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Transfer of Cd, Cr and Zn from zooplankton prey to mudskipper *Periophthalmus cantonensis* and glassy *Ambassis urotaenia* fishes

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ABSTRACT: Assimilation efficiency (AE) of metals from ingested food is critical for understanding trace metal accumulation and trophic transfer in aquatic animals. Most recent measurements of metal AEs have been on aquatic invertebrates, whereas relatively few studies have examined metal assimilation in fishes. In this study we determined the AEs of Cd, Cr and Zn in 2 fishes (pelagic glassy *Ambassis urotaenia*, Ambassidae, and the intertidal mudskipper *Periophthalmus cantonensis*, Gobiidae) feeding on 2 zooplankton prey (brine shrimp *Artemia* larvae and copepods). Zooplankton were radiolabeled either by feeding on radiolabeled phytoplankton or by direct exposure to radiotracers in the dissolved phase. Fishes were then fed with radiolabeled zooplankton prey for <1 h, and the retention of ingested metals in the fishes was followed for 2 d. The measured AEs of Cd, Cr and Zn were 14 to 33, 4 to 12, and 5 to 17% in glassy fish, and 10 to 26, 4 to 19, and 11 to 31% in mudskipper, respectively. Routes of radiolabeling in copepod prey did not affect metal AEs in either mudskipper or glassy, whereas metal AEs differed by up to 10-fold in glassy fish feeding on *Artemia* larvae labeled from different routes. There was little difference in the gut passage time of metals for different food types and metals or between fishes. AE was not significantly related to metal gut passage time or metal distribution in the soft tissues of zooplankton prey, for each metal. However, AE in mudskippers was significantly correlated with metal distribution in the prey's soft tissues when all 3 metals were considered. Our study demonstrated that marine fishes can appreciably assimilate trace metals, and trophic transfer should be considered as a source for metal accumulation in fishes.

KEY WORDS: Fish · Assimilation efficiency · Cadmium · Chromium · Zinc · Trophic transfer

INTRODUCTION

Aquatic animals are exposed to chemicals in both dissolved and particulate phases. The relative importance of each uptake pathway is critical for the setting of water quality criteria. Recently, there has been an increasing interest in the trophic transfer of metal contaminants in aquatic invertebrates (Fisher & Reinfelder 1995, Reinfelder et al. 1998, Wang & Fisher 1999a,b). These studies have demonstrated that uptake from ingested food can be a significant source for metal uptake in marine invertebrates, especially for animals having a high feeding activity or for metals having a high concentration in food particles. Recent advances in delineating the exposure pathways of metals have primarily been due to the development of experimental approaches in measuring metal assimilation efficiency (AE), a physiological parameter used to quantify metal bioavailability from ingested food (Wang & Fisher 1999b). In contrast to aquatic invertebrates, there are very few studies which considered metal AE in marine fishes (Reinfelder & Fisher 1994, Reinfelder et al. 1998).

The importance of trophic transfer in the bioaccumulation of several metals, including methylmercury (CH_3Hg) and Se, in fish is well recognized (Riisgard & Hansen 1990, Fisher & Reinfelder 1995, Wiener & Spry 1996). Although other metals such as Ag, Al, Cd, Co, Cu, Pb, Mn, Ni and Zn are not considered to biomagnify in fish (Amiard-Triquet et al. 1980, Douben 1989a), their occurrences in contaminated environments and potential toxicity to aquatic life may present an environmental

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hazard (Reinfelder et al. 1998). For most metals, there is a lack of consensus on the relative importance of dietary intake by fish (Dallinger & Kautzky 1985, Bendell-Young et al. 1986, Douben 1989a,b). Uptake from ingested food is generally not considered as a significant source for metal accumulation in fishes (e.g Bradley & Sprague 1985), although many previous studies have indicated that food can indeed be a major source for metal accumulation (e.g. Willis & Sunda 1984, Dallinger & Kautzky 1985, Dallinger et al. 1987). Mechanistic understanding of trophic transfer of metals in marine fishes requires measurements of the variability of metal AE from diverse food sources. Various techniques (tissue residue, kinetic modeling and radiolabeled pulse-chasing feeding) have been used to measure metal assimilation in fishes (Pentreath 1976, Harrison & Klaverkamp 1989, Reinfelder & Fisher 1994). It is however difficult to generalize metal assimilation in fishes based on a few studies that employed different methodologies. Mechanistic understanding of the processes controlling metal assimilation in fish is limited.

