

Significance of subcellular metal distribution in prey in influencing the trophic transfer of metals in a marine fish

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Abstract

We investigated how the subcellular metal distribution in prey affects metal dietary assimilation in a marine fish, the grunt *Terapon jarbua*. The assimilation efficiency (AE) of metals (Cd, Se, and Zn) in the grunt varied by prey, which included copepods, barnacles, clams, mussels, and fish viscera. The AEs were 3–9% for Cd, 13–36% for Se, and 2–52% for Zn. The AEs of Se and Zn were significantly correlated with the subcellular Se and Zn distributions in the prey, suggesting that the subcellular forms of Se and Zn in the fish's diet affected assimilation. Further experiments determined AEs using purified subcellular fractions of copepods and mussels as fish diets. AEs were higher in the grunts fed the heat-stable protein fraction or the heat-sensitive protein fraction than in those fed insoluble fractions. AEs were comparable in fish fed purified subcellular fractions from different prey, further indicating the importance of subcellular metal distribution in metal assimilation. AEs of Se and Zn but not Cd were significantly dependent on the ingestion rate of fish and gut passage times for metals, suggesting that fish had different digestive strategies to handle essential and nonessential elements. Assimilation of metals by marine fish is determined both by the subcellular metal distribution in the prey and by the feeding process of the fish.

There have been wide concerns about the accumulation of metals in fish because of their commercial values and health risks to humans due to consumption. Fish are exposed to various sources of metals, including from water and food. It has become increasingly clear that trophic transfer of metals contributes to (or dominates) overall metal accumulation in fish (Spry et al. 1988; Xu and Wang 2002; Zhang and Wang 2005). Understanding the dietary assimilation in fish has become important in modeling metal accumulation (Luoma and Rainbow 2005). Metal assimilation results from digestive processing of metals associated with ingested prey. The assimilation efficiency (AE) indicates the fraction of ingested metal remaining in the fish body after the undigested materials are evacuated. Many studies have determined the AEs of different metals in a wide range of aquatic organisms, including fish (Reinfelder and Fisher 1994; Zhao et al. 2001; Long and Wang 2005). However, mechanisms underlying the dietary assimilation of metals in marine fish are not yet clearly understood.

Assimilation is an interactive process between food and the digestive system of the consumer. It has been shown that different prey resulted in a variation of metal AEs in fish (Ni et al. 2000; Xu and Wang 2002), suggesting that different forms of metals in the food may influence the availability of metals. Some previous studies have indicated that the cytosolic distribution of metals in marine phytoplankton is important in dietary assimilation by

marine herbivores (Reinfelder and Fisher 1991; Wang and Fisher 1996; Chong and Wang 2000), but assimilation in marine predators appears to be more complicated. Recently, there has been an increasing awareness of the subcellular fate of metals in prey organisms and of how the subcellular metal forms may subsequently affect the trophic transfer to the next level (Wang 2002; Vijver et al. 2004). In these studies, using a metal subcellular partitioning method (Wallace et al. 2003; Wallace and Luoma 2003), the prey were separated into metal-rich granules (MRG), cellular debris, organelles, heat-denatured protein (HDP), and heat-stable protein (HSP) fractions. MRG and HSP (containing metallothionein [MT]) were considered to sequester and thereby detoxify metals. Furthermore, metals distributed in organelles, HDP, and HSP were considered to be trophically available (Wallace et al. 2003). A recent study used this subcellular partitioning method to determine the transfer of Cd from grass shrimps to mummichog fish, but the relationship between the metal speciation in the shrimps and the AE in the fish was not explored (Seebaugh et al. 2005).

Additionally, feeding in the predators including food ingestion and digestion also controls the assimilation of metals. Active ingestion is a common behavior in fish because of their good swimming abilities. After food ingestion, digestion occurs in the acidic environment of the stomach and the alkaline environment of the intestine by the action of enzymes in digestive fluids and the epithelial cells. Assimilation occurs mostly in the intestine in most fishes (Fänge and Grove 1979; Horn 1997). The ingestion rate (IR) and the gut passage time (GPT), reflecting the amount of ingested food and the movement of the food in the digestive tract, are the two most important parameters of feeding and therefore are closely related to assimilation. The dependence of metal assimilation on IR and GPT has been demonstrated in marine

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herbivores and deposit-feeding invertebrates (Wang and Fisher 1996; Selec et al. 1999; Xu and Wang 2001), but only a few convincing results have been obtained in fish (Xu and Wang 2002).

In this study, we conducted a series of experiments to investigate the influence of metal subcellular partitioning in different prey and the influence of IR and GPT on metal dietary assimilation in a marine fish, *Terapon jarbua*. We fed the predatory fish different prey, including crustaceans, mollusks, and other species of fish. We then fed the fish purified subcellular fractions of copepods and mussels to confirm the differing bioavailabilities. The influences of IR and GPT were determined in a single-pulse feeding experiment. The metals employed here were Cd, Se, and Zn for the indication that Cd may be biotransformed, whereas Se and Zn may potentially be biomagnified during their transfer to higher trophic levels in planktonic food chains in recent modeling studies (Wang 2002).

Materials and methods

Fish and prey—Juvenile grunts *T. jarbua* (3–4 cm in length) were obtained from a fish farm at Yung Shu Au, Hong Kong, and acclimated in aerated natural seawater (20°C, 30‰) and fed minced shrimp (from a local supermarket) at about 5% of their body weight daily under a 14:10 light:dark regime in the laboratory. Different prey types considered in this study of metal assimilation in fish included copepods, barnacles, clams, mussels, fish viscera, and brine shrimps. The barnacles *Balanus cirratus* and the prey fish *Acanthopagrus schlegeli* were collected from the same fish farm where the fish were from. The clams *Ruditapes philippinarum* were collected from sandy beach near Hong Kong University of Science and Technology. The green mussels *Perna viridis* were collected from Tolo Harbor. These four prey types were maintained in the laboratory for at least 1 week before the radiolabeling experiments. The copepods (predominantly *Acartia erythraea*) were collected by net tows from Port Shelter surface water 1 d before the radiolabeling. The brine shrimp (*Artemia*) nauplii were hatched from eggs (from Brine Shrimp Direct) in 0.22- μ m filtered seawater at 25°C for 48 h.

