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Availability of copper from phytoplankton and water for the bivalve *Macoma balthica*. II. Uptake and elimination from ^{64}Cu-labelled diatoms and water

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Abstract. The amount of copper taken up via algae and water by *Macoma balthica* from the Oosterschelde sea arm, S.W. Netherlands, was established using the radioisotope ^{64}Cu. As far as we know, this isotope has never been used before in marine food chain studies. As a model food source the marine diatom *Phaeodactylum tricornutum* was allowed to accumulate ^{64}Cu for 1 d. These labelled algae were fed to the clams in the presence of the complexing agent EDTA (0.27 m*M*). EDTA was added to prevent uptake of dissolved ^{64}Cu that could be leaking from the labelled diatoms. In control experiments, unlabelled diatoms were fed to *M. balthica* in the presence of dissolved ^{64}Cu (with and without EDTA) in order to assure a similar filtration activity. In repeated experiments with varying particulate/dissolved copper ratios, uptake through food always turned out to be at least as efficient as uptake from the water. It was concluded that Cu, associated with food, is well available for uptake by *M. balthica*.

Introduction

It is often suggested that filter-feeding bivalves be used as a biological monitor in estuarine areas. However, bivalves are exposed to various dissolved as well as particulate metal species in the waterphase. In addition to this, sediment-dwelling bivalves are also directly exposed to sediment-associated metals. On the relative contribution of food as a particulate metal species to the overall metal accumulation very little information is available. The sediment-dwelling deposit feeder *Macoma balthica* feeds by taking in algae, bacteria and detrital material through its siphons (Brafield and Newell 1961, Gilbert 1977). Like many bivalve mollusc species, *Macoma balthica* is able to ingest preferentially nutritious particles and reject non-nutritious particles like sediment grains as pseufofaeces (Morton 1973, Levinton 1989). The metal concentration in food particles is much higher than in the surrounding water (Luoma and Bryan 1982, Bryan 1985). As food material is digested, it seems logical to assume that uptake of metals through food is an important pathway for marine bivalves. However, the total quantity of metal to which a filter feeder is exposed via the water can be very large, considering the large water volumes with which they have contact during respiration. So far no conclusive evidence for the importance of either of the pathways is available.

For cadmium, various studies indicated that the contribution of food to the overall metal accumulation is low: Janssen and Scholz (1979) assessed a food contribution of only 10% with *Mytilus edulis* and Borchardt (1983) concluded that food contributed for not more than 0.2 to 0.5% to the Cd body burden of *Mytilus edulis*. Uptake of bacterially-associated ^{109}Cd by *Macoma balthica* resulted in 39% of a 14-d total uptake. Although it is an essential metal, ^{65}Zn from bacteria resulted in only 23% of the total uptake. ^{57}Co, on the other hand, accounted for 81.6% of the total uptake (Harvey and Luoma 1985). Data on Pb are contradicting: *Mytilus edulis* accumulated Pb from water and food in equal amounts (Schulz-Baldes 1974), whereas with oysters (*Crassostrea gigas*) direct uptake from water led to body burdens approximately 100 times higher than those reached after contaminated food ingestion (Amiard-Triquet et al. 1988). Accumulation of ^{75}Se by the clam *Puditapes philippnarum* was mainly from Se-labelled phytoplankton (Zhang et al. 1990). Particulate organo-Se was assimilated with 86% efficiency by *Macoma balthica* when the clam was fed ^{75}Se-labelled diatoms.

Cu has not been studied before in sufficient detail. A reason for the scarcity on uptake data is the lack of a suitable tracer. Radioactive tracers enable the use of low, ecologically realistic concentrations and often provide an opportunity to separate different routes of metal uptake. Cu radioisotopes, however, are either not easy to prepare (^{67}Cu) or have relatively short half-lives (^{64}Cu).

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In spite of the limitations described above, we have tried to assess the role of Cu uptake through food by means of a radioactive tracer study using ^{64}Cu. The objective of the present study was to measure ^{64}Cu uptake by *Macoma balthica* via food and water by separation of uptake pathways.

Materials and methods

The experimental conditions of the clams, the preparation of ^{64}Cu and ^{64}Cu-labelled algae are described elsewhere (Absil et al. 1993). The experiments were carried out in the dark because, in this situation, the gut evacuation time is comparable with the gut evacuation time of clams burrowed in sediment (Hummel 1985). After acclimatizing, the individuals were introduced in the experiment and received filtered seawater (FSW) with equal amounts of labelled or unlabelled algae. The algal density was kept similar to ensure a comparable filtration activity for the different experiments.

