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Effects of gut chemistry in marine bivalves on the assimilation of metals from ingested sediment particles

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ABSTRACT

Bioavailability and uptake of trace metals by benthic animals are often assumed to be limited by authigenic sulfide minerals because of their low metal solubilities and reactivities under sedimentary conditions. However, digestive processes and gut conditions such as Eh, pH, and enzyme or surfactant activity, can affect the release of ingested metals in the gut and control uptake. In a series of laboratory experiments with the deposit-feeding clam, *Macoma balthica* and the suspension-feeding mussel, *Mytilus edulis,* we assessed assimilation efficiencies (AE) of radioisotopes of Ag, Cd and Co associated with acid-volatile sulfide (AVS), iron oxide (re-oxidized AVS), and reduced and oxidized natural sediment. To evaluate controls on AE, we measured the gut passage time (GPT) of ingested particles, gut Eh, pH, and extraction of Ag, Cd, and Co from particles into "gut juice." In general, the overall trends of AEs and metal extraction were Co > Cd ≥ Ag. AEs, metal extraction, and GPTs were higher in *M. balthica* than in *M. edulis* in most cases. *M. balthica* tended overall to take up metals more readily from oxidized than reduced natural sediment, whereas *M. edulis* did the opposite for Co and Cd. AEs of metals in reoxidized AVS (Fe-oxides) were generally similar to oxic sediment (Ag being the exception for *M. edulis*). In *M. balthica,* there was no significant difference in AEs from AVS and Fe-oxide particles for Cd (14–20%) or Co (27–35%), but AEs for Ag from AVS particles were greater in large clams (28%) than small clams (15%). There were generally poor correlations between AEs of metals and metal release in gut juice. Low pH and moderate reducing conditions facilitated dissolution of AVS- and iron oxide-bound metal in the guts of both animals. The GPTs (64 h) for Co associated with AVS particles in *M. edulis* were an order of magnitude greater than for Ag and Cd, or for Co associated with other particle types. Overall, no single mechanism appears to control metal AE in marine bivalves and *in vitro* studies of metal dissolution in gut juice do not completely mimic the complex digestive processes operating *in vivo,* and thus cannot fully explain metal assimilation in these animals.

1. Introduction

Estuarine sediments constitute a large reservoir of trace metals in coastal areas. These metal-rich particles are commonly ingested by suspension and deposit-feeding animals

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(Campbell *et al.,* 1988). The degree to which sedimentary metals are bioavailable and toxic to animals can determine the extent to which contaminated deposits negatively impact benthic communities. Many studies have focused on the geochemical properties of sediments and how they influence bioavailability in organisms. Except for a thin oxidized surface layer and localized irrigated areas, the bulk of surface estuarine sediments are moderately to highly reduced, and trace metals, especially B-type metals, strongly associate with sulfides. In particular, acid-volatile sulfides (AVS), an operationally defined labile sulfide constituent, have been implicated as a key binding phase for metals and control on pore water metal concentrations (Morse, 1994; DiToro *et al.,* 1990). Uptake of metals from the ambient dissolved phase had been assumed to be the primary route of metals into benthic invertebrates in many studies; however, there are additional factors that can control bioavailability (Luoma, 1989). Recently, a number of field and laboratory studies have shown that for some trace elements, the predominant route of metal uptake for diverse benthic organisms is ingestion of food (Luoma *et al.,* 1992; Wang and Fisher, 1999a; Wang *et al.,* 1999; Lee *et al.,* 2000a).

Surface sediments are subjected to repeated passes through the digestive systems of detritus-eating organism. The geochemical properties within animal guts can differ substantially from sedimentary environments with regard to pH, Eh, and concentrations of DOC, enzymes, and surfactants. Gut geochemistry can influence the stability of mineral phases, trace metal partitioning and affect bioavailability (Plante and Jumars, 1992; Mayer *et al.,* 1996, 1997). For example, in the gut, dissolution of iron phases that bind many trace metals (both oxide and sulfide phases) may influence the assimilation of these metals in animal tissues. In general, it is thought that metals must be in the dissolved form before they cross the gut lining (Reinfelder and Fisher, 1991; Mayer *et al.,* 1996; Gagnon and Fisher, 1997), although finer particles can be selected and sent to the digestive gland of bivalve molluscs where they are phagocytized, digested intracellularly and assimilated (Purchon, 1971; Decho and Luoma, 1996).

In this study, we focus on the digestive environment of two marine bivalve molluscs, the mussel *Mytilus edulis* and the clam, *Macoma balthica,* and how properties of the gut, such as pH and Eh and gut processing time, affect the assimilation of metals bound to ingested sediment particles. *Macoma,* a facultative deposit feeder, feeds from the surface sediment or water column, depending on food availability (Brafield and Newell, 1961; Olafsson, 1986). *Mytilus* gains nutrition predominantly from the water column (Bayne and Newell, 1983) and consumes only small amounts of inorganic particles (Wang *et al.,* 1996). However in some areas *Mytilus* can be dependent on microphytobenthos in resuspended sediments as a food source (Smaal and Zurburg, 1997).

Assimilation efficiency (AE) reflects the bioavailability of a metal for a given animal under a specified set of conditions and is experimentally determined by measuring the fraction of ingested metal retained by an animal after it has emptied its gut. Although AE as operationally defined here may reflect total retention of a metal in various forms rather than physiological assimilation per se, such values can be compared among different organisms

and between trace elements, and have been used successfully in modeling metal accumulation in clams and mussels (Luoma *et al.,* 1992; Fisher *et al.,* 1996; Wang *et al.,* 1996). We used gamma-emitting radioisotopes to measure the assimilation in animals of Ag, Cd, and Co from ingested oxidized and reduced sediments, and from AVS and re-oxidized AVS (Fe_xS_y and FeOOH). To help interpret our assimilation data, we evaluated factors that might influence metal assimilation in the bivalves, including the gut Eh, the influence of gut passage time of ingested metals, and the influence of "gut juices" on metal release from ingested particles. The importance of sulfides in the diet of these two animals is unknown but a fraction of detritus consumed by *Macoma* likely contains sulfides. It is unlikely that *Mytilus* would regularly consume significant amounts of sulfides, however resuspension from storms could expose mussels to reduced particles as a pulse input.