Radiolabeling of prey—The prey were radiolabeled with ^{109}Cd ($t_{1/2} = 462$ d, specific activity = 135 kBq μg^{-1} , in 0.1 mol L^{-1} HCl, from New England Nuclear), ^{75}Se ($t_{1/2} = 120$ d, specific activity = 26 kBq μg^{-1} , in 0.1 mol L^{-1} HNO_3 , from Riso National Laboratory), and ^{65}Zn ($t_{1/2} = 244$ d, specific activity = 8,880 kBq μg^{-1} , in 0.1 mol L^{-1} HCl, from Brookhaven Science Associates). The copepods were exposed to 74 kBq L^{-1} ^{109}Cd , ^{75}Se , and ^{65}Zn for 36 h. The newly hatched brine shrimps (2 d old) were exposed to waterborne 74 kBq L^{-1} ^{109}Cd , ^{75}Se , and ^{65}Zn for 60 h. The barnacles were exposed to 37–74 kBq L^{-1} ^{109}Cd , ^{75}Se , and ^{65}Zn for 5 d. The clams and mussels were labeled in 7.4–14.8 kBq L^{-1} ^{109}Cd , ^{75}Se , and ^{65}Zn for 5 d. During the labeling periods, the five prey were also fed the diatom *Thalassiosira weissflogii* (exposed to 37 kBq L^{-1} ^{109}Cd , ^{75}Se , and ^{65}Zn for 4–6 d). Fish *A. schlegeli* were fed fresh

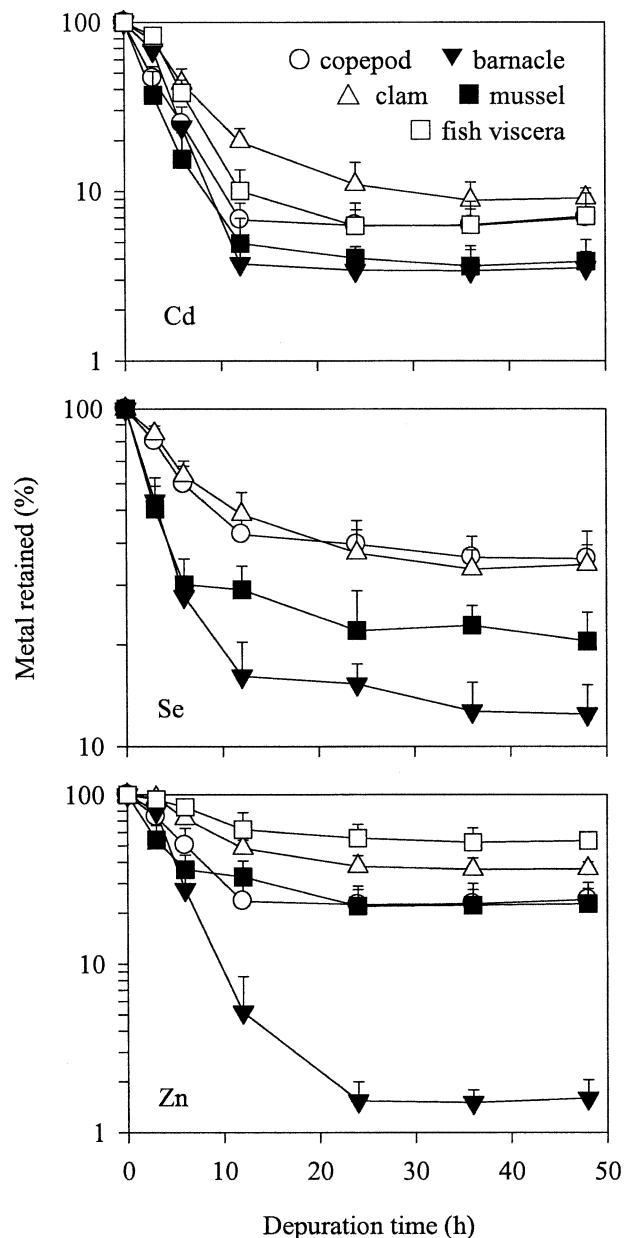


Fig. 1. Retentions of Cd, Se, and Zn in the predator fish *T. jarbua* following pulse feeding of five different prey. Mean \pm SD ($n = 8-10$).

shrimp mixed with 185 kBq L^{-1} ^{109}Cd and ^{65}Zn three times each day and depurated for 2 d. After the radiolabeling, the prey were rinsed with 0.22- μ m filtered seawater for 30 min. The copepods and brine shrimps were collected by mesh. The barnacles, clams, and mussels were dissected to obtain soft tissues. The fish were also dissected to obtain viscera.

Subcellular fractionation—The subcellular fractions of the six prey were measured using the method described by Wallace et al. (1998, 2003) with slight modification. The radiolabeled prey were homogenized with a tissue homog-

Table 1. Assimilation efficiencies (AEs) of Cd, Se, and Zn in the grunt *T. jarbua* fed different prey and purified subcellular fractions prepared from copepods and mussels. Mean \pm SD ($n=8-10$). IF, HDP, and HSP represent the insoluble fraction, the heat-denatured protein fraction, and the heat-stable protein fraction, respectively. AE of Se to fish viscera was not calculated because of the low ^{75}Se radioactivity ingested by the fish. Data with different letters are significantly different ($p<0.05$) for the same metal. Percentages in parentheses are the metal distribution in relevant fractions. NA=not available.