In the present study, we quantified the AEs of Cd, Cr and Zn in the pelagic glassy *Ambassis urotaenia* and the intertidal mudskipper *Periophthalmus cantonensis* from zooplankton prey. Both fish species are widely distributed in the Indo-Pacific region, especially in Hong Kong and southern China (Shen et al. 1993, Ni & Kwok 1999). In recent years, there has been considerable concern about metal contamination (such as Hg, methyl Hg, and Cd) in marine fishes in Hong Kong coastal waters (Blackmore 1998, Dickman & Leung 1998, Ong & Cheung 1998). However, the mechanisms and routes of metal accumulation in these local fishes are not well studied. The overall objectives of our study were to (1) determine the extent to which metals can be assimilated by fishes from ingested zooplankton diets and (2) examine the mechanisms controlling metal assimilation from different prey in fish. Three metals (Cd, Cr and Zn) were considered in this study, largely because of their environmental impact in Hong Kong coastal waters and the availability of their radiotracers for experimental studies. Among these metals, Cd and Zn are soft acid metals that have a higher binding stability constant with S ligands than with N or O ligands. Cr(III) is a hard acid metal that has a higher binding stability constant with O ligands than with N or S ligands.

MATERIALS AND METHODS

Choice of fishes. Two fish species with different ecological habitats, the pelagic glassy *Ambassis urotaenia* (Ambassidae) and the intertidal mudskipper *Periophthalmus cantonensis* (Gobiidae), were used in this study. The glassy *A. urotaenia* is mainly a marine filter-feeding fish, and can be commonly found in the surface waters of Hong Kong during winter and spring seasons. *P. cantonensis* is a common intertidal mudskipper in Hong Kong's marshy areas and tidal mud flats. This fish can breathe through its skin, especially when its body is exposed to the air (Graham 1997). It is a demersal fish and is limited to Japan, Korea and China. The length of fishes used in the experiments was 37 to 55 mm for mudskipper and 40 to 45 mm for glassy. Glassy were collected from the Clear Water Bay and mudskippers were collected from Lantau Island of Hong Kong. They were then maintained in the laboratory for about 1 wk prior to the experiments described below. During the acclimation period, fishes were fed with brine shrimp *Artemia* larvae or a copepod assemblage. The temperature and salinity of seawater used in all experiments were 23°C and 30 ppt, respectively.

Radiolabeling of phytoplankton and zooplankton. Radioisotopes ^{109}Cd (in 0.1 N HCl), ^{51}Cr(III) (in 0.1 N HCl) and ^{65}Zn (in 0.1 N HCl) were used as radiotracers of their respective metals. Two zooplankton were used as fish prey in the present study, including brine shrimp (*Artemia*) larvae and a copepod assemblage (dominated by *Acartia spinicauda*) collected by net tows from Clear Water Bay. Brine shrimp larvae were used as a model prey organism and to represent other sources of zooplankton food eaten by the fishes (other than copepods). The brine shrimp larvae and copepods were radiolabeled by being exposed to radioisotopes in the dissolved phase for 2 d or fed with radiolabeled diatoms for 2 d as described below.

To label the *Artemia* larvae or copepods with radioisotopes from the dissolved phase, about 1000 individuals were placed in 400 ml filtered seawater. Radioisotope additions were 185 to 370 kBq l^{-1} for ^{109}Cd (corresponding to 22–44 nM), 185 to 370 kBq l^{-1} for ^{51}Cr (corresponding to 0.5–0.9 nM) and 185 to 370 kBq l^{-1} for ^{65}Zn (corresponding to 22–44 nM). Seawater was changed after 1 d, and a new batch of radioisotopes was added. Following 2 d of radiolabeling, *Artemia* larvae or copepods were collected by a nylon mesh, rinsed with non-radioactive water, and placed in a small volume of water before being fed to the 2 fishes.

