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Exposure and potential food chain transfer factor of Cd, Se and Zn in marine fish *Lutjanus argentimaculatus*

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ABSTRACT: Radiotracer techniques were employed to quantify the rates of uptake from aqueous and dietary sources, and rates of elimination of Cd, Se and Zn by a marine predatory fish, the mangrove snapper *Lutjanus argentimaculatus*. The relative significance of bioaccumulation of metals by fish from water and food and the food chain transfer factor were then assessed using a kinetic modeling approach. Cd and Zn in the aqueous phase exhibited an approximately linear uptake pattern over a 1 to 2 d exposure, whereas Se exhibited a 2-compartmental uptake at a low ambient concentration, with a slow initial uptake followed by a rapid increase in Se influx. Most of the accumulated aqueous Se and Zn were incorporated into the muscles, whereas Cd was evenly distributed in the viscera and the remaining tissue, with a lower proportion in the gills. The influx rates were dependent on the ambient metal concentration and were tissue-specific for each metal. The assimilation efficiency of trace metals in fish ingesting different prey (copepods, *Artemia* sp. and clam tissue) ranged from 6 to 24 % for Cd, 32 to 68 % for Se and 15 to 46 % for Zn, and decreased with an increase in ingestion rate. The efflux rate constant of Cd in fish following uptake from the dietary phase (0.047 d^{-1}) was higher than that following aqueous uptake (0.025 d^{-1}), whereas the efflux rate constants of Se and Zn were comparable between these 2 exposure pathways. Our modeling calculations indicate that dietary uptake of Cd and Zn dominates their accumulation in fish when zooplankton are the main prey, whereas aqueous uptake may be the dominant pathway when planktivorous fish are the dominant prey for the predatory fish. Dietary uptake always dominates Se accumulation in these fish. The modeling results also indicated that the food chain transfer factor of Cd was <0.5 in the fish regardless of the ingestion rate and the assimilation efficiency, consistent with the results of field studies. However, Se and Zn may potentially be biomagnified when the ingestion rate and assimilation efficiency are at the high end of the range possibly encountered by the predatory fish.

KEY WORDS: Metals · Mangrove snapper · Exposure · Food chain · Uptake

INTRODUCTION

Human activities such as metal-related industries have greatly increased the input of trace metals into aquatic systems, where these metals are accumulated by aquatic organisms and may be further transferred up to the top trophic levels. It is well recognized that metal accumulation in these animals can result from both the dissolved and the dietary uptake (Reinfelder et al. 1998, Garnier-Laplace et al. 2000, Besser et al. 2001), with the gills and the alimentary tract being the main possible uptake receptors in the fish (Dallinger et al. 1987, Szebedinszky et al. 2001). In general, the gills, liver and kidney contain relatively high metal concentrations (Dallinger & Kautzky 1985, Hanson 1997, McGeer et al. 2000, Wong et al. 2001), indicating that they may be the major organs for metal uptake (gills) or metal final deposition (liver and kidney) (Dallinger et al. 1987, Thomann et al. 2000, Szebedinszky et al.

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2001). Organ specificity in metal accumulation may also be related to the metal speciation and the regulation of essential and non-essential metals (Hanson 1997, Baskin 2000, McGeer et al. 2000, Burgos & Rainbow 2001). Although most studies have quantified the metal concentrations in fish collected from the field, where they are exposed to metals both in the aqueous and in the dietary phases, it is difficult to quantitatively distinguish the metal uptake via food or water. Such information is useful for modeling metal transport within marine food chains and the interaction between fish and their environments.

Recently, the kinetic modeling approach has been developed to quantitatively evaluate the relative contribution of water and food to trace metal accumulation in fish (Reinfelder et al. 1998). Both laboratory and field evidence have confirmed that food can be a major source for metal bioaccumulation in fish (Milner 1982, Klaverkamp et al. 1983, Willis & Sunda 1984, Spry et al. 1988, Mount et al. 1994, Bowie et al. 1996), but the relative quantitative importance may vary with the type and the abundance of prey (Dallinger et al. 1987, Spry et al. 1988). Key parameters inherent in the model include the metal assimilation efficiency (AE), the metal uptake rate from the dissolved phase, the metal efflux rate and the feeding activity of the animals. Most of these parameters have generally been ignored in previous studies. Furthermore, the variability of these key physiological parameters under various environmental and biological conditions remains to be further studied, which is essential for understanding the bioaccumulation processes and the setting of proper water quality criteria.