Treatments	Assimilation efficiency (%)		
	Cd	Se	Zn
Different prey			
Copepods	6.3 \pm 1.6 ^a	36.2 \pm 5.6 ^a	22.6 \pm 4.9 ^a
Barnacles	3.4 \pm 1.4 ^b	12.7 \pm 2.7 ^b	1.5 \pm 0.3 ^b
Clams	8.8 \pm 2.5 ^{ac}	33.5 \pm 4.7 ^a	36.2 \pm 6.0 ^c
Mussels	3.6 \pm 0.9 ^b	22.8 \pm 3.3 ^c	22.2 \pm 7.7 ^a
Fish viscera	6.3 \pm 2.4 ^a	NA	52.3 \pm 11.4 ^d
Copepod's pure subcellular fraction			
IF	12.0 \pm 2.1 ^c (82%)	33.0 \pm 3.4 ^a (53%)	15.0 \pm 1.8 ^a (80%)
HDP	42.4 \pm 6.8 ^d (11%)	45.4 \pm 6.0 ^d (4%)	51.9 \pm 10.5 ^d (11%)
HSP	35.0 \pm 4.7 ^{de} (7%)	53.8 \pm 9.1 ^d (43%)	61.4 \pm 9.9 ^d (9%)
Mussel's pure subcellular fraction			
IF	9.0 \pm 2.9 ^{ac} (58%)	29.0 \pm 3.0 ^a (68%)	15.5 \pm 2.0 ^a (81%)
HDP	29.2 \pm 4.6 ^c (23%)	41.1 \pm 5.0 ^d (12%)	39.7 \pm 7.8 ^c (15%)
HSP	46.9 \pm 11.2 ^d (19%)	46.2 \pm 8.1 ^d (20%)	55.5 \pm 10.2 ^d (4%)

enizer (Ultra-Turrax T25 basic, IKA) on medium speed for 2×3 min in cold 20 mmol L⁻¹ Tris-HCl buffer (pH = 7.4, 10 mL g⁻¹ tissue) and centrifuged at $1,500 \times g$ for 15 min at 4°C. Thus, the pellets (P1) contained tissue fragments and cellular debris, and the supernatants (S1) contained organelles and soluble compounds. The pellets (P1) were then resuspended in 4 ml 1 mol L⁻¹ NaOH at 60°C for 10 min and centrifugation at $5,000 \times g$ for 10 min to be separated into metal-rich granules (MRG, P2) and cellular debris (S2). The supernatant (S1) was centrifuged at $100,000 \times g$ for 1 h at 4°C to yield organelles (P3) and cytosol (S3). Then, after being heated at 80°C for 10 min and ice-cooled for 1 h, the cytosol (S3) were further centrifuged at $30,000 \times g$ for 10 min at 4°C to separate the HDP as pellets and the HSP as the supernatant. Prey tissues were kept on ice throughout the fractionation procedure except heating steps. The sum of the radioactivity in the five fractions (MRG, cellular debris, organelles, HDP, and HSP) was 85–110% of the initial radioactivity in the untreated prey tissues, suggesting that metals were generally not lost during the fractionation procedure. The subcellular metal distribution was also calculated via the radioactivity in these five fractions.

Metal assimilation from different prey—The five radiolabeled prey (copepods, barnacles, clams, mussels, and fish viscera) were fed to 10 individual fish for 1 h. After the radioactive pulse feeding, the fish were rinsed with seawater (i.e., they were placed in nonradioactive water for 2–3 min) and were measured for radioactivity. They were subsequently depurated of their ingested prey in 5-L non-radioactive water for 48 h. Radioactivity of fish was measured at 3, 6, 12, 24, 36, and 48 h. The water was renewed every 12 h, and the feces were removed frequently. Because the radioactivity in the fish remained nearly constant beyond 36 h of depuration, the AE was calculated as the percentage of metals remaining in the fish after 36 h

of depuration:

$$AE = A_{36h}/A_{0h} \times 100\% \quad (1)$$

where A_{36h} is the radioactivity in the fish at 36 h and A_{0h} is the initial radioactivity in the fish.

Metal assimilation from purified subcellular fractions in the prey—The homogenized radiolabeled copepods and mussels were centrifuged at $100,000 \times g$ for 1 h at 4°C to yield insoluble fractions (IF, containing cellular debris, MRG, and organelles) as pellets and cytosol as the supernatant. The HDP and HSP were separated after heating and centrifugation as described previously. The three fractions (IF, HDP, and HSP) were freeze-dried and mixed with a gelatin solution to form gelatin cubes as food for the predator fish (as described by Wallace et al. 1998; Cheung and Wang 2005). After 1 h of feeding, the fish were depurated in clean seawater to determine the metal AEs.

Effects of IR and GPT on metal assimilation—Twenty-three individual fish were fed radiolabeled brine shrimps as prey for 1 h and depurated in clean seawater for 48 h. The fish were separated randomly to different tanks containing different food densities to result in large variations in IRs. The IR (g g⁻¹ d⁻¹) in different individuals was determined by the following equation:

$$IR = A_{0h}/(R \times W) \times 24 \quad (2)$$

where A_{0h} is the initial radioactivity in the fish after feeding (corrected count per minute [ccpm]), W is the dry weight of fish (g), and R is the radioactivity–dry weight ratio of brine shrimps (ccpm g⁻¹). After measurement of the initial radioactivity, fish were fed unlabeled food during the depuration period. To determine the GPT of metals in each individual fish, the feces were collected each hour during

