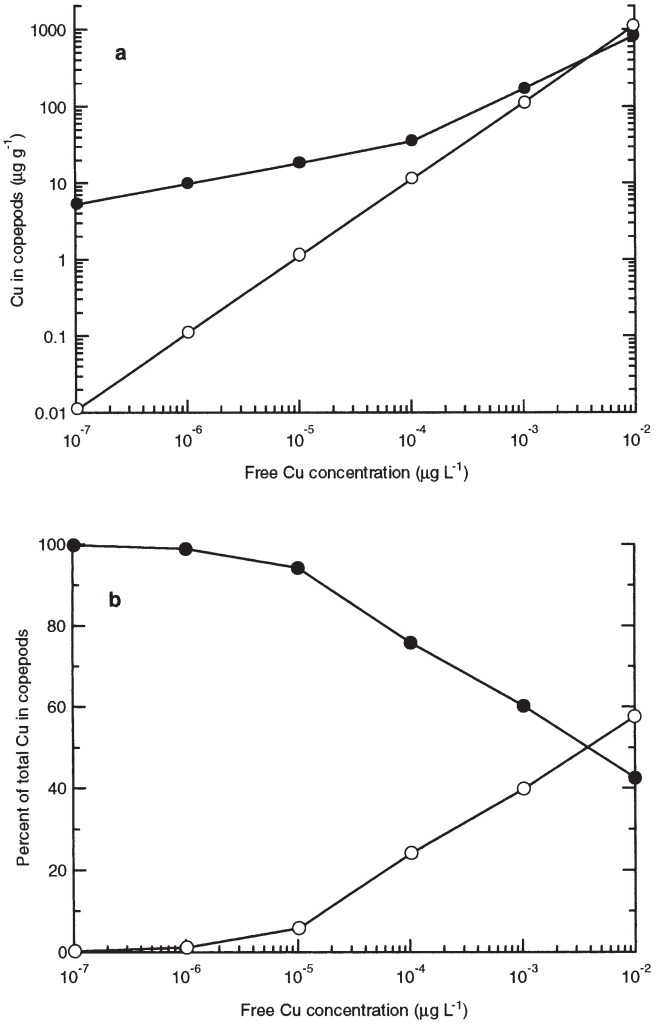

Fig. 3. (a) Model predicted Cu content and (b) percent total Cu in copepods attributable to accumulation from water (○) or food (●)

to low dissolved free-Cu ion concentrations (≤10^{-4} µg l^{-1} or $10^{-11.79}$ M Cu^{2+}). Thus, food accounts for >75% of overall Cu accumulation at free-Cu ion concentrations ranging from 10^{-7} to 10^{-4} µg l^{-1} ($10^{-14.79}$ to $10^{-11.79}$ M, Fig. 3b). As a result of the relatively shallow slope of the diatom-Cu–free-Cu concentration relationship (Chang & Reinfelder 2000), over the 1000-fold increase in free-Cu concentration from 10^{-7} to 10^{-4} µg l^{-1}, Cu accumulation in copepods from food increases by only a factor of 7 (Fig. 3a). Above a free-Cu ion concentration of 10^{-4} µg l^{-1}, Cu accumulation from food increases more sharply with increasing free Cu, but the fraction of total Cu accumulation from food decreased from 75 to 41% (Fig. 3b).

At free-Cu concentrations > 10^{-4} µg l^{-1} ($10^{-11.79}$ M), dissolved Cu is a significant (>20%) source of Cu to copepods (Fig. 3). It is well recognized that organic complexation dominates the speciation of Cu in coastal and oceanic surface waters and buffers the concentration of free dissolved Cu (Coale & Bruland 1988, 1990, Kozelka & Bruland 1998). However, small increases in total dissolved Cu can titrate Cu-complexing organic ligands, resulting in large increases in free-dissolved-Cu ion concentrations (Moffett 1995, Moffett et al. 1997). For example, free-Cu ion concentrations in polluted harbors can reach as high as 10^{-10} M (Ahner et al. 1997). Under such conditions, there could be a greater contribution of the dissolved phase than trophic transfer to Cu accumulation in copepods, especially if the increase in dissolved Cu occurs over a time scale that is shorter than that required for prey phytoplankton to reach steady-state with the dissolved phase. Eventually, elevated free-Cu concentrations will lead to higher phytoplankton Cu concentrations and higher Cu accumulation in consumer organisms such as copepods via trophic transfer.