The transfer of metals in aquatic food chains is important in predicting the concentration at higher trophic levels (Reinfelder et al. 1998, Chen et al. 2000). As a general rule, trace metal concentrations do not appear to be biomagnified along food chains in aquatic systems, with the exception of cesium and organometallic compounds such as methylated mercury. There has been an increasing interest in the trophic transfer of other metal contaminants such as Cd and Zn in aquatic systems (Stemberger & Chen 1998, Chen et al. 2000). A few examples of biomagnification are to be expected by chance, according to the particular bioaccumulation physiologies of the organisms at the different trophic levels, and these recent studies have found field evidence of the biomagnification of Zn in particular lake food chains. For other elements such as Se, there is a lack of consensus on the potential food chain transfer factor (TTF; e.g. concentration in the predator to concentration in the prey) in defined food chains (Saiki et al. 1993, Suedel et al. 1994), probably because the predator and prey organisms have different physiological responses to the bioavailability of metals. Therefore, quantitative study of the processes involved in the bioaccumulation of trace metals in aquatic food webs is important to predicting the environmental significance of metals in aquatic systems (Reinfelder & Fisher 1994).

Few studies have focused on metal bioaccumulation in marine fish (Pentreath 1977, Milner 1982, Willis & Sunda 1984, Burgos & Rainbow 2001), and the kinetic parameters quantifying metal bioaccumulation have not yet been well quantified. This study examined the bioaccumulation of Cd, Se and Zn in a marine predatory fish (the mangrove snapper *Lutjanus argentimaculatus*) from both the aqueous and the dietary phases. The influences of prey type and feeding rate on the AE of metals by the fish were also considered. A kinetic model was then employed to estimate the exposure and the potential TTF of metals in the fish under various physiological and environmental conditions.

MATERIALS AND METHODS

Fish and metals. Mangrove snappers *Lutjanus argentimaculatus* (2.0 to 3.0 cm) were purchased from a fish farm in Hong Kong. The fish were maintained in aerated artificial seawater (Instant Ocean®) (23°C, 30 psu) and fed fresh clam and/or frozen shrimp (obtained from a local supermarket) twice a day. All experiments were performed at this temperature and salinity. One day before the experiments, the fish were acclimated in natural seawater. The biokinetics of Cd, Se and Zn were studied using appropriate radiotracer techniques: ^{109}Cd ($t_{1/2}$ = 462 d, in 0.1 N HCl, from New England Nuclear), ^{65}Zn ($t_{1/2}$ = 244 d, in 0.1 N HCl, from New England Nuclear) and ^{75}Se ($t_{1/2}$ = 120 d, as selenite, in 0.1 N HCl, from Livermore National Laboratory).

Metal uptake from the dissolved phase over time and at different concentrations. Metal uptake by the fish was studied using radiotracers in the presence of stable metals. The radioisotope additions were 7.4 kBq l^{-1} Cd, 23.1 kBq l^{-1} Se and 12.0 kBq l^{-1} Zn. The radioisotopes and stable metals (2 µg l^{-1} of Cd and Se, and 5 µg l^{-1} of Zn) were equilibrated for 8 h before the uptake measurements. Fish were placed in 5 l of 0.22 µm filtered seawater containing both the radiotracers and the stable metals. At 8, 16, 24, 32, 40 and 48 h, 3 fish were removed from the radioactive medium and rinsed twice (transferred from one beaker to another) with filtered non-radioactive water. The radioactivity of the fish was counted non-destructively using a NaI gamma detector (Wallac 1480) at 88 keV for ^{109}Cd, 264 keV for ^{75}Se and 1115 keV for ^{65}Zn. The radioactivity in the seawater and in the particulate phase (>0.22 µm) was measured at the beginning and the end of metal uptake period. There was no significant decrease in the metal radioactivity of the water, nor were there

detectable metals in the particulate phase during the exposure period. After each radioactivity measurement, the 3 fish were dissected into 3 fractions: gills, viscera and the remaining tissues (including the bones). The radioactivity of each fraction was measured. Finally, the dry weight of each fraction was measured after drying at 80°C for >1 d. The concentration factor (CF) of trace metals, which can be regarded as a kinetic parameter, was calculated as the ratio of the radioactivity in the fish or in the different fractions of the fish (counts per minute [cpm] kg^{-1}) to the radioactivity in the water (cpm l^{-1}).