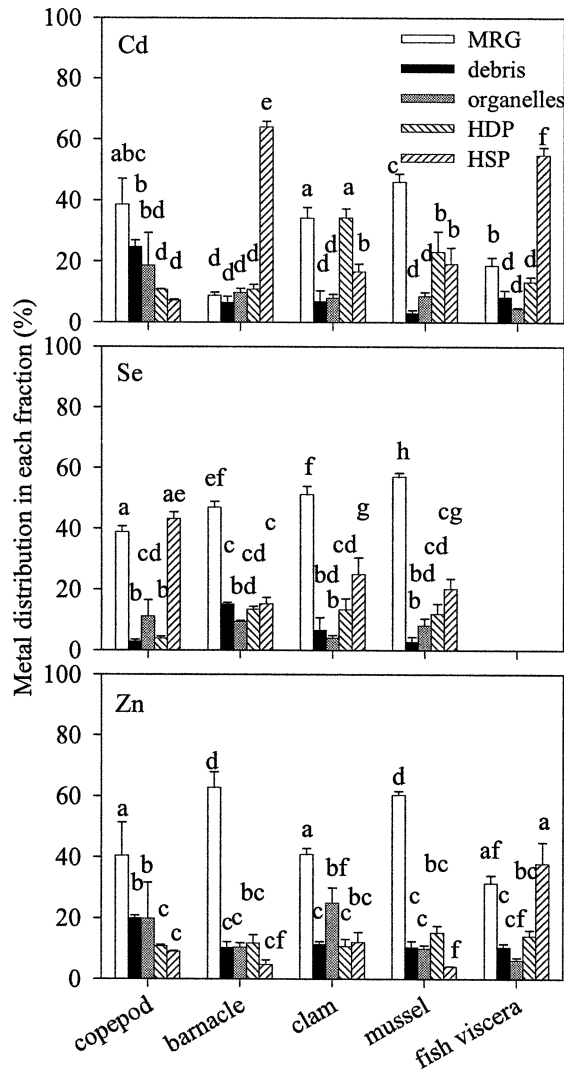


Fig. 2. Subcellular distributions of the radiolabeled Cd, Se, and Zn in the prey. Mean \pm SD ($n = 4$). Bars with different letters are significantly different ($p < 0.05$) for the same metal.

the first 15 h and less frequently afterward to measure their radioactivity. The GPT of metals was calculated as the time when 90% of unassimilated metals were ejected in the cumulative feces (Wang and Fisher 1996).

Radioactivity measurements and statistical analysis—The radioactivity of ^{109}Cd , ^{75}Se , and ^{65}Zn in all samples was measured using a Wallac 1480 NaI (T1) gamma counter (Wallac). The gamma emission of ^{109}Cd was detected at 88 keV, ^{75}Se at 334 keV, and ^{65}Zn at 1,115 keV. All counting times were adjusted to result in a propagated counting error $< 5\%$. Statistically significant differences among the treatments were detected through one-way analysis of variance using a least-significant-difference post hoc test ($p < 0.05$), and all the percentages were arcsine transformed before the statistical analysis.

Results

Metal assimilation from different prey—The depuration of ingested metals by the grunt varied for different metals and prey, but the metal retention in the fish remained stable during 24–48 h of depuration in all treatments (Fig. 1). The calculated AEs of Cd ranged from 3% to 9% and were significantly lower for mussel and barnacle prey than for the other three prey (Table 1). The AEs of Se ranged from 13% to 36% for different prey except the fish viscera. The values were similar for copepod and clam prey and higher than those measured for barnacles and mussels. The AEs of Zn varied from 1% (for barnacles) to 52% (for fish viscera) and were significantly different among different prey except for copepods and mussels.

The subcellular metal distributions in the prey were measured, and the results are shown in Fig. 2. HSP was the major binding site for Cd in barnacles (64%) and fish viscera (55%). MRG was the major binding site for Cd in copepods (39%) and mussels (46%) and was one of the two major binding sites in clams (34%). Cellular debris and organelles played a relatively minor role in binding of Cd ($< 10\%$) in all prey except copepods. More Cd (23% and 34%) existed in HDP in the bivalves than in the other prey (2–10%). MRG was the dominant pool for Se in barnacles (47%), clams (51%), and mussels (57%). HSP was the second most important Se-binding pool in clams (25%) and mussels (20%). In copepods, both MRG and HSP were important to bind Se (39% and 43%, respectively). In contrast, only a small fraction of Se was bound with cellular debris, organelles, and HDP ($< 10\%$ in all prey). MRG was the most important binding pool for Zn in copepods (40%), barnacles (63%), clams (41%), and mussels (60%). HSP was an important fraction for Zn binding in fish viscera (38%), and MRG was another important fraction (30%). About 10% Zn was in cellular debris or HDP in all prey except copepods.

The correlation of metal AE and their distributions in different subcellular fractions were analyzed, and significant correlations are shown in Fig. 3. The AEs of Se and Zn had positive relationships with the corresponding metal distribution in HSP, the soluble fraction (a combination of HDP and HSP), as well as the trophically available fraction (TAF) (combination of organelles, HDP, and HSP) in different prey. However, no significant correlation was observed for Cd.