Experimental setup

To assess the contribution of food-associated copper to the total copper uptake by *Macoma balthica*, four different experiments were carried out. With these experiments, *Macoma balthica* individuals were allowed to feed on (tracer-labelled) algae for a short period. After the feeding period, the retention of the tracer was measured during the depuration of the ingested food. This so called "pulse-chase" experimental setup has several advantages compared with other types of uptake experiments (Luoma et al. 1992). The major advantages in this case were a minimization of recycling of tracer in the experiment and fewer problems caused by altered animal behaviour in long-term experiments, e.g. decreased activity as a result of a deteriorated condition.

In Expt 1, clams were allowed to feed on algae (*Phaeodactylum tricornutum*), labelled with ^{64}Cu. 0.27 m*M* ethylenediaminetetraacetate (EDTA) was added to prevent the uptake of ^{64}Cu that could have been leaking from the labelled algae. This excess amount of EDTA has proved to be very efficient in minimizing the uptake of dissolved ^{64}Cu. In Expt 2, clams were allowed to feed on ^{64}Cu-labelled algae without EDTA. If differences between Expts 1 and 2 occurred, this would indicate that the algae were indeed leaking. The presence of dissolved ^{64}Cu would also be indicated by increased adsorption on the shell. In Expt 3, ^{64}Cu was dissolved in the water in the presence of 0.27 m*M* EDTA. Unlabelled (control) algae were added to this experiment to ensure a comparable filtration activity. This experiment functioned as a control: if EDTA were shown to be an effective inhibitor of Cu uptake, accumulation by *Macoma balthica* and adsorption into the shell should be negligible in this experiment. In Expt 4, again ^{64}Cu was dissolved in the water, but no EDTA was added. Here also fresh, unlabelled algae were added to stimulate the filtration activity. As dissolved Cu without EDTA is supposed to be readily available for uptake, considerable accumulation and adsorption were expected. The difference between uptake from ^{64}Cu-labelled algae in Expts 1 and 2, on the one hand, and ^{64}Cu-labelled water in Expts 3 and 4, on the other hand, should give more information on the relative importance of the different sources of Cu uptake.

Expts 1 through 4 were repeated three times, with different ratios between particulate and dissolved ^{64}Cu. Also the accumulation and elimination periods were varied. The experimental scheme is summarized in Fig. 1.

In the first series of Expts 1 through 4 (repetition 1), the algae were exposed to 790 n*M* ^{64}Cu for 48 h and rinsed with 5 μM EDTA for 18 h [for more details see Absil et al. (1993)]. After a 3-h feeding period in 2-litre beakers, the individuals were put in FSW for another 3 h to clean their guts. This time was considered to be sufficient, as the average gut passage time for *Macoma balthica* was reported to be approximately 90 min, irrespective of the temperature (range

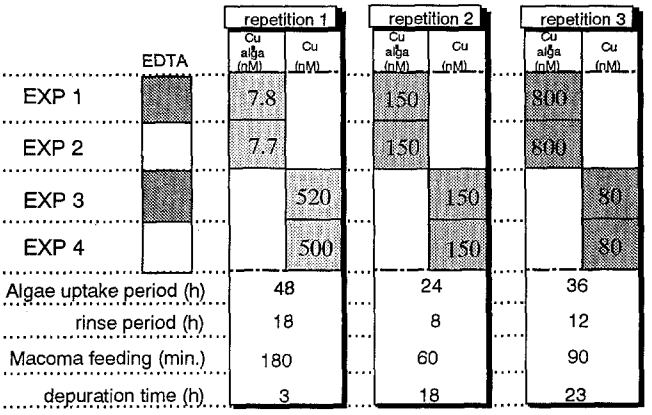

Fig. 1. Experimental setup of feeding Expts 1 to 4 with three repetitions. ^{64}Cu concentrations (in n*M*) in the experiments shown in shaded boxes

5.5 to 21 °C) (Hummel 1985). After a short freezing period, the individuals were dissected and the radioactivity of the shells and tissue was measured immediately. For each measurement, three individuals were taken. The measurements were carried out in triplicate.