The uptake of Cd, Se and Zn was also determined at 4 different ambient concentrations: 0.5, 2, 10 and 50 µg l^{-1} of Cd and Se (added as stable $CdCl_2$ and Na_2SeO_3); and 2, 5, 20 and 100 µg l^{-1} of Zn (added as stable $ZnCl_2$) using methods similar to those described above. Radioactivity additions were 13.9 kBq l^{-1} ^{109}Cd, 46.3 kBq l^{-1} ^{75}Se and 23.1 kBq l^{-1} ^{65}Zn, corresponding to concentrations of 0.85 nM Cd, 0.013 pM Se and 0.074 nM Zn. Twelve individual fish were exposed in 4 l of 0.22 µm filtered seawater at each concentration. At 4, 10, 16 and 24 h, 3 individual fish were removed and their radioactivity was counted. The fish were then dissected into gills, viscera and the remaining tissues.

AE of metals in fish. The AEs of metals were measured in separate experiments in fish feeding on different prey and at different densities of copepod prey. In the prey experiments, 3 prey diets were examined: the brine shrimp *Artemia* sp. (hatched under laboratory conditions for 1 d), the copepod *Acartia spinicauda* (collected from Port Shelter, Hong Kong) and the clam *Ruditapes philippinarum* (collected from Tolo Harbor, Hong Kong). The *Artemia* sp. and the copepods were radiolabeled with ^{109}Cd, ^{75}Se and ^{65}Zn in 0.22 µm filtered seawater (aqueous exposure). In addition, the copepods were radiolabeled with ^{109}Cd, ^{75}Se and ^{65}Zn through feeding on the radiolabeled diatom *Thalassiosira weissflogii* (dietary exposure). The clams were also radiolabeled by feeding on the radiolabeled diatoms. The diatoms had previously been radiolabeled with ^{109}Cd, ^{75}Se and ^{65}Zn in 0.22 µm filtered seawater enriched with f/2 levels of N, P, Si and vitamins, and f/20 levels of trace metals, but without Cu, Zn and EDTA, for 4 d. Uniformly radiolabeled diatoms were collected and fed to the clams and copepods. After 36 h exposure to radiotracers in the aqueous phase or feeding on the radiolabeled diatoms, the *Artemia* sp. and the copepods were collected by a mesh, rinsed with seawater and then fed to the fish. The clams were dissected, and only the foot and mantles were fed to the fish (the viscera was not fed to the fish to avoid any unassimilated metals in the clam's gut). Fish were fed the different radiolabeled food species for 90 to 120 min. There were 7 replicated individuals in each treatment.

In the prey density experiment, the copepods were radiolabeled by feeding on radiolabeled diatoms for 36 h (dietary exposure), as described above. The prey were then collected by mesh, rinsed with GF/C seawater and fed to the fish at different densities for 30 min: 375, 750, 1125, 1500 and 1875 ind. l^{-1}. There were 4 individual fish in each treatment. Copepods were added every 10 min to maintain a constant prey density. No radioactive feces were produced by the snappers during the radioactive feeding. After the radioactive feeding, the radioactivity of each fish was immediately measured. The fish were subsequently placed in non-radioactive water and allowed to be depurated of the ingested food materials for 72 h. Frozen shrimp was fed to the fish during the depuration period, and the seawater was changed every day. Any feces produced during the depuration period was removed every 8 h.