2. Materials and methods

Clams were collected from a mudflat in San Francisco Bay near Palo Alto Baylands (a site in which the clam population has been studied for more than 25 years) and mussels were gathered off a jetty at the entrance of Flax Pond (Wang *et al.,* 1995), a small, relatively pristine salt marsh located on Long Island in Old Field, NY. Small and large clams (shell lengths 14 $\pm$ 2 and 25 $\pm$ 3 mm, respectively) were used for the pure phase particle experiments and for iron reduction measurements in the guts, and medium-large clams (21 $\pm$ 1 mm) for the oxic/anoxic sediment AE experiments. Small, medium and large mussels (shell lengths 1.5, 3.0 and 5 cm) were used for the iron reduction measurements, and 3.0–3.5 cm mussels were used for all AE experiments. All natural sediments used in these experiments were collected from Flax Pond in the area where the mussels were collected. Surface sediments were carefully scraped off the top 0.5 to 1 cm, sieved to $<$63 μm in oxidizing conditions, and the fine sediments were kept for up to 2 months at 2–4°C before use.

To measure the oxidation state of iron in the guts of the two species, we used a colorimetric technique that utilizes ferrozine to measure reduced iron (Stookey, 1970). Initially, animals were fed a diet of the diatom *Thalassiosira pseudonana* for 1 d prior to the beginning of the experiment to minimize residual detritus-derived iron levels in the digestive system. Total iron concentrations in the gut contents (plankton) of animals were below detection ($\sim$2 μmol Fe g^{-1}) before feeding with sediment. Two batches each of 8 clams or 5 mussels were placed into well-stirred aquaria and fed oxidized sediment at a concentration of approximately 4 mg $liter^{-1}$. In a separate treatment, 8 clams were placed into sediment in an aquarium filled with 3 cm of sediment and 1 liter of aerated seawater (25 psu) and allowed to burrow in and feed on surface sediment. In all treatments the animals were removed after 2 or 6 h (*Mytilus* or *Macoma*), dissected, and sediment material in the stomachs (approximately 5–10 mg) was immediately placed into 2 ml of 6 N HCl and vigorously vortexed. Aliquots of the oxidized suspended sediment, *in situ* sediment (surface and 1 cm depth), and fecal material were also extracted and the amount of both total Fe and Fe(II) were measured. Suspended sediments had no measurable

reduced Fe, whereas *in situ* sediment had about 15% reduced Fe. After leaching for exactly 10 min in 6N HCl, the acid/particle mixture was centrifuged and 0.5 ml of the overlying liquid was pipetted into each of two vials and analyzed for Fe(II) or for total iron (Aller and Blair, 1996). This leach procedure releases approximately the same total quantity of iron and dissolves similar Fe phases (plus Fe-carbonate) as does the common oxalate leach for reactive iron oxides (Canfield and Berner, 1987; Aller and Blair, 1996). Iron results are reported as percentage of total reactive iron in the form Fe(II). Standards of 4 Fe concentrations were used to create a standard curve. Blanks containing 6N HCl were all below detection. Egested fecal pellets (collected within 10 min of egestion) and the stomach contents of animals fed on algae alone were also analyzed.

A separate radiotracer experiment was conducted to compare the AE of ingested radioactive Cd, Co and Ag associated with pure phase AVS (FeS) and iron(hydr)oxides. Pure phase FeS was precipitated onto 10 μm ashed, acid washed glass beads, and a re-oxidized FeS treatment was created following the methods of Lee *et al.* (2000a) and Schoonen and Barnes (1991). The chemical structure of the precipitate is initially amorphous but transforms within seconds to hours into mackinawite, a metastable iron sulfide mineral and a common component of AVS in anoxic sediments (Rickard, 1989; Morse and Arikaki, 1993). To co-precipitate the radioisotopes with the AVS, microliter additions of ^{110m}Ag (in 0.5 N HNO_3), ^{57}Co (in 0.1 N HCl) and ^{109}Cd (in 0.1 N HCl) were added to 1 ml of a 4.0 mM solution of $Fe(NH_4)_2(SO_4)_2$ in de-aerated, 30 psu filtered seawater to produce approximately 20 ng Ag mg^{-1} beads, 45 ng Cd mg^{-1} beads, and 300 pg Co mg^{-1} beads. Dilute NaOH was added to neutralize the acid additions. This mixture was stirred into 4 ml of 1.1 mM Na_2S in de-aerated, deionized water containing 80 mg of 10 μm glass beads, which served as a substrate for the instantaneously precipitated Fe(^{110m}Ag, ^{57}Co, ^{109}Cd)S. Additionally, approximately 5 kBq of ^{85}Sr contained within inert synthetic (Furan) beads (15 μm diameter) were also added to determine the rate of digestive processing of the bulk material. Similar methods were employed by Decho and Luoma (1991) in which inert ^{51}Cr-impregnated latex beads (also 15 μm diameter) were fed to *Macoma.* The sulfide precipitates were rinsed twice with de-aerated seawater and were cured for 18 h. To create the iron(hydr)oxide treatment, an aliquot of the sulfide particles was re-oxidized by gently bubbling with air for 18 h. Both AVS and re-oxidized AVS (termed here 'Re-ox') particles were resuspended into well-stirred aquaria and fed as a pulse (approx. 4 mg L^{-1} particle concentration—a concentration low enough to prevent the production of pseudofeces) to actively filtering mussels and clams for 5 or 15 min, respectively. Oxidation of FeS and the co-precipitated metal sulfides was negligible in the 15 min exposure, where $<10\%$ of the FeS would be expected to oxidize (Simpson *et al.,* 1998; DiToro *et al.,* 1996), indicating that animals ingested the metal sulfide phase.

In a fully oxic aqueous environment, re-oxidation of precipitated mono-sulfides probably occurs on particle surfaces, initially forming an oxide rind over deeper sulfide layers (DiToro *et al.,* 1996; M. Schoonen, pers. comm.) in which the ^{110m}Ag, ^{57}Co and ^{109}Cd,

initially associated with the FeS probably remained associated with the newly formed iron(hydr)oxide. This concept was substantiated in a preliminary experiment in which radiolabeled anoxic sediment was resuspended into oxic seawater at a particle load of 40 mg/liter. Over 99% of the metals remained bound to particles (> 0.2 μm) after the re-oxidation step and no appreciable loss of isotope into the dissolved phase was measurable at any time over a period of 2 d.