Sunda et al. (1987) suggested that the concentration of free Cu in some polluted estuarine waters (~10^{-11} M) is high enough to affect the survival and reproduction of copepods. We observed very little mortality of copepods (~5%) during 24 h exposure to high free-Cu ion concentrations (pCu = 9.79), which indicates that toxic effects on the survival of copepods may not result from short-term dissolved Cu exposures. Copper toxicity in copepods may result from chronic exposure to high dissolved Cu or from Cu obtained via trophic transfer as has been shown for Ag and Cd (Hook & Fisher 2001).

The importance of trophic transfer to the accumulation of metals (most notably Se and Zn) has been demonstrated in a number of marine herbivores (Wang et al. 1996, Fisher et al. 2000). The present results show that trophic transfer is also of major importance to the accumulation of Cu in marine copepods. This is so because the bioaccumulation of Cu in copepods is characterized by low efflux rates, a relatively high AE, and a negligible contribution of dissolved uptake at free-Cu ion concentrations that are typical of most natural waters.

Acknowledgements. This work was supported by Hatch/McIntyre-Stennis grant 07142 and NJ SeaGrant project R/E-2002 to J.R.R. We thank 3 anonymous reviewers for their comments.

LITERATURE CITED

Ahner BA, Morel FMM, Moffett JW (1997) Trace metal control of phytochelatin production in coastal waters. Limnol Oceanogr 42:601–608

Anderson DM, Morel FMM (1978) Copper sensitivity of *Gonyaulax tamarensis*. Limnol Oceanogr 23:283–295