Water samples were taken regularly during the feeding period to follow the decrease in algal density. For algal counts, the samples were fixated with 2% formalin. Algal densities were measured with a particle counter (Coulter Multisizer). In order to follow further filtration behaviour, nine individuals per experiment were allowed to continue filtering after the 3-h accumulation period. The accumulation results were corrected for the possible differences in pumping rate: the metal accumulation rate is supposed to be directly dependent on the amount of water that has passed the gills. This depends on the pumping activity of *Macoma balthica*. The amount of water that had passed the gills was assessed by measuring the decrease in algal density, relative to the number of individuals during the experiment.

In the second run of Expts 1 through 4 (Fig. 1), four *Macoma balthica* individuals were kept per 100-ml beaker. Total ^{64}Cu concentration on the labelled algae was similar to the dissolved concentration with unlabelled algae: 150 n*M*. Because of the short half-life of ^{64}Cu, the loading procedure of the algae was shortened to 24 h with 7.9 μM ^{64}Cu spiking and 8 h rinsing to be able to measure elimination as long as possible. The clams were allowed to feed on labelled algae and on unlabelled algae with dissolved ^{64}Cu for 60 min. After the feeding period, the clams were transferred to glass scintillation vials, each containing 20 ml FSW and unlabelled algae. The algae were added to continue digestive activity. While digesting, the total ingested material was determined by whole body counts on the living individuals. After 90 min, the individuals were transferred to other vials with FSW and unlabelled algae and allowed to depurate for another 90 min. The first series of vials with defecated material was measured for radioactivity. After a second transfer, the individuals were left overnight. Using this method, elimination from the individual *Macoma balthica* could be measured without losses. This series was carried out in duplicate, revealing 2×4 individuals for each experiment.

The third run of the experiments was comparable with the second, but now the transfers to a fresh vial were more frequent, in order to detect any pattern in the elimination. The transfers were carried out every 60 min for the first 3 h, every 90 min for the following 10 h and finally after 2 h. The transfers were continued until the radioactivity of the depuration products was near the detection limit (caused by a decrease of the elimination and the decay of ^{64}Cu). The individuals were subsequently dissected, and the remaining radioactivity of the shells and tissue were counted. This series was carried out in triplicate, revealing 3×3 individuals for each experiment.

With the elimination data of the second and third runs, the half-life of ^{64}Cu in the ingested food was calculated. The elimination of ^{64}Cu can be described by the exponential function:

$$A_t = A_o e^{-\lambda t}, \quad (1)$$

where A_o = ^{64}Cu at time o; A_t = ^{64}Cu at time t; t = time since feeding; λ = loss rate constant.

The half-time for the ingested material is:

$$T_{1/2} = \frac{\ln 2}{\lambda}. \quad (2)$$

As a measure for the part of the radionuclide from the food that is retained by *Macoma balthica*, the absorption efficiency is calculated by comparing the total ingested and total eliminated radioactivity:

$$F = \frac{I-E}{I} 100, \quad (3)$$

where F = apparent absorption efficiency; I = ingested material; E = eliminated material.

Results

In the first run of Expts 1 and 2, the concentration of ^{64}Cu associated with the algae was 7.8 and 7.7 n*M* (see Fig. 2). The ^{64}Cu concentration in the water with unlabelled algae (Expts 1 and 2) was 520 and 500 n*M*. Because the initial ^{64}Cu concentrations in the media were very different, the accumulation results in Fig. 2 were related to the ^{64}Cu concentration in the water. Surprisingly, the uptake of ^{64}Cu by *Macoma balthica* from labelled algae was considerable (Fig. 2b). The low amount of ^{64}Cu on the shell indicates that if any Cu had been lost from the algae, this was effectively complexed by EDTA (Fig. 2a). Without EDTA in the water, the sorption on the shells was a little higher, indicating that a small amount of ^{64}Cu had been leaking from the labelled algae. The difference in accumulation between Expts 1 and 2 was not significant. In Expts 3 and 4 with unlabelled algae and dissolved ^{64}Cu, overall sorption on shells and uptake in tissue was very low in the presence of EDTA. This indicates again that dissolved ^{64}Cu was effectively complexed by EDTA. It was also clearly demonstrated in Expt 3 that Cu-EDTA was not accumulated. Without EDTA, uptake was considerable (as expected). Sorption on shells was even higher than uptake. This pattern is also seen in uptake experiments without algae (Absil et al. 1993). The total decrease of algae in Expt 3 was comparable with Expts 1 and 2 (Fig. 3). The filtration rate in Expt 4 was distinctly lower than in the other test beakers (Fig. 3).