Diatoms *Skeletonema costatum* were radiolabeled with Cd, Cr and Zn as described in Wang et al. (1999a). Briefly, diatom cells were filtered and resuspended in 150 ml of 0.2 µm filtered seawater enriched with *f*/2 levels of N, P, Si and vitamins, and *f*/20 levels of trace metals minus EDTA, Cu and Zn. The initial cell density in the medium was 20000 cells ml^{-1}. Radioisotope additions were 123 kBq l^{-1} for ^{109}Cd (corresponding to 14.8 nM), 123 kBq l^{-1} for ^{51}Cr (corresponding to 0.3 nM) and 123 kBq l^{-1} for ^{65}Zn (corresponding to 15 nM). After 4 to 5 d of growth, the cells were filtered and resuspended twice in non-radioactive water to remove

loosely bound radioisotopes before being fed to the brine shrimp larvae or copepods. Zooplankton were fed 4 times a day for 2 d, during which the water was not changed. The animals were then collected, rinsed, and placed in a small volume of water before being fed to the fishes. The distributions of radioisotopes in the soft tissues of zooplankton prey before being fed to the fish were determined as described in Wang & Fisher (1998a). Briefly, zooplankton was rinsed with 100 mM EDTA to remove the weakly sorped metals, and then extracted with 4 ml 0.2 N NaOH at 65°C for 4 h, and filtered through 10 µm polycarbonate membrane to remove the exoskeleton. Radioactivity in the soft tissues and exoskeleton was then measured.

Pulse-chase feeding. Radiolabeled brine shrimp larvae or copepods (about 100 individuals) were then fed to individual fish held in 200 ml filtered seawater, for 30 to 60 min, until most of the zooplankton was consumed. There were 6 replicate individual fish for each treatment. No radioactive feces were produced during the course of radioactive feeding. Following the radioactive feeding, each individual fish was rinsed with non-radioactive water and their radioactivity was immediately counted (described in the following section). They were then placed individually in 700 ml of non-radioactive water for a period of 50 h, during which brine shrimp larvae were provided as food. The radioactivity retained in the fishes was monitored every 4 to 8 h. Water and food were renewed during measurement of radioactivity in fishes. Any feces produced by the fish during the initial 12 to 24 h of depuration were removed every 1 to 2 h and their radioactivity was analyzed, thus minimizing radioisotope desorption from the feces into the dissolved phase. After 24 h, egested feces contained negligible amounts of radioactivity, and the feces were collected less frequently.

AE was calculated by both the mass balance method and the *y*-intercept method (Wang & Fisher 1999b). In the mass balance method, assimilation was assumed to be complete within 24 h of depuration (see 'Results'), and AEs were calculated as the percentage of ingested metals retained in fish at 24 h. In the *y*-intercept method, the percentage of ingested radioisotope remaining in fishes was modeled by the following equation:

$$y = AE \times e^{-bt}$$

where y is the percentage of ingested radioactivity retained in the fish between 24 and 50 h, AE is the assimilation efficiency, which was calculated as the *y*-intercept of the linear regression between the natural log of y and time of depuration (t), and b is the depuration rate constant.

Radioactivity measurements. Radioactivity of ^{109}Cd, ^{51}Cr and ^{65}Zn in the samples was measured by a Wallac 1480 NaI(Tl) gamma detector. The radioactivity counts were corrected for radioactive decay and spillover from a higher energy window to a lower energy window. Counting time was adjusted to yield a propagated counting error <5%. The gamma emission of ^{109}Cd was detected at 22 keV, of ^{51}Cr at 320 keV, and of ^{65}Zn at 1115 keV.

RESULTS

A bi-phasic depuration pattern, including an initial rapid loss within the first day and then a slower loss, was evident in both fish species (Fig. 1). The depuration pattern was similar for all food types for each