The methods for labeling the oxic and anoxic sediment, and the protocol used in this study for conducting AE experiments are described in detail elsewhere (Griscom *et al.,* 2000). Briefly, retention of the radioisotopes in the live individual animals and in the egested fecal pellets was monitored for 4 d by noninvasive gamma counting of the whole animal. Fecal pellets from each individual animal were collected every 15 to 20 min during the first 2 h after the appearance of radioactive fecal pellets, every 2 h for the next 8 h, and then every 6–12 h for the remainder of the experiment. Once the gut was emptied of the initial pulse of radioactive food (3 d), the radioactive metal remaining in the animal was considered assimilated; gut passage time (GPT) was calculated as described in Wang *et al.* (1995). GPT, defined operationally as the time at which 90% of the total fecal radioactivity is lost from an individual, is positively correlated with the AEs of some metals in mussels feeding on phytoplankton (Wang and Fisher, 1996). Thus, AEs as determined here, in which metals retained in the animals are measured after gut passage is complete, include metals physiologically bound to tissue, metals that may remain within the gut fluids, and other pools of metals not eliminated by the animals during defecation. AEs of Cd, Co, and Ag measured in 2-wk and 4-d depuration periods produced comparable values (Griscom, unpubl.), indicating that the AEs measured with the 4-d method used here probably reflect assimilated metal rather than entrained unassimilated metal in the bivalves.

To simulate the desorption of metal from particles in the guts of the two bivalves, experiments were performed by resuspending the AVS and Re-ox particles into extracted clam and mussel gut juice. The gut juice was collected from large clams (24–30 cm, n = 100) and mussels (> 5 cm, n = 40) by centrifuging the contents of dissected stomachs and digestive glands. A total of 4.5 ml was extracted from the clams and mussels, and the pH was measured immediately. The juice was then filtered (0.2 μm) under a N_2 atmosphere. One batch of gut juice was kept anoxic and another batch (clam gut juice only) was gently bubbled with air for 1 d. 10 mg of AVS or Re-ox particles were each resuspended into 200 μl of oxic or anoxic clam gut juice, anoxic mussel gut juice, or pH 5.5 seawater. Desorption of metal from the particles was measured periodically (Fisher and Teyssié, 1986; Fisher *et al.,* 1991) over 8 h. Additions of fresh gut juice followed by centrifugation and removal of equal amounts of supernatant fluid were performed every hour. This method of multiple extraction allows for the use of small amounts of liquid and minimizes desorption artifacts that may be caused by concentration equilibria between the liquid and the particle at each time step (M. Ahrens, pers. comm.). The pH of the gut and seawater solutions remained stable throughout the metal desorption experiments. The glass pH

electrode (Ag/AgCl reference; VWR Scientific) was calibrated against NIST buffers (pH 4, 7, 10).

In addition to measuring Fe reduction directly into the gut, we also used tetrazolium salts as a second measure to bracket the gut Eh (Altmann, 1976). Tetrazolium salts are relatively colorless and are soluble in water. They are designed to provide an irreversible color indication when exposed to a specific Eh (or mV). In this study we used three tetrazolium salts: nitroblue tetrazolium (−50 mV, pH 7.2) iodonitrotetrazolium violet (−90 mV, pH 7.2), and neotetrazolium (−170 mV, pH 7.2), to estimate the reducing potential of the gut juice (Altman, 1976; Ahrens and Lopez, 2001). The Eh values of tetrazolium reductions were corrected for measured gut pHs (Eh of tetrazolium reduction varies inversely with pH and is ~50 mV more positive in the pH range 5—5.6, assuming a single reduction step to formazan). The salts were added directly into freshly extracted gut juice under oxic and anoxic conditions. Color indication was evident within 15 min and progressively deepened during the following hour.

Radioanalyses of whole organisms were determined using a Canberra deep well NaI(Tl) gamma detector, while activities in all other samples (water and filters) were determined with an intercalibrated LKB Wallac 1282 Compugamma counter. The gamma emissions of ^{110m}Ag were determined at 658 keV, of ^{57}Co at 122 keV, of ^{109}Cd at 88 keV, and of ^{85}Sr at 514 keV. Counting times were adjusted so that propagated counting errors were <5%. All samples were counted with radioactive standards and were corrected for background, inter-element interference and for decay where necessary.

3. Results

Both the clams and mussels ingested the radiolabeled pure-phase particles and sediment particles. After the feeding periods on radioactive particles, the depuration patterns of the ingested radiolabeled metals obtained from AVS-coated glass beads (AVS) and re-oxidized AVS-coated beads (Re-ox) were typically an initial rapid egestion (especially in *Mytilus*), followed by a slower loss rate (Fig. 1). Release of inert ^{85}Sr beads showed that egestion of the unassimilated radioactive material was essentially complete after approximately 2 d in *Mytilus* and that the loss patterns were the same for the two pure-phase particle types. In *Macoma,* 3–8% remained in the animals after 3 d.

Metal AE values, calculated as the percent of ingested metal remaining in the animal's tissues at 72 h of depuration (Wang *et al.,* 1995), ranged from a low of 14% for Cd in small clams to a high of 35% for Co in large clams and, in *Mytilus,* from a low of ~3% for Ag to 32% for Cd (Table 1). In *Macoma,* AE values for Cd, Co, and Ag from ingested natural anoxic sediment were approximately 2-fold lower than from either oxic sediment or pure phases, and were similar (9–15%) among all three metals (Table 1). Large clams assimilated Ag from AVS at a value almost 2 times higher than small clams, otherwise there were no significant differences ($p > 0.05$) in the AE values between the pure phase AVS and Re-ox particles for a given metal or size class of clams. With the exception of AVS—Ag in the small clams, the metal AE values did not differ statistically between