In the second run, the particulate ^{64}Cu concentration (associated with the algae) was similar to the dissolved concentration: 150 n*M*. Because in the first run the limited depuration time could have masked real ^{64}Cu accumulation through a large portion of undigested algae, depuration time was extended. Although the total ^{64}Cu concentration in all four experiments was similar, the uptake results were very different (Fig. 4). The difference in ingestion between individual clams in the same treatment could be more than 100% because of the different feeding activities of the individual clams. However, in spite of this large variation, differences between the treatments were always significant (Anova: $R^2 > 0.75$ and $P < 0.001$). The ingestion of ^{64}Cu by *Macoma balthica* through the labelled algae was high, and the presence of EDTA did not cause a decreased uptake. In contrast, accumulation of ^{64}Cu from the water (in the presence of

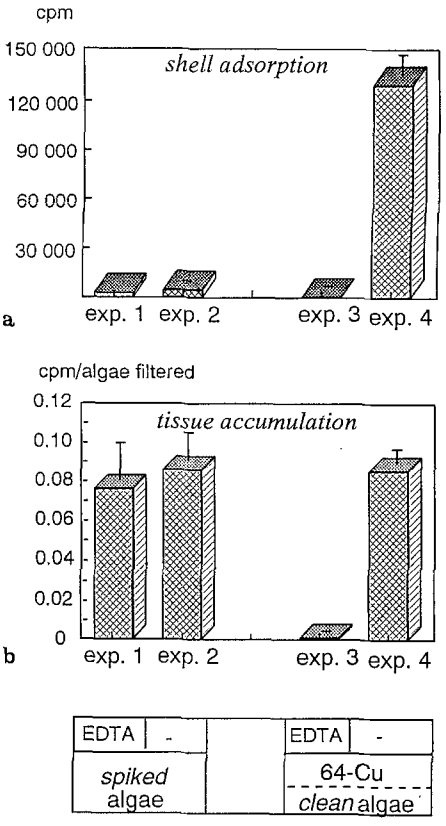

Fig. 2. *Macoma balthica*. (a) Shell adsorption and (b) tissue accumulation after 3 h feeding on ^{64}Cu-labelled and unlabelled algae in Expts 1 to 4. Accumulation plotted in relation to the administered ^{64}Cu concentration: *Y*-axis label = (cpm clam/cpm 1 ml water) 100. Depuration period was 3 h (repetition 1). Each bar represents the average of nine clams

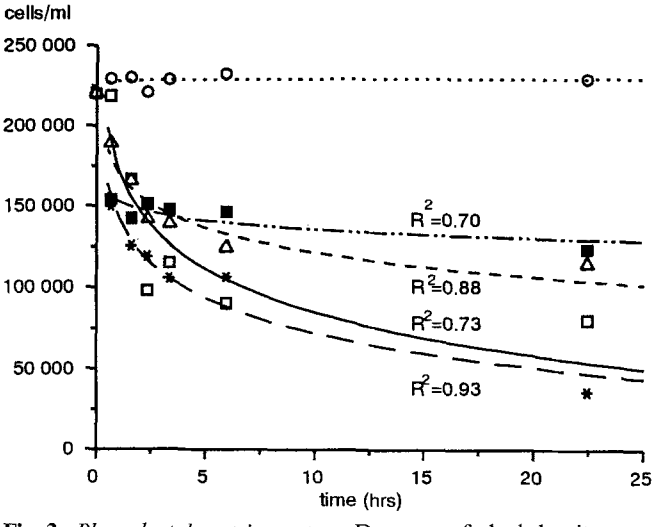

Fig. 3. *Phaeodactylum tricornutum*. Decrease of algal density as a result of feeding *Macoma balthica* in repetition 1 of Expts 1 to 4. Expt 1 (□); Expt 2 (△); control (no clams) (○); Expt 3 (*); Expt 4 (■)