Metal efflux rate from the fish after aqueous and dietary exposure to metals. Fish were exposed to ^{109}Cd, ^{75}Se and ^{65}Zn both in the aqueous phase and in the dietary phase. In the aqueous exposure treatment, 12 individual fish were exposed to ^{109}Cd, ^{75}Se and ^{65}Zn in the aqueous phase for 7 d, as described above. In the food exposure treatment, 12 individual fish were placed in 5 l seawater and fed both radiolabeled and non-radioactive copepods. The water was renewed each day. The fish were fed under these conditions for 7 d. Afterwards, they were placed in non-radioactive water for 12 h to evacuate the ingested radiolabeled food from the gut. The fish were then rinsed twice (transferred from one beaker to another) with filtered non-radioactive water, and the radioactivity of each fish was immediately measured. Two individuals were dissected to determine the distribution of metals among the gill, viscera and remaining tissues. Radioactivity was measured and the tissues were dried at 80°C for >1 d. The remaining fish were then divided into 2 groups for each treatment and depurated in natural seawater (5 l) for 31 d. The seawater was renewed every 2 d, and the fish were fed frozen shrimp meat twice a day. The radioactivity in individual fish was measured at frequent time intervals. After 22 d of depuration, 2 individuals, or at the end of depuration, all the remaining fish were dissected to determine the distribution of metals among the gill, viscera and remaining tissue. Radioactivity was measured and the tissues dried at 80°C for >1 d before the dry weight measurements.

Modeling the exposure and TTF of metals in the snappers. Under steady-state conditions, Cd, Se and Zn accumulation in fish can be calculated by the following equations (Thomann 1981, Wang & Fisher 1997):

$$C_{ss} = (k_u \times C_w)/k_{ew} + (AE \times IR \times C_f)/k_{ef} \quad (1)$$

where C_{ss} is the total metal concentration in the fish (μg g^{-1}), k_u is the metal net uptake rate constant from the aqueous phase ($l g^{-1} d^{-1}$), C_w is the metal concentration in the dissolved phase (μg l^{-1}), k_{ew} is the efflux rate constant following uptake from the dissolved phase (d^{-1}), AE is the metal assimilation efficiency, IR is the fish feeding rate ($g g^{-1} d^{-1}$), C_f is the metal concentration in the ingested prey (μg g^{-1}), and k_{ef} is the efflux rate constant following uptake from food (d^{-1}). The growth rate constant was ignored in the calculation.

Assuming that C_f can be predicted based on the bioconcentration factor of metals (BCF, under equilibrium assumption) in the prey and on C_w, the fraction of metals (f) coming from the dietary phase can be calculated from Eq. (1) (Wang & Fisher 1999):

$$f = [(AE \times IR \times BCF)/k_{ef}]/[(AE \times IR \times BCF)/k_{ef} + (k_u/k_{ew})] \quad (2)$$

TTF can be calculated as follows (Reinfelder et al. 1998, Wang & Fisher 1999):

$$TTF = (AE \times IR)/k_{ef} \quad (3)$$

RESULTS

Metal uptake from the aqueous phase

Over the 2 d exposure period, Cd and Zn exhibited an approximately linear pattern of uptake (Fig. 1). Se uptake by the gills appeared to be 2-compartmental, including an initial linear uptake within the first 24 h followed by a faster uptake. The *y*-intercepts of the linear regressions between the CF (defined as the radioactivity in the fish divided by the radioactivity in water) and the time of exposure were not significantly different from zero. Generally, the calculated CFs were higher for the gills and the viscera, whereas the remaining tissues and the whole body had lower CFs. After 2 d exposure, the CF of the gill tissue was 1.7 to 2.0× higher than the CF of the viscera. Among the different metals, fish gill and viscera had the highest CFs for Cd and the lowest CFs for Se, whereas the remaining tissue and the whole body had the highest CFs for Zn and the lowest CFs for Se. Cd was evenly distributed between the viscera and the remaining tissue, with a lower distribution in the gills (22 to 31%),