Metal assimilation from purified subcellular fractions—The effects of subcellular metal forms in the prey on metal AE were further determined in the experiments where the fish were fed separated pure subcellular fractions (IF, HDP, and HSP) from copepods and mussels (Fig. 4). MRG, cellular debris, and organelles were combined as a single fraction (IF) to result in measurable radioactivity in the fish after the pulse radioactive feeding. The AEs of the metals measured at 36 h of depuration were similar in the grunts fed the HSP fraction between the two different prey (40%, 50%, and 55% for Cd, Se, and Zn, respectively; Table 1). The AEs were also similar from the IF fractions between copepods and mussels and significantly lower than

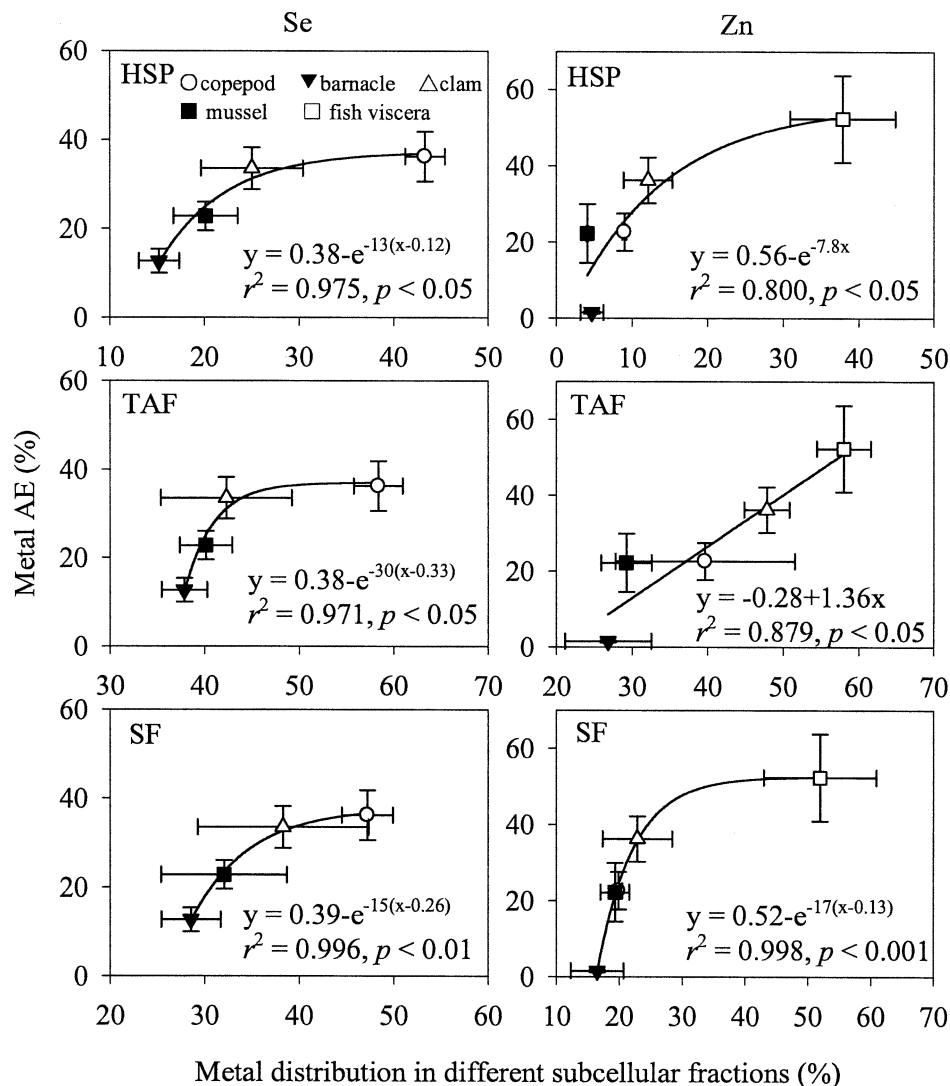


Fig. 3. Correlations between the assimilation efficiency (AE) of Se and Zn in the fish *T. jarbua* and the Se and Zn distributions in the subcellular fractions of prey. HSP, heat-stable protein fraction; TAF, trophically available fraction; SF, soluble fraction (heat-stable protein and heat-denatured protein fractions). Mean \pm SD ($n = 8-10$ for AE and 4 for subcellular distributions).

the ones corresponding to HDP and HSP. Additionally, most AEs from HDP were comparable to those from HSP, except the AEs of Cd and Zn from the mussel prey.

Effects of IR and GPT on metal assimilation—The measured IRs ranged from 0.01 to 0.55 g g⁻¹ d⁻¹ among the individual fish fed brine shrimps. The calculated GPTs were 7–26 h, 15–34 h, and 13–26 h for Cd, Se, and Zn, respectively. The measured AEs of Cd, Se, and Zn were 6–28%, 22–70%, and 13–78%, respectively, among the individual fish. The AEs of Se and Zn were significantly correlated with the IRs (via a power function) and the GPTs (via a linear function) (Fig. 5). However, the AE of Cd was not correlated with either IR or GPT (mean of 16%).

Discussion

Fish have a relatively advanced extracellular digestive system in which ingested food materials are digested in the stomach and intestines mainly via an enzymatic process and subsequently assimilated mainly in the intestine. By examining the individual response of AE within a continuous IR range, this study demonstrated that the feeding process (IR and GPT) in fish strongly controlled the assimilation of Se and Zn. The maximum AEs of Se and Zn were as high as 70% and 78%, respectively, at IR of 0.01 g g⁻¹ d⁻¹. The AEs became higher at a lower IR, suggesting a somewhat more complete digestion in the fish. One possible mechanism was the lower amount of food in the gut (lower IR) facilitating digestive juice contacts and