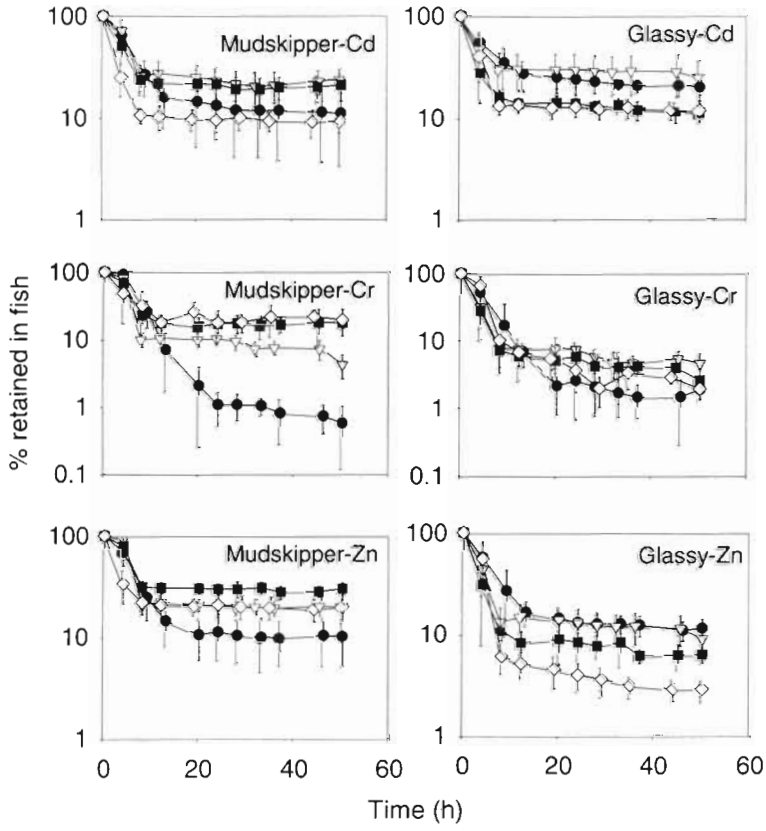

Fig. 1. Retention of Cd, Cr and Zn in mudskipper *Periophthalmus cantonensis* and glassy *Ambassis urotaenia*, following a pulse feeding on different prey. ●: *Artemia* larvae radiolabeled with metals in the dissolved phase for 2 d; ▽: *Artemia* larvae fed with radiolabeled diatoms for 2 d; ■: copepods radiolabeled with metals in the dissolved phase for 2 d; ◇: copepods fed with radiolabeled diatoms for 2 d. Means ± SD (n = 6)

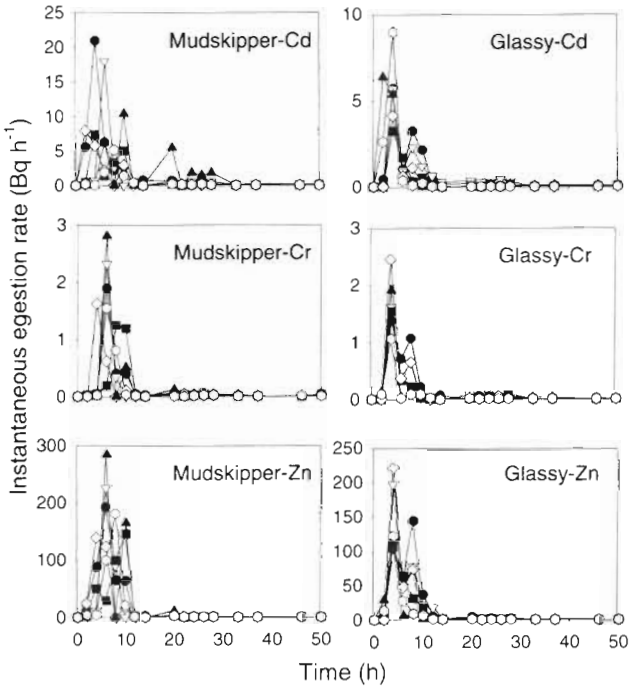

Fig. 2. Instantaneous egestion rate of Cd, Cr and Zn in mudskipper *Periophthalmus cantonensis* and glassy *Ambassis urotaenia*. Each symbol represents an individual fish. Only data for fishes feeding on *Artemia* larvae radiolabeled with metals in the dissolved phase are shown

metal. Unassimilated radioactive feces were found 2 h after the depuration, indicating that fishes processed ingested food materials and metals rapidly. Similar patterns of egestion for Cd, Cr and Zn were observed in mudskipper and glassy fishes (Fig. 2). In general, any unassimilated metals were egested within the first 12 h, after which there was negligible loss of metals by feces egestion. Maximum egestion was observed between 4 and 10 h for mudskipper and between 2 and 8 h for glassy.