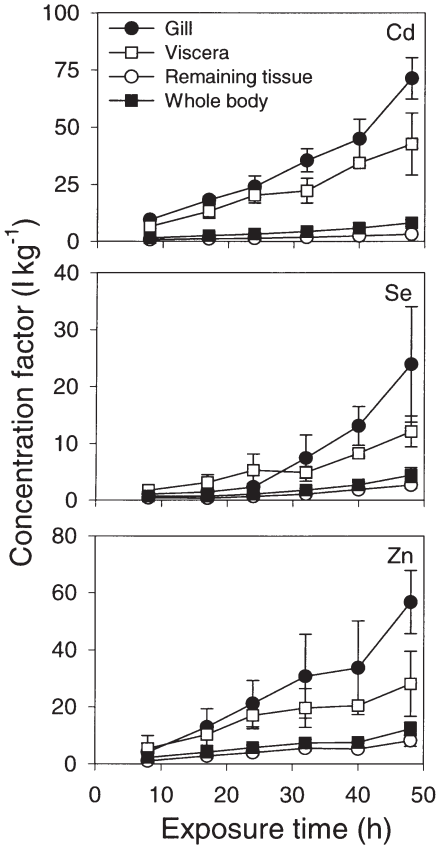

Fig. 1. *Lutjanus argentimaculatus*. Calculated concentration factors for Cd, Se and Zn in mangrove snappers during the 2 d exposure to metals in the dissolved phase. Values are mean ± SD (n = 3)

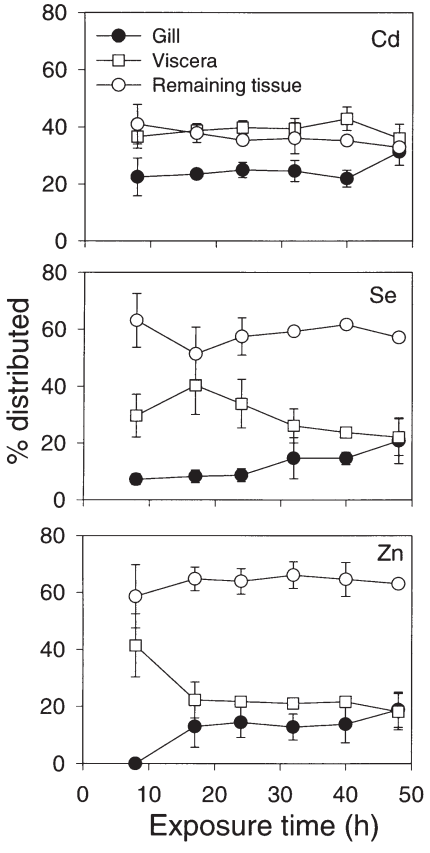

Fig. 2. *Lutjanus argentimaculatus*. Distribution of Cd, Se and Zn in mangrove snappers during the 2 d exposure to metals in the dissolved phase. Values are mean ± SD (n = 3)

whereas most Se and Zn (57 to 66%) were distributed in the remaining tissue, followed by the viscera and the gills (Fig. 2). The relative distribution in different tissues was approximately constant during the 2 d exposure period.

The net uptake of Cd, Se and Zn was quantified at different metal concentrations for up to 1 d. Generally, the CFs at different metal concentrations increased linearly with time for Cd and Zn (Fig. 3), with a few exceptions noted. Se uptake followed a 2-compartmental pattern at the 2 lowest Se concentrations (0.5 and 2 µg l^{-1}). After 1 d exposure, the CFs of Cd and Zn in the whole fish at the highest concentrations were 1.6 to 2.1× lower than the CFs measured at the lowest concentration, whereas there was no major difference in the calculated CFs between the different concentration treatments for all 3 metals within the first 16 h of exposure. For Se, the highest CFs were found at 2 µg l^{-1} for all the body fractions. The influx rate of metals was calculated as the slope of the linear regression between the CF and the time of exposure (4 to 24 h) multiplied by the metal concentration in the dissolved phase. For Se at the 2 lower concentrations, the influx rate was calculated based on the first 16 h of exposure (i.e. the first compartment of uptake). The influx rate increased linearly in a log-log relationship with increasing ambient metal concentration (with a regression coefficient > 0.94; Fig. 4). The power coefficient of the relationship between metal influx rate and metal concentration in the dissolved phase was 0.79 to 1.14 for the different metals. The uptake rate constant for the whole fish (the intercept of the log-log relationship between the influx rate and the metal concentration in the dissolved phase) was 0.0051 l g^{-1} d^{-1} for Cd, 0.0008 l g^{-1} d^{-1} for Se and 0.0100 l g^{-1} d^{-1} for Zn. The magnitude of the uptake rate constants was ordered gill > viscera > the remaining tissue for all 3 metals. Among the 3 metals, the influx rate constants for Cd and Zn were 1 order of magnitude higher than that for Se.