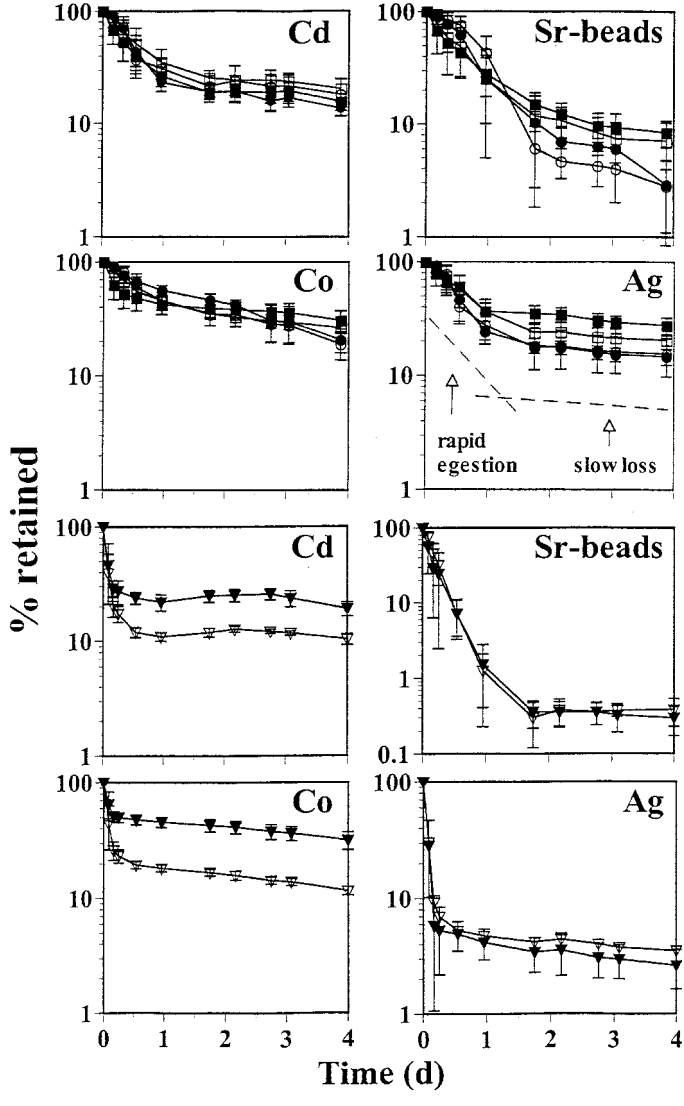

Figure 1. Depuration patterns of radioactive metals in whole animals following pulse feeding with radiolabeled particles. Filled symbols represent acid-volatile sulfide (AVS) particles and open symbols represent re-oxidized AVS (Re-ox) particles; ○ = small (14 mm) clams; □ = large (25 mm) clams; ▽ = mussels. Error bars represent standard deviations around the mean (5 replicates, each consisting of 4 individuals for *M. balthica* and 1 individual for *M. edulis*).

either the AVS or oxidized pure-phase particles and natural oxic sediments (Table 1). For *Mytilus,* the AE values of Cd and Co were about 2-fold higher from the reduced AVS particles than from Re-ox particles (19% v. 10% and 32% v. 12%), whereas the assimilation of Ag from the two pure phase particle types was virtually indistinguishable

Table 1. Metal assimilation efficiency (AE) (%), gut passage time (GPT) (h), and dissolution (%) in gut juice (GJ) of *M. balthica* and *M. edulis* or pH 5.5 seawater (SW) for AVS, Re-ox, oxic sediment, and anoxic sediment particles. Small and large *M. balthica* individuals are compared. nd: not determined.

	Ag				Cd				Co			
	AVS	Re-ox	Oxic sed	Anox sed	AVS	Re-ox	Oxic sed	Anox sed	AVS	Re-ox	Oxic sed	Anox sed
M. balthica												
% AE (small)	15 ± 2	16 ± 6	nd	nd	14 ± 2	18 ± 6	nd	nd	30 ± 8	27 ± 7	nd	nd
% AE (large)	28 ± 4	21 ± 5	20 ± 5	10 ± 2	16 ± 3	21 ± 5	21 ± 2	9 ± 3	35 ± 7	29 ± 4	28 ± 6	15 ± 5
GPT (small)	21 ± 5	23 ± 4	nd	nd	19 ± 6	20 ± 5	nd	nd	22 ± 5	26 ± 8	nd	nd
GPT (large)	30 ± 10	23 ± 7	18 ± 6	22 ± 20	17 ± 8	24 ± 11	16 ± 6	14 ± 5	36 ± 20	26 ± 10	18 ± 5	19 ± 16
% diss oxic (GJ)	14	25	nd	nd	27	54	nd	nd	94	83	nd	nd
% diss anox (GJ)	21	26	nd	nd	30	30	nd	nd	75	82	nd	nd
% diss oxic (SW)	1.7	1.6	nd	nd	2.5	1.9	nd	nd	52	54	nd	nd
% diss anox (SW)	5.4	1.5	nd	nd	7.2	0.7	nd	nd	27	12	nd	nd
M. edulis												
% AE	2.6 ± 0.4	3.5 ± 0.6	10 ± 4	3 ± 2	19 ± 3	10 ± 1	15 ± 3	33 ± 6	32 ± 6	12 ± 1	9 ± 2	15 ± 3
GPT	1.4 ± 1.2	2.2 ± 1.4	9 ± 7	7 ± 2	1.2 ± 0.4	1.8 ± 1.2	1.3 ± 0.3	1.8 ± 1.5	64 ± 5	5.9 ± 5	10 ± 8	21 ± 10
% diss anox (GJ)	0.9 ± 1	4.9 ± 4.2	nd	nd	1.4 ± 0.5	2.8 ± 2	nd	nd	>47	33 ± 16	nd	nd
% diss anox (SW)	0.2	0.01	nd	nd	0.1	0.7	nd	nd	27	12	nd	nd

and low (AE $\sim$ 3.5% v. 2.6%) (Table 1). In contrast to surface deposit-feeding clams, the AE values for Cd and Co were significantly greater from natural anoxic sediment than from oxic sediment: for Cd the AE was 35% from anoxic sediment compared to 16% from oxic sediment, the Co AE was 16% from anoxic sediment and 9% for oxic sediment. For Ag, the AE was greater from oxic sediment (13%) than from anoxic sediment (5%) and consistently 2–5 times less than for *Macoma* regardless of carrier phase (Table 1). Relative trends in the metal AEs from anoxic and oxic sediments were similar to the pure phase AVS and Re-ox particles: higher assimilation of Cd and Co and lower or similar assimilation of Ag associated with reduced compared with oxidized particles.

Gut passage time for all metals in individual *Macoma* and *Mytilus* ranged from 4 to 59 h and 0.6 to 72 h, respectively, and followed the same relative trend as AEs (Co $>$ Cd $\geq$ Ag) (Fig. 2). Good correlations of gut passage time (GPT) with AEs were found in both animals for Ag bound to pure phase AVS particles ($r^2 = 0.59$ and 0.63 for small and large *Macoma,* $r^2 = 0.35$ for *Mytilus*) (Fig. 2). GPT and Co AE in *Mytilus* correlated well ($r^2 = 0.91$) only when the pure phase AVS (GPT mean 64 h) and Re-ox particle (GPT mean 5.9 h) results were combined. For Cd in *Mytilus* there was a significant difference in the Cd AE between the two particle types ($p < 0.01$; two-way ANOVA) but no difference in the GPT ($p > 0.05$). No significant differences in GPTs were noted between the different size classes of clams or between different metals ($p > 0.05$).