The calculated AEs of metals for different food types in mudskipper and glassy fishes are shown in Table 1. There was no major difference in AEs calculated by the mass balance method and the *y*-intercept method. Food composition had a considerable effect on Cd, Cr and Zn assimilation in fishes. In general, AEs calculated by the *y*-intercept method varied by a factor of 2.4 to 2.5 for Cd, 2.7 to 4.5 for Cr and 2.8 to 3.3 for Zn in both fishes. AEs in mudskipper were comparable to or somewhat higher than the AEs in glassy (except Cd labeled to *Artemia* larvae from the dissolved phase), particularly when the copepods were used as prey. The route of radiolabeling (water vs diatom food) in copepod prey appeared to have no major influence on metal AEs in either mudskipper or glassy. In contrast, metal AEs differed by up to 10-fold in glassy feeding on *Artemia* larvae labeled by 2 different routes.

The gut passage time (GPT) of metals was calculated as the time at which 90% of unassimilated radioisotopes were recovered in the cumulative feces, assuming a 100% egestion of unassimilated metals at 24 h (Wang & Fisher 1996) (Table 1). Unfortunately we did not measure the GPT in fishes feeding on *Artemia* larvae radiolabeled from the dissolved phase, because the radioactivity used in this experiment was too low to allow meaningful measurements. In general, the GPT was within 12 h, indicating that metals associated with the food particles passed through the digestive system rapidly. Metal GPTs were comparable between the 2 fishes, among the metals and among different food types. When all metals and food types were considered, no relationship between metal AE (calculated by the *y*-intercept method) and GPT was found in either fish species.

Because individual fish were multi-radiolabeled with 3 metals, it was possible to examine the interrelationships of metal AEs among different individuals. No significant relationship of AEs among metals was found in the mudskipper, whereas the AEs of Cd and Zn were significantly correlated in glassy (Fig. 3).

Following 2 d radiolabeling, about 26 to 86% of Cd, 7 to 50% of Cr and 12 to 89% of Zn were found in the soft tissues of zooplankton (Table 1). Route of exposure did not affect the distribution of Cd and Cr in the soft tissues of copepods. More metals were found in the soft tissues of *Artemia* larvae when metals were obtained from ingested diatoms. For each metal, the AEs in both fishes were not significantly related to the metal distribution in the prey's soft tissues (Fig. 4). However, there was a significant relationship between metal AE and metal distribution in the prey's

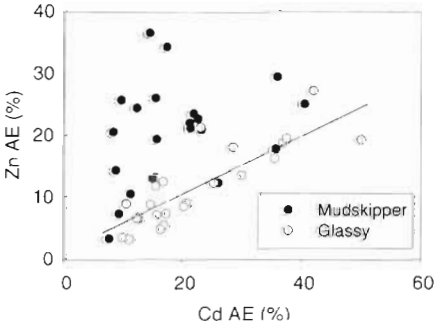

Fig. 3. Relationship between the assimilation efficiencies (AE) of Cd and Zn in the mudskipper *Periophthalmus cantonensis* and the glassy *Ambassis urotaenia*. Each data point represents 1 individual. Regression line describing the relationship of AEs between Cd and Zn in glassy was $y = 0.946 + 0.485x$ ($r^2 = 0.706$, $p < 0.001$). No significant relationship of AEs between Cd and Zn was found in mudskipper

Table 1. Calculated assimilation efficiencies (AE, %), gut passage time (GPT, h) of Cd, Cr and Zn in mudskipper *Periophthalmus cantonensis* and glassy *Ambassis urotaenia* fishes. Distribution of metals in the soft tissues of radiolabeled zooplankton prey is also presented. The route of metal uptake in prey is indicated in parentheses (water or diatom). Data are given as mean ± SD (n = 6 for AE and GPT, and 2 for tissue distribution). AEs were calculated by both the mass balance method (MB) and the *y*-intercept method; see 'Materials and methods' for further explanation. Statistically significant differences (by *t*-test) in metal AEs between the mudskipper and glassy fishes for each prey type are indicated by $^{*}(p < 0.05)$ and $^{**}(p < 0.01)$ (nd: not determined)