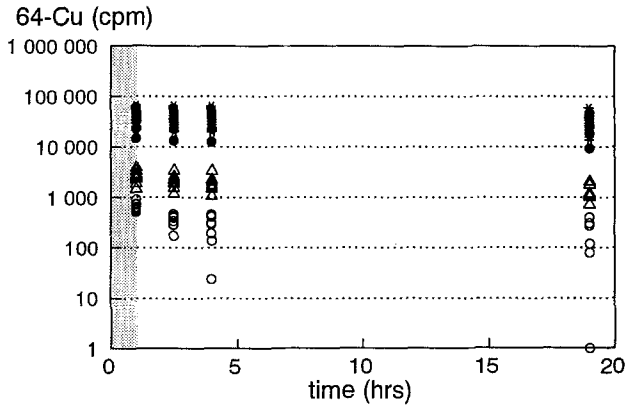

Fig. 4. *Macoma balthica.* Ingestion and depuration of ^{64}Cu in repetition 2 of Expts 1 to 4. Total (dissolved or particulate) ^{64}Cu concentration equal in all test situations. Expt 1 (*); Expt 2 (•); Expt 3 (o); Expt 4 (Δ). Shaded part indicates feeding period. Data points at $t=1$ h indicate ingested ^{64}Cu. Data points at $t=2.5$, $t=4$ and $t=19$ h indicate ingested ^{64}Cu minus the sum of depurated material at that time

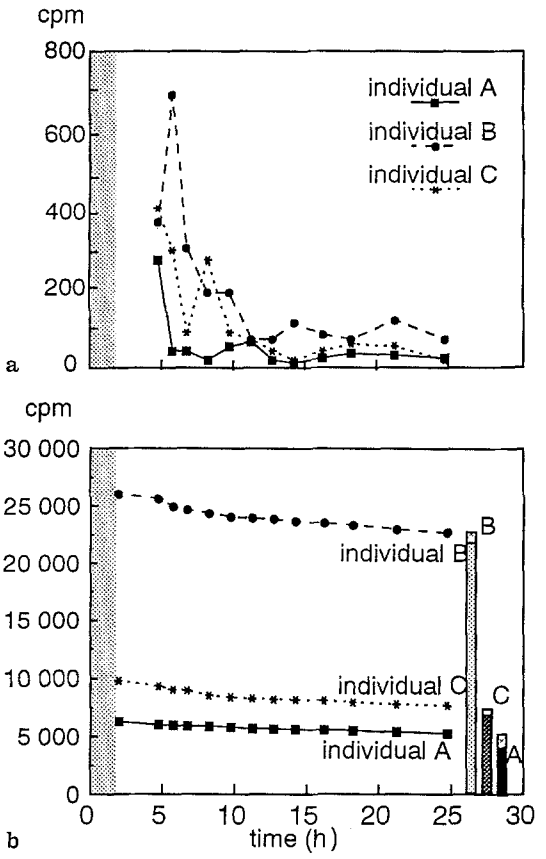

Fig. 5. *Macoma balthica.* (a) Typical elimination patterns of ingested ^{64}Cu-labelled algae in three clams (repetition 3 of Expt 1). (b) Ingestion and depuration of ^{64}Cu in the individual clams (see 5a). Shaded part indicates feeding period. First data point indicates ingested ^{64}Cu. Following data points indicate ingested ^{64}Cu minus the sum of depurated material at that time. Bars represent ^{64}Cu content of the dissected clam at the end of the depuration time. Crossed part stands for the proportion adsorbed on the shell

unlabelled algae) in Expt 4 was much lower, although algal density and total ^{64}Cu present were comparable. The presence of EDTA reduced uptake of dissolved ^{64}Cu to amounts near the detection limit (Expt 3). The biological half-life of the ingested ^{64}Cu-algae was 49.3 ± 22.6 h in Expt 1 and 54.6 ± 16.9 h in Expt 2 (without EDTA). The half-life of the ingested dissolved ^{64}Cu in Expt 4 (without EDTA) was 6.3 ± 2.4 h. In the situation with dissolved ^{64}Cu and EDTA (Expt 3), no reliable half-lives could be calculated. The elimination from the clams that accumulated ^{64}Cu from the water was difficult to measure: counts were near background measurements. Counting error was more than 20% in this case. In spite of the difficult elimination measurement, it can be concluded that based on the large differences in accumulation results from Expts 1 through 4, ^{64}Cu uptake from labelled algae was more efficient than uptake of dissolved ^{64}Cu from seawater.