The pH of the gut juice was 5.0 and 5.6 for *Macoma* and *Mytilus,* respectively. Generally, the extraction of metals in the gut juice of *Macoma* was greater than in that of *Mytilus,* and both exceeded metal release into pH 5.5 seawater (Fig. 3). Inter-specific differences in desorption in the two animal gut juices were greatest for Cd and Ag. Overall rankings of dissolution of metals from AVS and Re-ox particles for both gut juice treatments and seawater were Co $>$ Cd $\geq$ Ag, following the trend in metal AEs for *Mytilus* from both pure phase particles (Table 1). Metal AE results from pure phase particles in *Macoma* also followed the same trend as dissolution of the metals in gut juice except that the Co AE was only significantly higher than the Cd AE in the clams ingesting AVS; there was no difference between the two size classes for Cd and Co AEs from Re-ox particles (Table 1).

The *in vitro* dissolution experiments were shorter (maximum 8 h) than the GPT of all metals in *Macoma,* but all metal GPTs for *Mytilus* were within the time frame of the dissolution experiments except AVS Co (Fig. 2). For these GPTs $<$ 8 h, the *in vitro* metal dissolution was determined over the period of the individual animal's GPT; otherwise the metal dissolution at 6 h was used (Table 1, Fig. 4). For AVS Ag, individual mussel AEs ranged from 2.5 to 3.5%, which corresponded with approximately 0.2–3% dissolved Ag in *Mytilus* gut juice (GPT 0.6–4 h); Re-ox Ag AEs ranged from 2.8 to 4.3% corresponding to approximately 2–13% dissolved Ag (GPT 1.3–5 h) (Table 1, Fig. 4). AVS Cd AE in *Mytilus* ranged from 20–30% corresponding to 1–2% dissolved Cd (GPT 0.7–2 h); Re-ox Cd AE ranged from 10–12.5% corresponding to approximately 2–7% dissolved Cd (GPT 1–4 h). Overall, in *Mytilus* significant ($p < 0.05$) but small increases in Ag AE from the

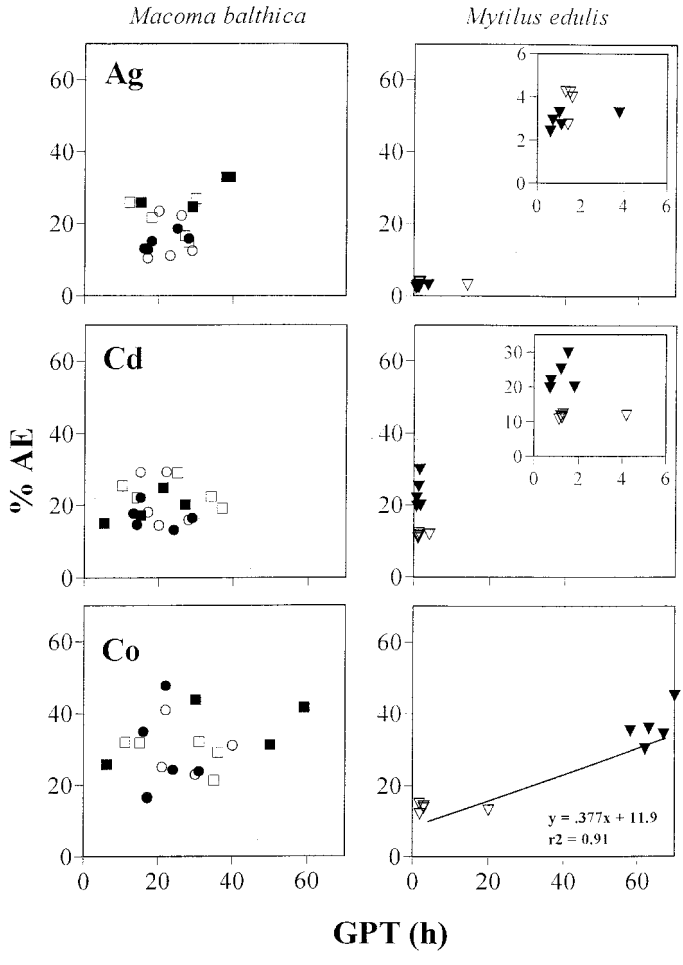

Figure 2. Gut passage time and AE of Ag, Cd and Co in *M. balthica* and *M. edulis*. Filled and open symbols represent AVS-bound and Re-ox bound metals, respectively. ○ = small (14 mm) clams; □ = large (25 mm) clams; ▽ = mussels.

Re-ox particles were also associated with slightly longer GPT and 3 to 4 times higher dissolution of Ag associated with Re-ox particles (Table 1, Fig. 4). In contrast, the Cd AE was two-fold higher from AVS but there was no significant difference ($p > 0.05$) in the GPT or dissolution between the two particle types. Trends in Co AE and metal dissolution in *Mytilus* for the two particles types were opposite but the four-fold longer GPT of the Co-AVS corresponded with a two-fold higher Co AE from the AVS particles (Fig. 2). Correlation of metal dissolution with AE (Fig. 4) indicates that more Co (1.5 to > 4 times) was dissolved than was assimilated in both animals. *In vitro* dissolution of Cd in *Mytilus* gut juice was 5 to 10 times lower than the AE, but in *Macoma* it was approximately 2 times higher than the AE.

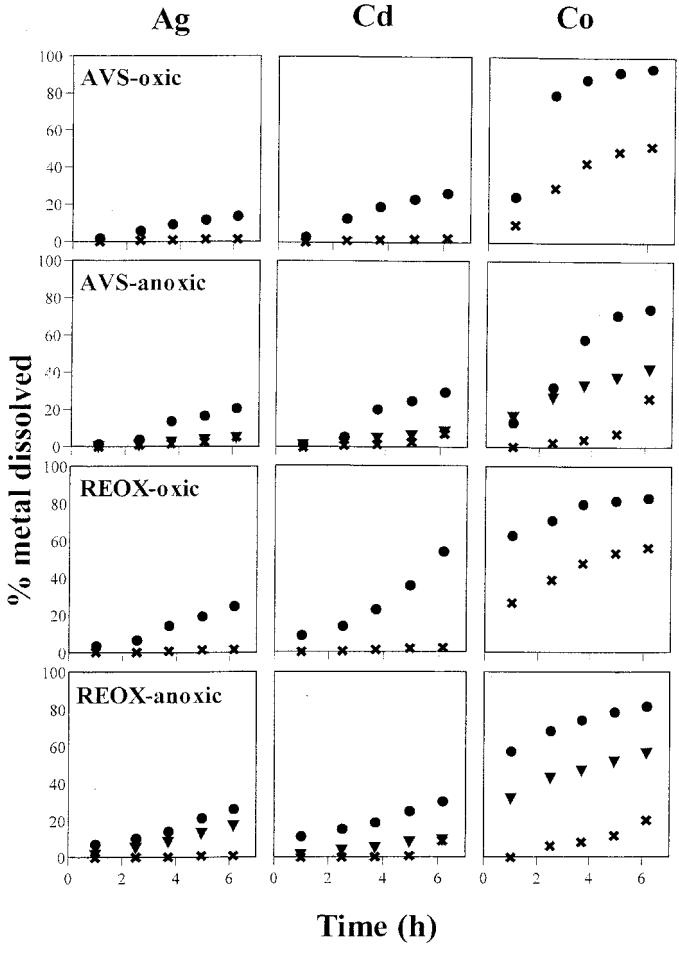

Figure 3. Dissolution of particle-bound metals in oxic and anoxic gut juice extracted from *M. balthica* and *M. edulis* and pH 5.5 seawater. ● = clams; ▼ = mussels. **x** = pH 5.5 treatment.