Food type	Metal AE		GPT	Metals in prey's soft tissues (%)
	MB	*y*-intercept		
Cd				
Mudskipper				
Artemia larvae (water)	13.1 ± 7.1*	14.7 ± 6.6**	nd	25.5 ± 14.0
Artemia larvae (diatom)	23.2 ± 7.7	26.0 ± 8.7	10.7 ± 4.0	85.9 ± 1.0
Copepod (water)	21.3 ± 7.3	21.8 ± 8.3	5.8 ± 1.4	68.9 ± 4.4
Copepod (diatom)	9.4 ± 2.3	10.4 ± 2.7	5.0 ± 1.4	71.7 ± 2.1
Glassy				
Artemia larvae (water)	23.7 ± 3.6*	26.9 ± 4.9**	nd	25.5 ± 14.0
Artemia larvae (diatom)	29.6 ± 13.2	32.6 ± 13.4	7.6 ± 3.1	85.9 ± 1.0
Copepod (water)	13.8 ± 1.7	15.0 ± 1.9	4.3 ± 0.9	68.9 ± 4.4
Copepod (diatom)	12.7 ± 2.6	13.7 ± 2.8	6.4 ± 1.1	71.7 ± 2.1
Cr				
Mudskipper				
Artemia larvae (water)	1.1 ± 0.6	4.2 ± 3.6	nd	7.1 ± 2.2
Artemia larvae (diatom)	10.4 ± 2.0	16.3 ± 3.9	8.1 ± 1.4	50.5 ± 1.6
Copepod (water)	16.9 ± 6.2*	16.6 ± 5.6**	10.0 ± 3.4	45.8 ± 6.6
Copepod (diatom)	17.6 ± 8.6*	19.1 ± 7.2	12.6 ± 5.4	38.2 ± 3.9
Glassy				
Artemia larvae (water)	2.5 ± 1.9	4.7 ± 2.9	nd	7.1 ± 2.2
Artemia larvae (diatom)	7.4 ± 2.3	9.8 ± 5.9	7.5 ± 1.7	50.5 ± 1.6
Copepod (water)	5.7 ± 2.6*	4.4 ± 2.6**	6.5 ± 2.2	45.8 ± 6.6
Copepod (diatom)	3.6 ± 1.9*	11.8 ± 7.3	8.4 ± 1.2	38.2 ± 3.9
Zn				
Mudskipper				
Artemia larvae (water)	11.2 ± 5.3	11.2 ± 5.5	nd	12.5 ± 4.7
Artemia larvae (diatom)	20.1 ± 3.2*	21.2 ± 2.3	8.3 ± 1.4	89.2 ± 0.1
Copepod (water)	29.6 ± 4.0**	30.9 ± 5.0**	5.3 ± 1.7	35.9 ± 3.1
Copepod (diatom)	20.7 ± 5.1**	21.2 ± 4.4**	5.3 ± 1.0	68.8 ± 2.7
Glassy				
Artemia larvae (water)	12.8 ± 2.7	15.1 ± 4.0	nd	12.5 ± 4.7
Artemia larvae (diatom)	12.9 ± 4.7*	17.1 ± 6.5	7.9 ± 2.1	89.2 ± 0.1
Copepod (water)	8.2 ± 2.7**	8.7 ± 2.5**	4.6 ± 0.9	35.9 ± 3.1
Copepod (diatom)	3.9 ± 1.2**	5.2 ± 2.0**	6.2 ± 1.0	68.8 ± 2.7

soft tissues in mudskippers when all 3 metals were considered.

DISCUSSION

Our measurements of Cd AEs (10 to 26% in mudskipper and 14 to 33% in glassy) were generally higher than previously published measurements. For example, Reinfelder & Fisher (1994) showed that the AEs determined for 2 marine fishes (*Menidia menidia*, *Menidia beryllina*) were only 2.7% for Cd and 6.2% for Zn. Very low AEs were also found for Cd in other fishes such as rainbow trout (0.5 to 5.4%, Kumada et al. 1980). For Zn, Pentreath (1976) fed the radiolabeled worm *Nereis diversicolor* to plaice for 1 h and then depurated the fishes in non-radioactive water for 34 d. AE of Zn measured after 5 d of depuration was 18%, comparable to our present measurements in 2 marine fishes feeding on zooplankton prey (11 to 31% in mudskipper and 5 to 17% in glassy). Among the 3 metals examined in this study, uptake from food sources has been demonstrated for Cd (Williams & Giesy 1978, Ramamoorthy & Blumhagen 1984, Douben 1989a,b, Harrison & Klaverkamp 1989, Wicklund-Glynn et al. 1992), and Zn (Ramamoorthy & Blumhagen 1984, Spry et al. 1988).