In the third run of the feeding experiments, elimination was followed in more detail. Total ^{64}Cu concentration on the labelled algae in Expts 1 and 2 was 800 n*M*. Total dissolved ^{64}Cu in the water in Expts 3 and 4 was 80 n*M*. In Fig. 5a, a typical example of the elimination in Expt 1 is given. The three individuals had the same treatment, but differed in ^{64}Cu accumulation (as in the second run). However, the elimination pattern was comparable. The ^{64}Cu concentration in the faeces was high initially, but declined progressively with time. The clams were dissected after 25 h depuration time. In Fig. 5b, elimination is compared with the ingested concentration (after the feeding period) in the clams (as in Fig. 4). The total ^{64}Cu content in shells and tissue of the dissected clams (bars) was the same as the initial ingested amount minus the sum of the elimination products at $t=25$ h. Very little ^{64}Cu was adsorbed into the shell, compared with the situation when ^{64}Cu was accumulated from the water (see Table 1). As in the first and second run, the difference in accumulation between Expts 1 and 2, on the one hand, and Expts 3 and 4, on the other hand, was remarkable.

The biological half-life of ^{64}Cu, accumulated from labelled algae in Expt 1 was 94.5 ± 20 ($n=7$) (Table 1). The counts in individuals 1.3 and 1.5 were very low, compared with other individuals in the same treatment. Considering that these individuals were probably in bad condition, they were not used in the calculation of the average half-life. In Expt 2, the half-life was 126.2 ± 50.8. The half-life of accumulated ^{64}Cu from water (Expt 4) was 61.5 ± 11.2. Because accumulation and elimination in Expt 3 were near background values, they were not used in Table 1.

Dissolved ^{64}Cu concentrations in the water in Expts 3 and 4 were kept very low, in order to prevent toxic effects, and thus to achieve a maximal uptake efficiency from the dissolved phase. In Table 1 it can be seen that the relationship between ingested and available ^{64}Cu was comparable for Expts 1 and 4. The relationship was slightly better in Expt 2. Possibly in this repeat of Expt 2, more ^{64}Cu had been leaking from the algae than in the first and second runs. In Expt 1, this leaked ^{64}Cu was immediately complexed by EDTA. In Expt 2 as well a slightly higher percentage of ^{64}Cu was found in the shell, compared with experiment 1 (Table 1).

Table 1. Ingestion (I), elimination (E) and apparent absorption efficiency (F) of ^{64}Cu from labelled algae and water in repetition 3 of Expts 1, 3 and 4. $t_{1/2}$: biological half-life of ingested Cu

Expt Treatment	Clam no.	I (cpm)	$I-E$ (cpm)	F (%)	Tissue (cpm)	Tissue + shell (cpm)	In shell (%)	Ingested/ available	$t_{1/2}$ (h)
Expt 1	1.1	22 400	17 330	77	11 820	13 640	13.3	28.0	67
800 n*M* Cu in algae + EDTA	1.2	13 260	11 380	86	9 710	10 660	8.9	16.6	119
	1.3	520	270	52	n.m.	n.m.		0.6	
	1.4	8 110	6 660	82	4 200	5 250	20.1	10.1	95
	1.5	550	250	45	n.m.	n.m.		0.7	
	1.6	22 740	19 190	84	17 630	18 600	5.2	28.4	89
	1.7	6 320	5 310	84	2 850	4 060	29.7	7.9	100
	1.8	26 040	22 730	87	21 380	22 440	4.7	32.5	122
	1.9	9 770	7 720	79	7 270	7 680	5.3	12.2	70
Expt 2	2.1	44 230	38 480	87	19 910	25 440	21.8	55.3	115
800 n*M* Cu in algae − EDTA	2.2	11 470	10 310	90	8 440	10 040	15.9	14.3	231
	2.3	10 310	8 950	87	6 870	8 230	16.6	12.9	128
	2.4	13 900	11 590	83	6 530	9 310	29.8	17.4	90
	2.5	55 710	49 960	90	44 670	48 430	7.8	69.6	197
	2.6	19 630	16 790	86	12 680	16 400	22.7	24.5	105
	2.7	9 880	7 740	78	3 720	5 760	35.4	12.4	75
	2.8	34 560	29 360	85	24 830	27 760	10.5	43.2	119
	2.9	3 690	2 920	79	1 460	2 530	42.3	4.6	75
Expt 4	4.1	1 274	993	78	74	1 137	53.3	15.9	86
80 n*M* Cu in water − EDTA	4.2	306	267	87				3.8	
	4.3	862	648	75	373	646	42.2	10.8	60
	4.4	295	208	71		229	114	3.7	
	4.5	1 020	735	72	307	645	52.2	12.8	50
	4.6	1 124	810	72	442	559	19.7	14.1	53
	4.7	301	236	78	78	318	75.4	3.8	68
	4.8	862	627	73	342	423	19.2	10.8	58
	4.9	284	208	73	36	220	84	3.6	56

Expt 3 (unlabelled algae with ^{64}Cu and EDTA) resulted in a very low accumulation. The elimination data were around the detection limit, so they were not used for further calculations.