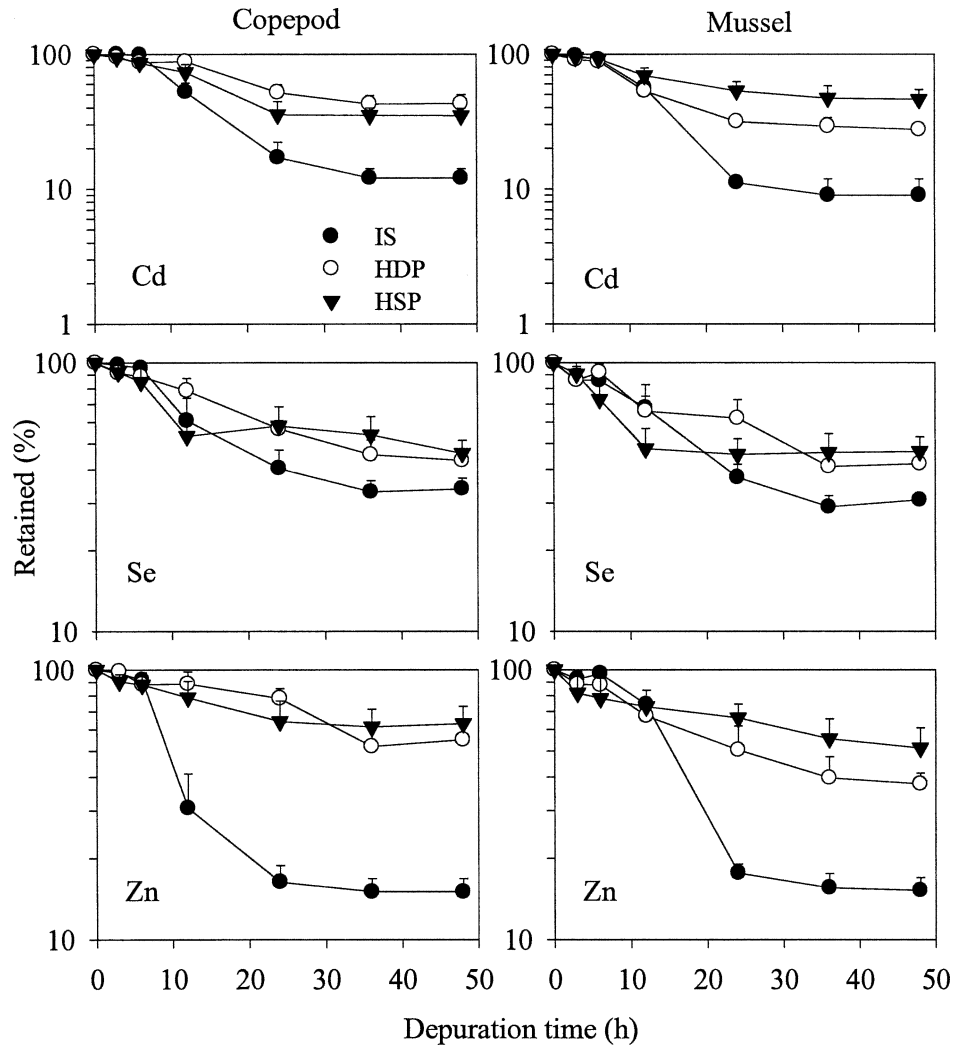


Fig. 4. Retentions of Cd, Se, and Zn in the fish *T. jarbua* following pulse feeding of insoluble fraction (IF), heat-denatured protein (HDP) fraction, and heat-stable protein (HSP) fraction purified from copepods and mussels. Mean \pm SD ($n = 10$).

reactions with digestive enzymes, leading to more efficient digestion. Metals may thus be more efficiently assimilated by the limited number of transporters on the intestine epithelium. Moreover, less food increased the GPT of metals and thus the digestive period. It was observed that the IR and the GPT of Se and Zn were negatively correlated. In contrast, the relationship of IR and AE of Cd was not significant, presumably because the AEs of Cd remained at a low level (see the following discussion).

According to the bioenergetic-based kinetic model, metal uptake from the dietary phase (I_f) can be described by the following equation:

$$I_f = AE \times IR \times C_f \quad (3)$$

where C_f is the metal concentration in food. Since the AE may be dependent on the IR, a better expression of this equation is to use the rate constant of uptake from the

dietary phase k_f , which is the combination of AE and IR:

$$I_f = k_f \times C_f \quad (4)$$

In this study, the AEs of Se and Zn were negatively correlated with IR, thus mitigating the increase of Se and Zn bioaccumulation caused by the increase in IR. In contrast, the Cd bioaccumulation should increase proportionally with increasing IR because of the constant AE. Therefore, it is necessary to distinguish the relationship between AE and IR when modeling the metal accumulation in fish.

Other than demonstrating the control of feeding, this study also demonstrated that subcellular metal distribution in food could influence metal assimilation in fish. Several studies have investigated the effects of metal partitioning within prey on metal assimilation in predators (Reinfelder