AEs of Cd and Zn measured in the 2 fishes were somewhat lower than the typical AEs measured in planktivorous or carnivorous invertebrates (Wang & Fisher 1999b). For example, AEs of Cd and Zn were 72 to 88 and 93%, respectively, in the barnacle *Balanus*

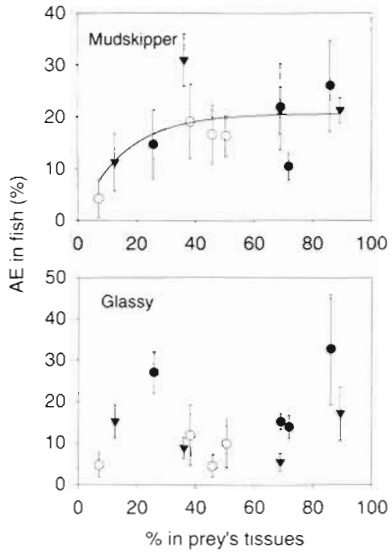

Fig. 4. Calculated AE of Cd, Cr and Zn in mudskipper *Periophthalmus cantonensis* and glassy *Ambassis urotaenia* in relation to their distribution in the prey's soft tissues. (●: Cd; ○: Cr; ▼: Zn). AE was calculated by the *y*-intercept method (mean ± SD, n = 6). Equation describing the relationship between metal AE (y) and metal distribution in prey's soft tissues (x) was: $y = 20.7[1-\exp(-0.06x)]$ ($r^2 = 0.373$, $p < 0.05$)

amphitrite feeding on copepod prey (Wang et al. 1999a,b). In the seastar *Marthasterias glacialis* feeding on radiolabeled mussels, the AEs of Cd and Zn were 73 and 78%, respectively (Fowler & Teyssie 1997).

Differences in metal AEs among different studies may result, in part, from the difference in the experimental approaches. For example, Reinfelder & Fisher (1994) radiolabeled the copepod prey from the nauplii stage and allowed the nauplii to develop into adults before being fed to the fishes. It was assumed that the prey was uniformly radiolabeled. They found that the majority of metals (including Cd and Zn) were distributed in the exoskeleton of copepods following radiolabeling. In our study, we found that a significant fraction of radioisotopes was in the soft tissues of prey following 2 d exposure, comparable to a previous measurement in another calanoid copepod, *Temora longicornus* (Wang & Fisher 1998a). Recent studies also demonstrated that the duration of radiolabeling (2 vs 6 d) did not significantly affect the distribution of metals in the exoskeleton and soft tissues of copepods (Wang & Fisher 1998a,b). Munger et al. (1999) compared the distribution of Cd in the cladoceran *Ceriodaphnia dubia* following 1 d and lifetime exposure. Their results indicated that both the amount and the tissue distribution were the same, independent of short- or long-term exposure. In our radiolabeling, it is possible that metals may have desorbed from the diatoms and been accumulated by the zooplankton from the dissolved phase, but this was not checked.

In our study, we were able to continuously monitor the percentage of ingested metals retained in the fishes following pulse ingestion of radiolabeled prey. Our results indicated that digestion was completed within 1 d, after which there was little loss of radioisotopes from the fish bodies. In contrast, Pentreath (1976) showed that a complete digestion of Zn occurred only after 3 to 4 d of depuration in the flatfish plaice, presumably due to the lower temperature used in the experiments (7.5°C). Douben (1989a,b) and Metayer et al. (1992) also indicated that increasing temperature can considerably increase the uptake and elimination rate of metals (Cd, Cu, Pb and Zn) in fishes.

Appreciable assimilation (4 to 19%) of Cr was found for both the mudskipper and glassy fishes. We are not aware of other measurement of Cr AEs in marine fishes. Cr has been considered as an inert tracer of food passage because of its very low assimilation in many aquatic invertebrates (Bricelj et al. 1984, Wang & Fisher 1996, Selec et al. 1999), but appreciable assimilation has also been found in other invertebrates such as clams (Decho & Luoma 1994, Chong & Wang 2000), barnacles (Wang et al. 1999a) and green mussels (Chong & Wang 2000). One possible explanation for the appreciable assimilation of Cr is the high penetration of Cr into the soft tissues of zooplankton prey. For example, we found that up to 50% of Cr was distributed in the soft tissues of prey. Only *Artemia* larvae labeled with Cr from the dissolved phase contained a small fraction (7%) in their soft tissues, and the AEs were considerably lower than the AEs measured for other foods in fishes.