The shorter biological half-life of dissolved ^{64}Cu (Expt 4) could be due to the fact that the major part of the Cu was reversibly bound to the shell. Absorption efficiency (F) was 87.9 ± 3.0 ($n=7$) in Expt 1. In Expt 2 it was $89.6\% \pm 3.7$ ($n=9$). In Expt 4 it was $80.1\% \pm 3.8$ ($n=9$). From the accumulated ^{64}Cu, a major part will be adsorbed on the shell.

Discussion and conclusion

Food density or even food absence might influence metal uptake from solute as well as from particulate sources for the following reasons: firstly, because metals are possibly accumulated from the food particles; secondly, because food quantity influences filtration activity and consequently the amount of dissolved metal that passes the gills (Janssen and Scholz 1979, Riisgård et al. 1987); thirdly, food availability will influence the physiological condition of organisms, which also determines the uptake rate of metals (Luoma 1983). To compare uptake via food and uptake from the water a similar pumping (or filtration) rate is required. This was achieved by adding the same amount of (unlabelled) algae to ^{64}Cu-labelled seawater in Expts 1 to 4. However, dissolved Cu has a tendency to adsorb to particulates, including algae. Adding unlabelled algae might reduce the dissolved Cu concentration and increase the particulate concentration, with unknown effects on the experimental results. Spiking experiments (Absil et al. 1993) showed that only a minor fraction of dissolved ^{64}Cu could become adsorbed on the algae during the time period of the feeding experiment. The filtration rate in Expt 3 was distinctly lower than in the other experiments (Fig. 3). Possibly, dissolved ^{64}Cu concentrations were high enough to inhibit filtration rate in these treatments. If Fig. 2b was corrected for the difference in filtration rate, still a large uptake from the labelled algae series would be seen. As in the subsequent runs of the experiments, dissolved ^{64}Cu concentrations were much lower, filtration activity was expected to be unaffected.

The calculated half-life of the ingested ^{64}Cu in the third run was rather different from the second. An explanation for the differences noticed might be the manipulation in the third run. The frequent transfer of the clams to fresh vials could have caused certain stress that retarded the digestive process. Another explanation can be found in the time of the year during which the experiments were carried out: the second run took place in June, whereas the third run was carried out in February. The clams used here were kept in a field station under ambient seawater temperatures. They are likely to have adapted their feeding activity to the seasonal food availability (Hummel 1985). Difference in feeding activity could therefore have influenced the half-life of the ingest-

ed material. Nevertheless, both runs of Expts 1 through 4 indicated that uptake of ^{64}Cu, associated with algae, might be at least as efficient as uptake of dissolved ^{64}Cu.

It can be argued that the conditions in these experiments are never met within natural waters, because the algae were exposed to much higher Cu concentrations for the accumulation than were the bivalves in the feeding experiments. However, because the spiking period was limited, a high concentration had to be used to obtain sufficiently labelled algae. The total Cu concentrations and the ratio between dissolved ^{64}Cu and food-associated ^{64}Cu in these experiments were realistic for estuarine and coastal environments. Although the distribution of Cu over the dissolved and particulate phase in estuarine waters varies considerably, generally dissolved and particulate fractions are of the same magnitude with a partition coefficient (K_d) between 1 and 2 (Valenta et al. 1986, Baeyens et al. 1987, Golimowski et al. 1990).

In a fjord with dissolved Cu concentrations varying from 0.3 to 4.0 $\mu g\, l^{-1}$ (4.8 to 64 n*M*), the Cu concentration in *Phaeodactylum tricornutum* (exposed in dialysis bags) varied between 6 and 54×10^{-9} $\mu g\ cell^{-1}$ (Eide and Jensen 1979). Considering a moderate algal bloom with 2×10^7 cells l^{-1}, the amount of Cu associated with the algae would be 0.12 to 1.08 $\mu g\, l^{-1}$. (In that particular situation, a *Macoma balthica* individual would have received the major part of its Cu through the food).

The results from the present study cannot be compared with other aquatic accumulation studies using ^{64}Cu, because as far as we know, this isotope has not previously been used.