Bryan GW (1984) Pollution due to heavy metals and their

compounds. In: Kinne O (ed) Marine ecology, Vol 5. John Wiley & Sons, Chichester, p 1289–1430
Byrne RH, Lump LR, Cantrell KJ (1988) The influence of temperature and pH on trace metal speciation in seawater. Mar Chem 25:163–181
Chang SI, Reinfelder JR (2000) Bioaccumulation, subcellular distribution, and trophic transfer of copper in a coastal marine diatom. Environ Sci Technol 34:4931–4935
Coale KH, Bruland KW (1988) Copper complexation in the northeast Pacific. Limnol Oceanogr 33:1084–1101
Coale KH, Bruland KW (1990) Spatial and temporal variability in copper complexation in the North Pacific. Deep-Sea Res 47:317–336
Depledge MH, Bjerregaard P (1989) Haemolymph protein composition and copper levels in decapod crustaceans. Helgol Meeresunters 43:207–223
Fisher NS, Reinfelder JR (1995) The trophic transfer of metals in marine systems. In: Tessier A, Turner DR (eds) Metal speciation and bioavailability in aquatic systems. IUPAC, Wiley, Chichester, p 363–406
Fisher NS, Stupakoff I, Sañudo-Wilhelmy S, Wang WX, Teyssié JL, Fowler SW, Crusius J (2000) Trace metals in marine copepods: a field test of a bioaccumulation model coupled to laboratory uptake kinetics data. Mar Ecol Prog Ser 194:211–218
Hook SE, Fisher NS (2001) Reproductive toxicity of metals in calanoid copepods. Mar Biol 138:1131–1140
Kozelka PB, Bruland KW (1998) Chemical speciation of dissolved Cu, Zn, Cd, Pb in Narragansett Bay, Rhode Island. Mar Chem 60:267–282
Landrum PF, Lee H, Lydy MJ (1992) Toxicokinetics in aquatic systems: model comparisons and use in hazard assessment. Environ Toxicol Chem 11:1709–1725
Langston WJ, Spence SK (1995) Biological factors involved in metal concentrations observed in aquatic organisms. In: Tessier A, Turner DR (eds) Metal speciation and bioavailability in aquatic systems. Wiley, Chichester, p 407–478
Lee BG, Griscom SB, Lee JS, Choi HJ, Koh CH, Luoma SN, Fisher NS (2000) Influence of dietary uptake and acid-volatile sulfide on bioavailability of metals to sediment-dwelling organisms. Science 287:282–284
Lonsdale DJ, Cosper EM, Kim WS, Doall M, Divadeenam A, Jonasdottir SH (1996) Food web interactions in the plankton of Long Island bays, with preliminary observations on brown tide effects. Mar Ecol Prog Ser 134:247–263
Luoma SN, Johns C, Fisher NS, Steinberg NA, Oremland RS, Reinfelder JR (1992) Determination of selenium bioavailability to a benthic bivalve from particulate and solute pathways. Environ Sci Technol 26:485–491
Moffett JW (1995) The spatial and temporal variability of copper complexation by strong organic ligands in the Sargasso Sea. Deep-Sea Res 42:1273–1295
Moffett JW, Brand LE, Croot PL, Barbeau KA (1997) Cu speciation and cyanobacterial distribution in harbors subject to anthropogenic Cu inputs. Limnol Oceanogr 42:789–799
Munger C, Hare L (1997) Relative importance of water and food as cadmium sources to an aquatic insect (*Chaoborus puntipennis*): implications for predicting Cd bioaccumulation in nature. Environ Sci Technol 31:891–895
Price NM, Harrison GI, Herring JG, Hudson RJ, Nirel PM, Palenik B, Morel FMM (1988/89) Preparation and chemistry of the artificial algal culture medium Aquil. Biol Oceanogr 6:443–461
Reinfelder JR, Wang WX, Luoma SN, Fisher NS (1997) Assimilation efficiencies and turnover rates of trace elements in marine bivalves: a comparison of oyster, clams, and mussels. Mar Biol 129:443–452
Reinfelder JR, Fisher NS, Luoma SN, Nichols JW, Wang WX (1998) Trace element trophic transfer in aquatic organisms: a critique of the kinetic model approach. Sci Total Environ 219:117–135
Ritterhoff J, Zauke GP (1997a) Evaluation of trace metal toxicokinetics in Greenland Sea copepod and amphipod collectives from semi-static experiments on board ship. Polar Biol 17:242–250
Ritterhoff J, Zauke GP (1997b) Trace metals in field samples of zooplankton from the Fram Strait and the Greenland Sea. Sci Total Environ 199:255–270
Ritterhoff J, Zauke GP (1997c) Bioaccumulation of trace metals in Greenland Sea copepod and amphipod collectives on board ship: verification of toxicokinetic model parameters. Aquat Toxicol 40:63–78
Sanders BM, Jenkins KD, Sunda WG, Costlow JD (1983) Free cupric ion activity in seawater: effects on metallothionein and growth in crab larvae. Science 222:53–55
Sharp AA, Stearns DE (1997) Sublethal effects of cupric ion activity on the grazing behaviour of three calanoid copepods. Mar Pollut Bull 34:1041–1048
Sokal RR, Rohlf FJ (1981) Biometry, 2nd edn. Freeman, San Francisco
Sunda WG, Guillard RRL (1976) The relationship between cupric ion activity and the toxicity of copper to phytoplankton. J Mar Res 34:511–529
Sunda WG, Huntsman SA (1983) The effect of competitive interactions between manganese and copper on cellular manganese and growth in estuarine and oceanic species of the diatom *Thalassiosira*. Limnol Oceanogr 28:924–934
Sunda WG, Huntsman SA (1995) Regulation of copper concentration in the oceanic nutricline by phytoplankton uptake and regeneration cycles. Limnol Oceanogr 40:132–137
Sunda WG, Tester PA, Huntsman SA (1987) Effects of cupric and zinc ion activities on the survival and reproduction of marine copepods. Mar Biol 94:203–210
Thomann RV, Mahony JE, Mueller R (1995) Steady-state model of biota sediment accumulation factor for metals in two marine bivalves. Environ Toxicol Chem 14:1989–1998
Turner DR, Whitfield M, Dickson AG (1981) The equilibrium speciation of dissolved components in freshwater and seawater at 25°C and 1 atm pressure. Geochim Cosmochim Acta 45:855–881
Wang WX, Fisher NS (1997) Modeling metal bioavailability for marine mussels. Rev Environ Contam Toxicol 151: 39–65
Wang WX, Fisher NS (1998) Accumulation of trace elements in a marine copepod. Limnol Oceanogr 43:273–283
Wang WX, Fisher NS, Luoma SN (1996) Kinetic determinations of trace element bioaccumulation in the mussel *Mytilus edulis*. Mar Ecol Prog Ser 140:91–113
Westall JC, Zachary JL, Morel FMM (1976) MINEQL, Computer program of thermodynamic calculation. Tech Note 18, RM Parsons Laboratory, MIT, Cambridge, MA
White SL, Rainbow PS (1985) On the metabolic requirement for Cu and Zn in molluscs and crustaceans. Mar Environ Res 160:215–229
Williams RJP (1981) Physico-chemical aspects of inorganic element transfer through membranes. Phil Trans R Soc Lond B Biol Sci 294:57–74
Zamuda CD, Sunda WG (1982) Bioavailability of dissolved Cu to the American oyster *Crassostrea virginica*. I. Importance of chemical speciation. Mar Biol 66:77–82

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: April 2, 2001; Accepted: November 8, 2001
Proofs received from author(s): March 26, 2002