In fish, Reinfelder & Fisher (1994) suggested that AE was directly related to the percentage of metal in the nonexoskeleton fraction of the copepod prey, i.e. fish only absorbed the soft tissues of the copepods and egested the chitinous exoskeleton and its associated metals. For example, they found that very little Cd and Zn were in the exoskeleton, and the measured AEs were lower (<6%). Such a tight correlation between AE in fish and elemental distribution in the soft tissue of copepods appeared to be mainly due to the higher assimilation of major elements such as C, S and P, which were also considered in the study. When only metals (Cd, Co and Zn) were considered in that study, no relationship between metal assimilation and metal distribution in the soft tissues of prey was evident.

In our study, we did not find a relationship between any of the individual metals and the different food types (2 prey labeled from the food source or the dissolved phase) for either fish species. When all 3 metals and the different food types were considered together,

no significant relationship between AE and metal distribution in the soft tissues of prey was evident for the glassy, whereas in the mudskipper there appeared to be a significant correlation between AE and metal distribution in prey's soft tissues. Thus, distribution of metals in the soft tissues of prey may explain the variability of AEs among different metals in mudskippers. It is however difficult to conclude from our study that fishes assimilated only metals bound to the soft tissues. In a recent study, Wang et al. (1999b) demonstrated that barnacles assimilated a considerable fraction of metals (Cd and Zn) bound to the chitinous exoskeleton of zooplankton prey.

Our study showed that the gut passage of metals in fish was either comparable to or faster than that observed in marine invertebrates. For example, the gut passage of unassimilated metals in marine mussels and copepods was in the range of 4 to 48 h (Wang & Fisher 1996, 1998a). In contrast to marine mussels and polychaetes (Wang & Fisher 1996, Selec et al. 1999), gut passage time in our experimental fishes did not significantly affect metal assimilation. In fish, digestion was characterized by a 1-phase pattern, in contrast to marine bivalves, which are characterized by a biphasic digestion pattern (e.g. extracellular and intracellular digestion, Decho & Luoma 1991). In addition, the positive relationship of AEs between Cd and Zn in glassy indicated that their digestive processes were similar, presumably because these 2 metals tend to bind to similar ligands, such as SH-containing compounds. Assimilation of Cr was apparently decoupled from the assimilation of Cd and Zn.

Several previous studies have demonstrated the significance of trophic transfer of metals in fishes. For example, Pentreath (1973, 1976) suggested that food constituted the major pathway of Zn and Mn accumulation in the plaice *Pleuronectes platessa*. Willis & Sunda (1984) estimated that food ingestion represented 78 to 82% of the total Zn accumulation in 2 species of fishes, *Gambusia affinis* and *Leiostomus xanthurus*. Dallinger et al. (1987) emphasized that the transfer of metals through food chains can result in high concentrations in the fish tissues. Development of generic models to predict the relative importance of food ingestion versus water in the overall metal accumulation would require measurements of metal AEs in fishes. A higher AE may potentially lead to a higher trophic transfer factor. Among the 3 metals considered in this study, there is no evidence for their biomagnification (Fisher & Reinfelder 1995), but a trophic transfer factor of >1 has been reported for Zn in fish collected from the Loire estuary in France (Amiard-Triquet et al. 1980). Appreciable assimilation of Zn may have been responsible for the high trophic transfer factor found in the field study.

In summary, our study suggested that metals can be appreciably assimilated by the mudskipper and glassy fishes collected from Hong Kong waters. Trophic transfer, which is dependent on the metal AE and the animal's feeding rate, can be a significant source for the overall metal bioaccumulation in fishes. There is considerable variation of metal AEs in fishes feeding on different food sources. Our study also suggests that both the gut physiology (e.g. gut passage time) and the metal localization in prey tissues are less critical in accounting for the variation of metal AEs in fishes compared to marine invertebrates. Further studies are required to develop realistic models to predict the exposure pathways of metals under the diverse ecological conditions likely encountered by fishes.

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