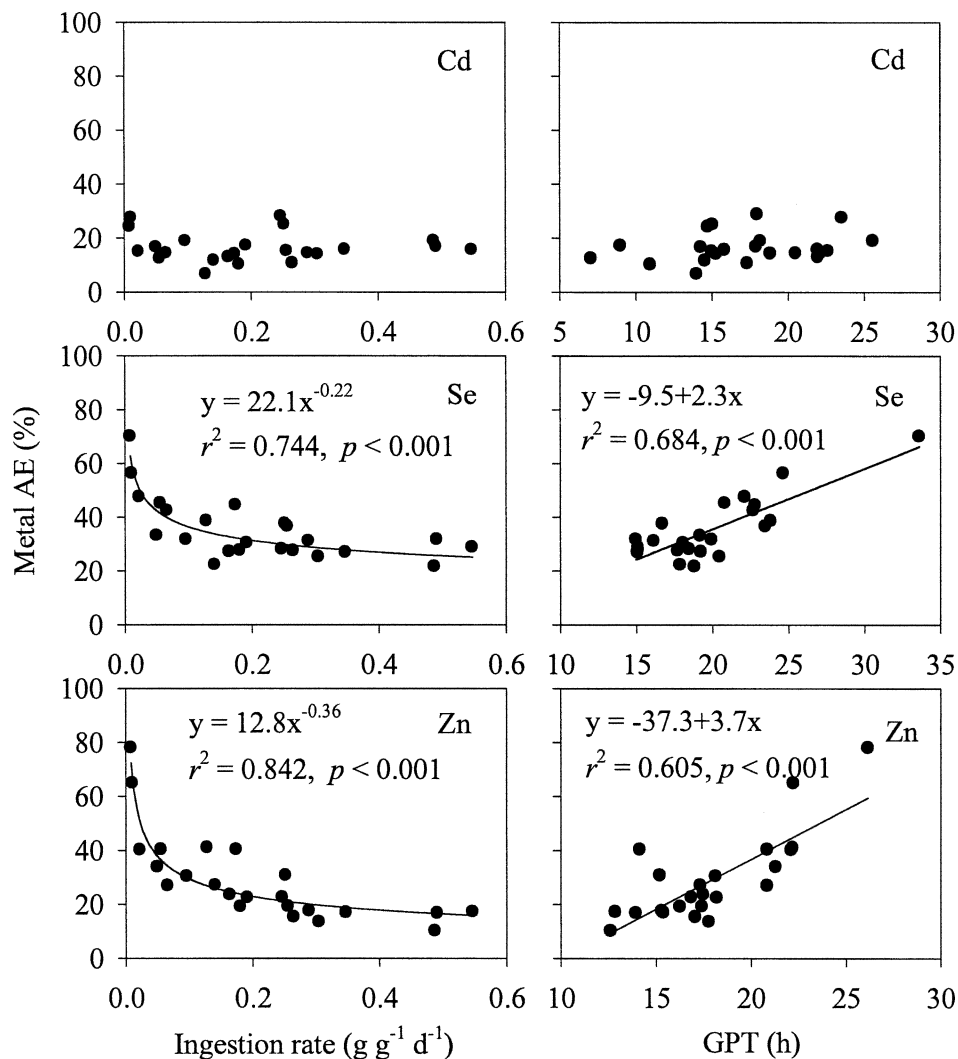


Fig. 5. Correlations of the assimilation efficiencies (AEs) of Cd, Se, and Zn and the ingestion rates (IRs) or the gut passage times (GPTs) of food in the fish *T. jarbua* fed brine shrimps. Each dot represents one individual fish.

and Fisher 1994; Wallace and Luoma 2003; Seebaugh and Wallace 2004). Reinfelder and Fisher (1994) quantified the assimilation of C, Cd, Co, P, S, Se, and Zn in two marine fish, *Menidia menidia* and *M. beryllina*, fed the copepod *Acartia* spp. They suggested that the AEs of elements in fish were directly correlated with the metal distribution in the nonexoskeleton fraction of the copepod prey. The correlation was based on a variety of elements with contrasting physiological and geochemical behaviors, but it was not observed in the two fish *Periophthalmus cantonensis* and *Ambassis urotaenia* (Ni et al. 2000). Recently, Wallace and Luoma (2003) introduced the concept of trophically available metal (TAM) (combination of organelles, HDP, and HSP) and indicated that about 100% of the TAM could be assimilated by predators. In their study, 1:1 relationship was established between the AE of Cd in the predator grass shrimp *P. pugio* and the Cd-TAM of invertebrate prey, including the oligochaete *Limnodrilus hoffmeisteri*, the bivalve *Potemocorbula amur-*

ensis, and the brine shrimp *A. franciscana* (Wallace et al. 1998; Wallace and Luoma 2003; Seebaugh and Wallace 2004).

In this study, we chose a different approach by using a variety of prey with contrasting subcellular metal distributions to examine if there are particular relationships for specific metals. No relationship was found for Cd to its AEs with any of the subcellular fractions (or a combination of different fractions), presumably because the AEs of Cd were low in the fish fed different prey types. In our study, the AEs of Cd were independent of either the prey type or the prey amount, suggesting that other factors control Cd assimilation. As a nonessential metal, Cd is considered to be taken up through the Ca transport pathway in fish gills (Niyogi and Wood 2003), and at least part of this pathway is in the fish intestine (Schoenmakers et al. 1992; Franklin et al. 2005). Thus, Cd assimilation could be inhibited by competition of Ca, as the Cd concentration in organism tissues is usually negligible compared with the Ca

concentration (Franklin et al. 2005). The low AE of Cd was unlikely caused by excretion, which was very low in marine fish ($<0.1 \text{ d}^{-1}$; Xu and Wang 2002). In contrast to Cd, the AEs of Se and Zn were positively correlated with the percentages of metals distributed in the soluble cytosolic fraction (combined HDP and HSP), suggesting a higher bioavailability of Se and Zn from these fractions. However, there appeared to be an upper limit of metal assimilation by the fish with a further increase in the metal distribution in this fraction.

To demonstrate the influence of metal subcellular partitioning in prey on metal assimilation in the predator, the purified subcellular fractions embedded in a gelatin were directly used as food. The AEs of each metal associated with the HSP fraction were similar, implying that HSP was a conserved trophically available fraction. The HSP was considered to be dominated by MT-like protein, which is a family of low-molecular-weight, cysteine-rich, metal-affinitive protein that can bind to essential metals and sequester toxic metals (Nordberg 1998; Wallace et al. 2003). The AEs in the fish fed HDP from copepods and mussels were similar to those fed HSP in most cases, suggesting that they were also highly bioavailable. The feeding experiment with the pure subcellular fraction demonstrated that metals distributed in the cytosolic protein fractions (HDP and HSP) could not be assimilated completely by the fish. Recently, Seebaugh et al. (2005) reported that a TAM-Cd of 68–69% in prey grass shrimp *P. pugio* was associated with only 3–19% of AEs of Cd in the predator fish *Fundulus heteroclitus*, also indicating that TAM may not be totally bioavailable to fish. The AEs of metals in the fish fed the IF were also similar and were much lower than those associated with HSP and HDP, indicating a lower bioavailability when associated with the IF. Overall, the purified subcellular feeding experiment indicated that the AEs of each subcellular fraction may be constant regardless of the prey species. Similar results were obtained by Cheung and Wang (2005) in studying the trophic transfer of Ag, Cd, and Zn from the barnacle *B. amphitrite*, the snail *Monodonta labio*, and the oyster *Saccostrea glomerata* to the predator snail *Thais clavigera*.