Results of the ferrozine experiments showed that reducing conditions were more intense or of greater reducing capacity in the larger clams than in small clams as indicated by the extent of reduction of Fe(III) from fully oxidized suspended sediment: 24% and 78% for small and large clams, respectively (Fig. 5). When compared to clams feeding on suspended particles, small clams that were placed in the sediment and fed on surface sediment reduced significantly more Fe (37% compared with 23%) but large clams reduced significantly less Fe (59% compared with 79%) (Fig. 5). In comparison, the guts of *Mytilus* reduced > 90% of the ingested Fe(III) in all three size classes of mussels (Fig. 5). No Fe(II) was measured in the recently egested fecal pellets of either species, indicating that all Fe that was reduced in the gut was completely re-oxidized. Ferrozine determined oxidation

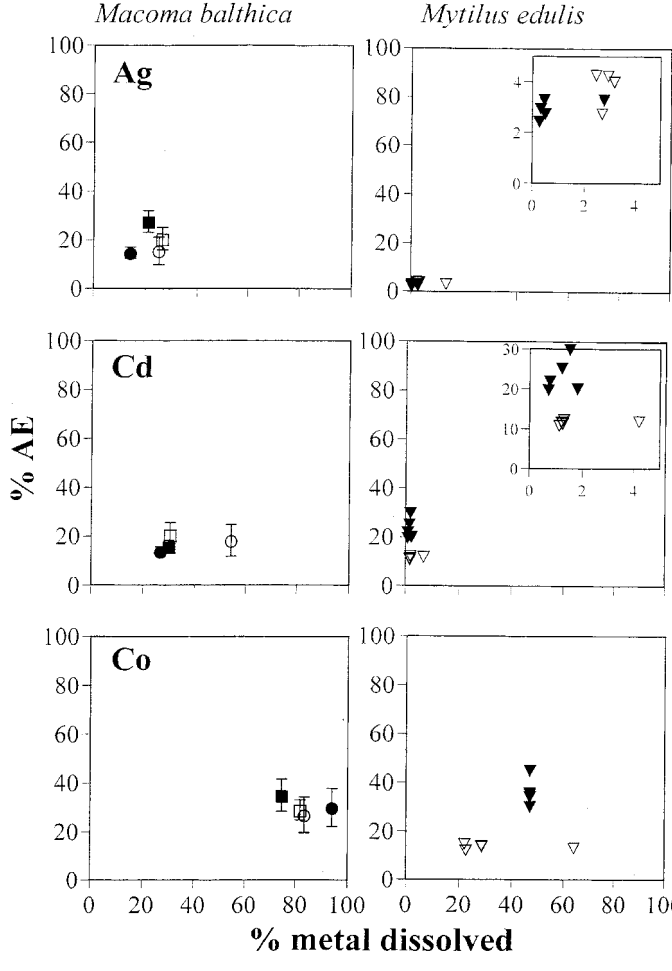

Figure 4. Ag, Cd and Co dissolution vs. % AE in *M. balthica* and *M. edulis*. Filled symbols represent AVS, open symbols represent Re-ox. ○ = small (14 mm) clams; □ = large (25 mm) clams; ▽ = mussels. Insert graph is a smaller scale detail of the larger graph. Dissolution of metal for *M. edulis* was determined for specific GPT of an individual if GPT was < 8 h. Otherwise, metal dissolution at 6 h was compared with AE for both animals.

states compared well with redox conditions inferred from the tetrazolium salts. Redox potentials were low enough to produce reduced iron from solid phase Fe oxide (tetrazolium indication between ~0 and ~ −40 mV) in large clams. Tetrazolium results suggested that smaller clams have less reducing guts, consistent with the ferrozine results. The gut Eh in all size classes of *Mytilus* was low enough to reduce Fe oxide but probably not low enough to produce sulfides (tetrazolium indication between ~ −40 and ~ −120 mV). However this method of bulk gut Eh can not resolve local sites within the gut where sulfides could

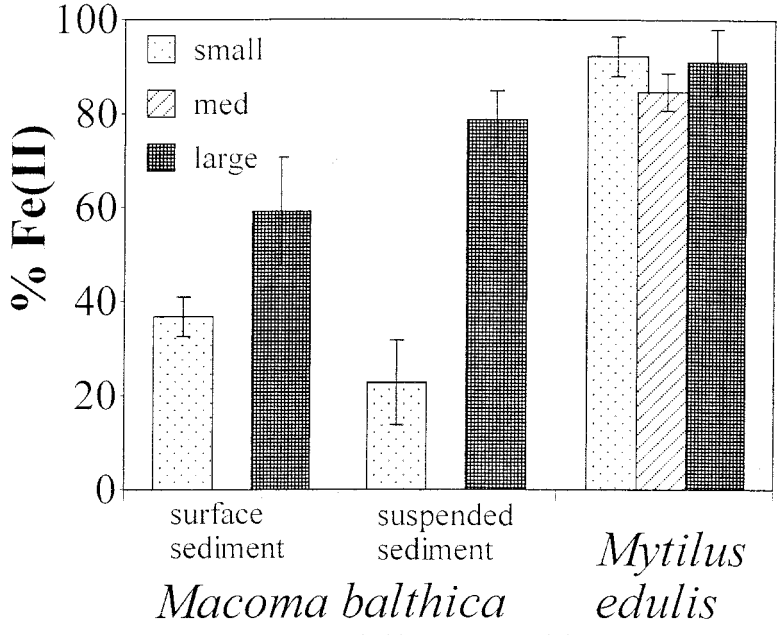

Figure 5. Percent Fe (II) in the guts of *M. balthica* and *M. edulis* fed well-oxidized suspended sediment (both species) or partly oxidized (< 15% Fe(II)) surface sediment (*M. balthica* only). Fe(II) in the stomach contents is measured as a percent of the total iron using the ferrozine method of Stookey (1970). Bar patterns represent different sized animals. Small and large *M. balthica* were 14 ± 2 and 25 ± 3 mm, respectively. Small, medium and large *M. edulis* were 1.5, 3 and 5 cm long.

potentially be produced. In general, gut Eh appears to have had little effect on metal AE when comparing large and small clams except possibly for AVS-Ag for which there was greater release of Ag into reduced gut juice (21%) compared with oxic gut juice (14%).