The only data on the contribution of food-associated Cu are known for young oysters (*Crassostrea gigas*): Cu contaminated algae induced poor growth and high mortalities in grazing larvae (Wikfors and Ukeles 1982). Amiard-Triquet et al. (1988) found a retention rate of phytoplankton-associated Cu of around 42%. Body burdens induced by exposure to Cu-contaminated seawater or contaminated water plus food were at least ten times higher than those registered in oysters exposed via phytoplankton. These results cannot be compared directly with our observations because no mention was made of the actual amount of copper that was associated with the algae. In our experiments it was shown that, even with relatively low particulate Cu concentrations, accumulation from food was considerable.

It is recognized that food particles will increase metal accumulation, because the filtration activity is stimulated (Borchardt 1983, Martincic et al. 1987). On top of this effect, we measured considerable Cu uptake from algae. A possible explanation can be found in the difference between deposit and suspension feeding. Suspension feeders have to concentrate a very dilute food source, whereas deposit feeders select from a concentrated source (Gilbert 1977). For several *Macoma balthica* species it is known that their pumping rates are far inferior to those of suspension feeders and average about 10% of the latter (Hughes 1969). If less water per unit of time is passing the gills, metal accumulation from the water phase is probably less important for deposit feeding bivalves when compared with suspension feeding bivalves. This could explain the discrepancy between the data on Cd for the filter feeding *Mytilus edulis* and the deposit feeding *Macoma balthica* (see "Introduction").

Will the ingested ^{64}Cu actually be accumulated? This depends on the digestive process. After ingestion, the first step in particle selection involves the gills: "quality" particles are passed to the labial palps. Unwanted particles are removed by ciliae and ejected as pseudofaeces via the inhalant opening. At the labial palps, further sorting takes place. Finally, particles of a suitable size are passed to the mouth and stomach (Gilbert 1977). In the stomach, extracellular digestion takes place through enzymes, released by the crystalline style. Finer particles are sent to the tubules of the digestive gland. Digestive cells phagocytize the particles and digest them intracellularly (Morton 1973). Food particles of suitable size can enter the stomach. In the gut of deposit feeders, pH is around 6 to 7. This level is lower than the surrounding seawater, and it can be expected that some weakly bound Cu species will be stripped from the algae. At this moderate pH level, carrier molecules will still be efficient in complexing the metal for transport (Luoma 1983). It is assumed that with microalgae the majority of Cu is in a readily exchangeable form, namely, associated with carboxyl groups on the cell wall.

For our experiments, it was necessary to have an idea about the duration of the different stages of digestion. Decho and Luoma (1991) assessed the time courses for ingestion, retention and release of microbial food and associated ^{51}Cr. Our experimental setup was comparable in some ways, but had the drawback of the limited time for measuring elimination. With the microbial food, Decho and Luoma found a gut passage time of 9.6 h. This was rather different from the 1.5 h gut passage time when feeding on diatoms, mentioned by Hummel (1985). The large difference between these figures can possibly be caused by the food source: a food-specific gut passage time is reported by several authors (Calow 1975, Taghon et al. 1978, Bricelj et al. 1984). As our food source more resembled the case with 1.5 h gut passage time, this was assumed to be a more realistic figure for our experiment.

The elimination pattern of the clams (Fig. 5 a) showed a peak in the first 2 h of elimination and a steady decline of tracer radioactivity in the subsequent fecal products. No distinction was made between faeces or pseudofaeces production, but the elimination in the first 2 h of the depuration time was considered to be pseudofaeces, as the gut passage time was supposed to be 1.5 h. Although we could not make a distinction between intestinal or glandular digestion (Decho and Luoma 1991), we considered the ingested material that remained after the gut passage time to be subjected to intracellular digestion and available for uptake. If the elimination pattern with 9.6-h gut passage time was chosen, a considerable portion of the ingested ^{64}Cu would still be adsorbed from the food particles.

From the above it can be concluded that although the radiotracer ^{64}Cu has a rather short half-life, assessment of the accumulation of Cu via labelled algae or water was possible. A main advantage of ^{64}Cu in comparison with "cold" Cu (^{63}Cu) is the possibility of assessing accumula-

tion in short-term experiments at environmentally realistic concentrations. From the results with feeding experiments it was obvious that Cu associated with food particles was very available for accumulation by *Macoma balthica*. The actual contribution of food-associated Cu to the overall Cu accumulation by *Macoma balthica* will depend on factors like Cu content in the food, feeding behaviour (suspension or deposit), food availability and the nutritive value of the ingested material.

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