It is possible to predict the AE (AE_p) from the whole prey by the following equation:

$$AE_p = \sum AE_i \times (M)_i \quad (5)$$

where AE_i is the AE in the predator fed a purified subcellular fraction i and $(M)_i$ is the metal distribution in the relevant fraction i .

Figure 6 compares the predicted and observed AEs in the copepods and mussels using the information determined from this study. The AEs of Se and Zn in the grunts fed on the two prey could be reasonably predicted on the basis of the measurements of metal subcellular distribution and assimilation from each pure fraction. However, the predictions appeared to overestimate the actual AE of Cd. It is possible that this overestimation was caused by the subcellular partitioning treatments. For example, a homogenization step may have facilitated the digestion of Cd by breaking the prey tissues into small

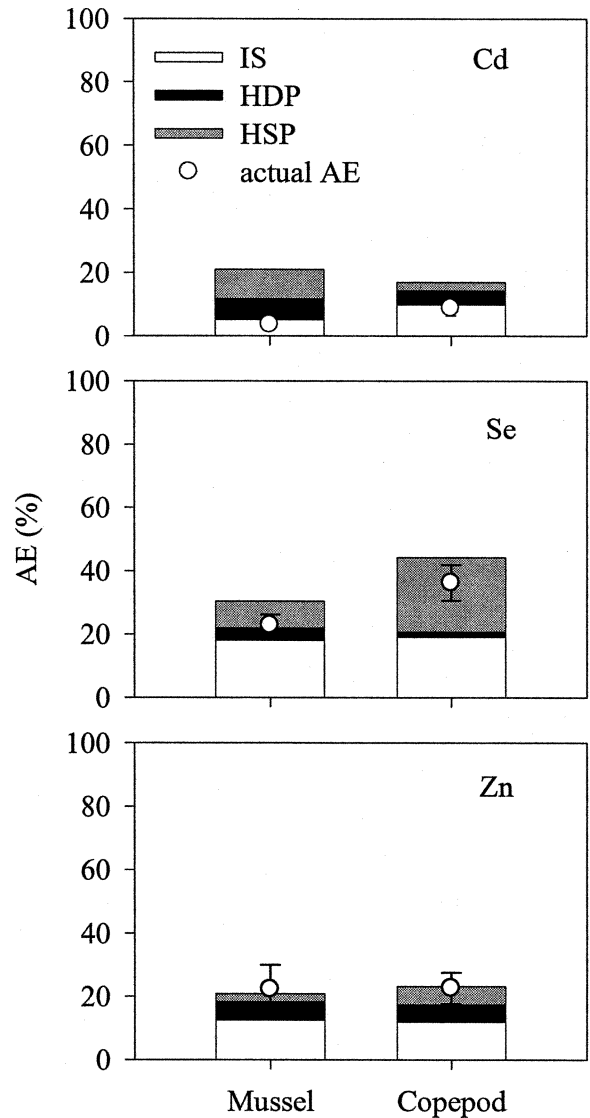


Fig. 6. Comparison of the predicted and actual AEs in the fish *T. jarbua* fed copepods and mussels. The predicted AEs were calculated from the AEs determined from insoluble fraction (IF), heat-denatured protein (HDP), and heat-stable protein (HSP) and the subcellular distributions of metals in the prey. Mean \pm SD ($n = 8$).

pieces, and addition of buffers may have increased the Cd bioavailability by weakening the binding affinity of Cd.

Apart from metal assimilation in marine fish, this study also investigated the trophic transfer of metals along marine food chains. Aquatic organisms have different strategies in handling incoming toxic metals, such as excreting the metals or storing them in physiologically inactive pools (Rainbow 2002). Different strategies of metal sequestration result in different metal distributions in the subcellular fractions (Wallace and Luoma 2003). MRG and HSP (containing MT) are considered as the two major metal sequestration sites available for metal storage or excretion (Rainbow 1993; Campbell et al. 2005). The five prey in our study employed different strategies. Fish used HSP as the main Cd- and Zn-binding sites, as demonstrat-

ed in earlier studies where MT was the predominant Cd- and Zn-binding ligand in fish (Hogstrand and Wood 1996; Campbell et al. 2005). The two bivalves distributed dominant metals in the MRG fractions (especially for Se and Zn), suggesting that both MRG plays an important role for sequestering metals under this radiolabeling condition, which had low metal concentrations. Similarly, other previous studies demonstrated that Cd and Zn were bound to MRG in mussels and clams after exposure (Shi and Wang 2004; Cheung and Wang 2005). The barnacle *B. cirratus* sequestered Se and Zn in MRG, consistent with previous studies on another species *B. amphitrite* (Rainbow et al. 2004; Cheung and Wang 2005). The copepods stored Cd and Zn in their cellular debris and MRG and stored Se in their MRG and HSP. This study further illuminated that the strategies of metal sequestration in the prey determined the availability of accumulated metals to upper trophic levels. For example, the relationship between Se and Zn distributions in HSP and the AEs in predator suggested that metals sequestered in MT were more trophically available.

In conclusion, the assimilation of metals by a predator fish was determined both by subcellular metal distribution in the prey and by the feeding process of the fish. The AEs in the grunt varied for different prey and were correlated with the subcellular metal distributions in the prey. Metals distributed in the HSP fraction and HDP fraction are more bioavailable to predator than those in the insoluble fraction. The AEs of fish fed purified subcellular fraction were relatively constant among different prey. We further showed that it is possible to predict the overall dietary metal assimilation in marine fish on the basis of measurements of metal distribution in different subcellular fractions of prey and assimilation from each subcellular pool.

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