4. Discussion

Both *Macoma* and *Mytilus* ingested the experimental particles (natural sediments and iron oxide- and sulfide-coated glass beads). Particle-bound Cd, Co, and Ag were measurably assimilated by animals from all particle types including pure AVS. The AE values in both species were comparable to AEs from several other sediment types but were 2 to 4 times lower than Ag and Cd AEs from phytoplankton, diatom-augmented sediments or bacteria (Wang and Fisher, 1996; Decho and Luoma, 1996; Reinfelder *et al.,* 1997; Lee and Luoma, 1998; Griscom *et al.,* 2000). In an experimental study by Gagnon and Fisher (1997), Cd and Co AEs in *Mytilus* from surface labeled iron oxide particles were similar (6% and 9%) but lower than our results. Co AEs from other food types including phytoplankton were similar to values observed here. In general, there is a trend of higher metal AEs when metals are associated with living matter than natural sediments (Lee and

Luoma, 1998; Wang and Fisher, 1999b; Griscom *et al.,* 2000). However, AEs of some metals that are adsorbed to particles (such as calcium carbonate particles) are similar to or greater than metal AEs from phytoplankton, probably because more surface-bound metals than metals within the matrix of a living cell desorb in the low pH gut environment and become available for diffusion across the gut lining. When compared with other metal AEs in mussels from natural oxic and anoxic sediments, Cr (III) tends to be very low (1 and 4%, respectively), and Se (14 and 21%) and Zn (22 and 32%) are moderate, but again these elements generally exhibit slightly lower AEs from sediment than from algae or bacteria (Griscom *et al.,* 2000; Wang and Fisher, 1999b; Decho and Luoma, 1991, 1994).

The AE values determined in this study were a function of different mechanisms or conditions governing uptake of each of the three metals in the gut. For natural sediment particles, *Macoma* tended to take up metals preferentially from oxidized relative to reduced material. In contrast, *Mytilus* tended toward preferential uptake from reduced material (Co,Cd) or showed only a small difference (Ag). Of the two species, *Mytilus* also effectively discriminated against Ag uptake. To evaluate these differences, we examined, in addition to pure phase behavior, the *in vitro* measurement of metals released into the dissolved phase during the period of digestion, the amount of time that the particulate or dissolved metals remained in the gut, the approximate reducing potential in mussels and small and large clams, and gut pH in the two organisms. No clear and systematic relationship was observed between GPT or metal dissolution from particles in extracted gut juices and assimilation in whole animals. In some cases (e.g., Co in *Macoma*), potential release far exceeded assimilation, whereas in other cases (e.g., Cd and Ag in *Mytilus*), assimilation greatly exceeded percentage dissolution. Wang and Fisher (1996) found that Co had the longest GPT in *Mytilus* of five metals but that the longer retention was not associated with higher assimilation. In general, longer retention of food increases the efficiency of digestion and absorption (Willows, 1992) and might be expected to be associated with higher metal assimilation, however the uptake of Co, an essential trace element, across the gut lining appears to be proportionally lower and may be partly regulated, similar to ingested Zn (Amiard-Triquet *et al.,* 1986; Wang *et al.,* 1995). We hypothesized that the amount of metal dissolved in the gut juice over the GPT period should correlate directly with metal AE, as previously shown for these metals where pH 5 seawater mimicked gut juice (Gagnon and Fisher, 1997). Comparison of these two sets of results may be misleading because in our study metals dissolved from a sulfide- or oxide-based matrix whereas in the study by Gagnon and Fisher metals desorbed from organic-coated particle surfaces. Furthermore, the regressions comparing the fraction desorbed with AE did not follow a 1:1 relationship as the proportion desorbed varied from 1 to 7 times $>$ the AE (Gagnon and Fisher, 1997).

The use of inert Sr beads as tracers of particle processing in these bivalves appears to have some limitations. While these beads were essentially eliminated entirely by *M. edulis* within 3 d, in *M. balthica* 6–8% of these ingested beads were retained by large clams and 3–5% were retained by small clams, suggesting that not all particles were eliminated by

this species within 3 d. However, it is noteworthy that in both bivalve species (most evident in *M. edulis*: see Fig. 1) metals were released at rates faster than the Sr beads, probably reflecting the fact that the Furan beads were less dense than the ingested sediment particles and hence were not ideal tracers of ingested particulate matter. In a similar experiment in which inert radioactive beads, also less dense than sediment particles, were used to trace digestive processing in *Macoma* (Decho and Luoma, 1991) egestion of the beads was completed between 3–4 d. In *Macoma* (as well as the clam, *Potamocorbula amurensis*) metals were initially released at rates faster than the beads, similar to our results, and a significant proportion (44%) of the lightweight beads were lost during the more prolonged egestion period. Still, if *M. balthica* AEs are conservatively corrected to take into consideration the Sr bead processing (by subtracting 8% and 6% from metal AEs for AVS and Re-ox particles, respectively for large clams, and 5% and 3% for small clams) no different conclusions or trends become evident in the resulting AEs. By doing so, AEs for large and small clams would become even closer to one another and AEs for Cd, for example, would be lowered to 8 and 9% for large and small clams feeding on AVS particles.

Low pH and "intermediate" Eh conditions in the gut facilitated sulfide and trace metal dissolution, favoring the presence of dissolved Fe species and preventing metal co-precipitation with oxide or sulfide species (Rickard, 1989). In our study it is apparent that greater amounts of Cd, Co and Ag dissolved in the gut juices than in pH 5.5 seawater, presumably because high concentrations of organic ligands as well as surfactants can enhance dissolution (Mayer *et al.,* 1997). Consistently higher Ag AEs in *Macoma* partly reflect the lower gut pH in the clam (5.0, compared to 5.5 in *Mytilus*) but AEs may also be enhanced by higher concentrations of organic ligands in the clam, typical of deposit feeders. In a study of sediment-bound Cu bioavailability in marine deposit feeders with higher gut pH (~7) than the animals in our study (Chen and Mayer, 1999), AVS-bound Cu in contaminated sediment did not dissolve appreciably during a 4 h *in vitro* extraction in gut juice. Additionally, there was lower bioaccumulation in the animals of AVS-bound Cu compared with non-AVS bound Cu. In another experimental study (Lee *et al.,* 2000b) negligible bioaccumulation of sulfide-bound Cd, Ni, or Zn was found in the sulfide-consuming, head-down deposit feeder, *H. filiformis* (neutral gut pH), consistent with field studies (Ahn *et al.,* 1995). Yonge (1949) postulated that, in general, low pH conditions in the guts of bivalves aid in digestion by creating an optimum pH for extracellular digestion of carbohydrates. Acidic conditions also lower the viscosity of mucous-bound food (*Mytilus* transports food from the labial palps to the stomach by way of mucous strings [Ward *et al.,* 1993]) which would facilitate diffusion of released nutrients (Yonge, 1949) and enhance uptake of released metals. Previous studies of *Macoma* measured less acidic guts (pH 6.5–6.8) than in our study (pH 5.0) and found that the pH varied spatially (lowest pH in the digestive diverticula $\leq$ 6.0) and temporally (variable gut pH of approximately 1 pH unit over a tidal cycle) (Purchon, 1971). Previous measurements of *Mytilus* guts were similar to our measurements (pH 5–5.5, Owen, 1974). However, it should be noted that

marine infaunal invertebrates exhibit a wide range of gut pHs (Ahrens and Lopez, 2001), and consequently metal AE's may vary accordingly in these animals.

The difference in Ag AEs between large and small clams ingesting AVS particles could be the result of greater dissolution of Ag in the reduced gut juice, longer GPT, or lower Eh in the larger clams. Our results suggest that gut Eh plays a minor role in influencing metal AEs in marine bivalves, however in sediment, Eh is an important influence on the release and dissolution of metal from Fe-oxide and sulfide carrier phases. Wang and Fisher (1997) measured AEs of Cd and Co in 3 sizes of *Mytilus,* all of which have the same "bulk" Eh, as indicated by ferrozine and tetrazolium salt measurements, and found that Cd AE decreased but Co AE increased with increasing body size, suggesting that other size-dependent mechanisms influence AE.

Dissolution of AVS-bound metals into oxic or anoxic seawater were nearly an order of magnitude lower than into *Macoma* gut juice and 2 to 4 times lower in *Mytilus* gut juice. In all treatments the overall trend in dissolution and assimilation of AVS-bound metals (Co $>$ Cd $\geq$ Ag) was the same. Solubility (and extractability) of pure phase Cd, Co and Ag sulfides may be significantly different than sulfides precipitated within a matrix of iron monosulfide (AVS). AVS resuspended into oxic seawater dissolved in 4 h at pH 7, however pure phase CdS and AgS particles were resistant to dissolution for days to weeks in seawater across a range of salinities and pHs as low as 6.0 (DiToro *et al.,* 1996; Simpson *et al.,* 1998). In our study, AVS Cd, Co, and Ag may be subjected to more rapid dissolution as the FeS matrix is rapidly destroyed, as suggested by Simpson *et al.* (1998). The individual metal sulfide molecules, no longer "protected" within the matrix, would then be exposed to the acidic gut conditions of the two animals. Silver, Cd and Co bound to, or precipitated in the matrix of marine iron sulfides are bioavailable and are highly vulnerable once ingested and subjected to chemical attack in the digestive tract in *Mytilus* and *Macoma* (Lee *et al.,* 2000a; these results). The present results do not follow previous concepts about trace metal bioavailability to benthic invertebrates from sulfides in which feeding and metal assimilation were not considered (DiToro *et al.,* 1990, 1996; Berry *et al.,* 1996).

What are the effects of redox reactions and low pH conditions within bivalve guts on iron carrier phases? Geochemical attack and subsequent release of metals associated with reactive iron species (e.g., Co in *Macoma* gut juice) interferes with the progressive formation of more stable and resistant species that trap trace metals, rendering them less bioavailable. Subsequently, a greater level of disequilibrium is maintained within surface sediments. The total reductive flux of reactive sedimentary iron (mg Fe(II) m^{-2} d^{-1}) by *Macoma* can be roughly estimated. *Mytilus,* an active filter-feeder, is capable of reducing much larger quantities of Fe than *Macoma* due to 2 orders of magnitude higher filtration rates (Bayne and Newell, 1983) as well as near complete reduction of Fe in the guts. However inorganic sediment, especially sulfides, is rarely an important component of the *Mytilus* diet (Smaal and Zurburg, 1997) compared with phytoplankton. The total amount of sediment processed by 750 *Macoma* individuals in a m^2 was 32.5 g d^{-1} (Bubnova, 1972).

Total reductive flux was calculated with the following parameters: % Fe in sediment (1–3%), % Fe reduced in *Macoma* gut (30–70%); amount of sediment reprocessed by *Macoma* in m^2 (20–80 g d^{-1}). The estimated reductive flux by *Macoma* using these values ranges from 0.06 to 1.68 g Fe reduced $m^{-2} d^{-1}$, or 21.9 to 613 g Fe reduced $m^{-2} y^{-1}$, in a typical intertidal mud flat.

In conclusion, metals associated with either reduced or oxidized particles are assimilable by both deposit and suspension-feeding species. From natural sediment, *Macoma* took up Co > Cd ~ Ag and, overall, accumulated metal more from oxidized sediment than from reduced material. *Mytilus* took up Cd > Co from both reduced and oxidized sediment, whereas Ag assimilation (which was low) was slightly greater from oxidized sediment. Metal uptake results from pure phase carriers (e.g., AVS, oxidized Fe) were most consistent with these patterns but cannot explain them entirely. Sulfide solubility constants, dissolution rates in seawater, and the concept of unidirectional stabilization of sulfide-bound trace metals do not exclusively determine the bioavailability of AVS-bound metals or metals in other carrier phases in or derived from surface sediments. Since the two bivalve species we examined assimilate metals from reduced and oxidized particles in divergent ways, it is clear that biological controls on metal processing can overcome geochemical controls. In the bioturbated and resuspension zones of marine deposits, particles are repeatedly consumed, depurated, and subjected to high-frequency oscillating pH and redox conditions, causing AVS and metal hydroxides to be continuously created and destroyed (Aller, 1994). Within this dynamic context, the gut environment of sediment-consuming organisms is clearly a separate and important condition with respect to metal bioavailability and metal biogeochemical cycling in general (Chen and Mayer, 1998).

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