In this concentration experiment, Cd was evenly distributed between the viscera and the remaining soft tissues, and most of the Zn was distributed in the remaining soft tissues. Distribution of Se in the gills increased and distribution in the viscera decreased with time of exposure. In contrast, the distribution of Cd and Zn was relatively constant within the first day of exposure (Fig. 5). After 1 d exposure, metal distribution in the gills was higher at the lower metal concentration. Distribution of metals in the remaining tissues was not affected by differences in metal concentration.

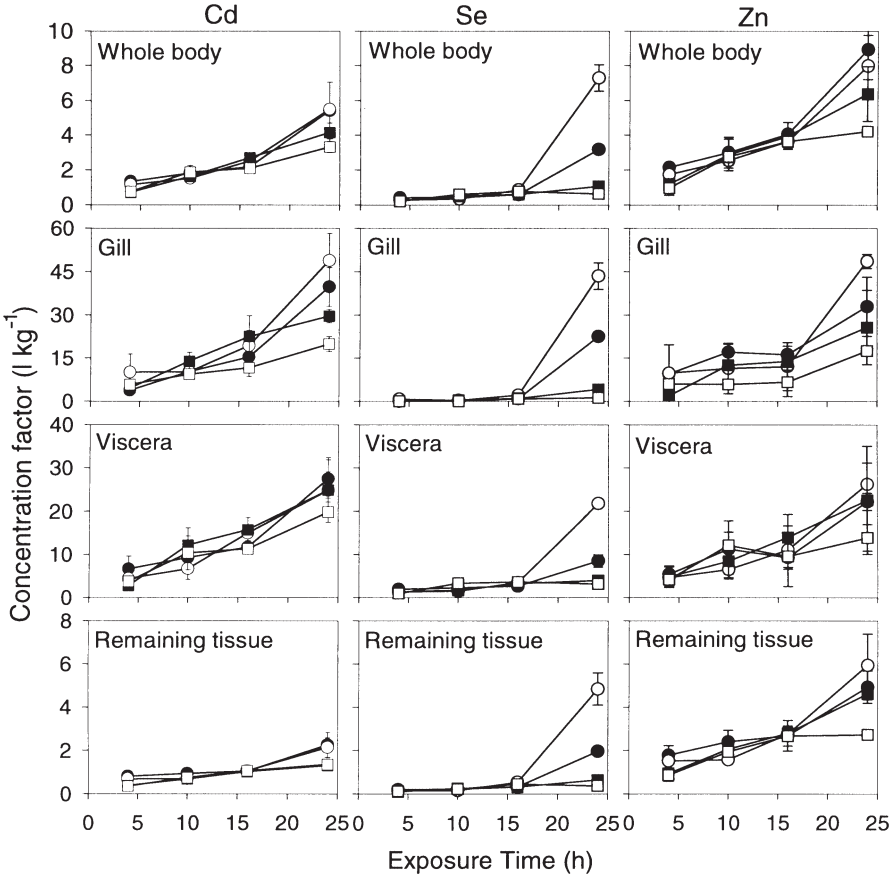

Fig. 3. *Lutjanus argentimaculatus*. Calculated concentration factors for Cd, Se and Zn in mangrove snappers during 1 d exposure to metals at different dissolved concentrations. (●) 0.5 µg l^{-1} of Cd and Se, 2 µg l^{-1} of Zn; (○) 2 µg l^{-1} of Cd and Se, 5 µg l^{-1} of Zn; (■) 10 µg l^{-1} of Cd and Se, 20 µg l^{-1} of Zn; (□) 50 µg l^{-1} of Cd and Se, 100 µg l^{-1} of Zn. Values are mean ± SD (n = 3)

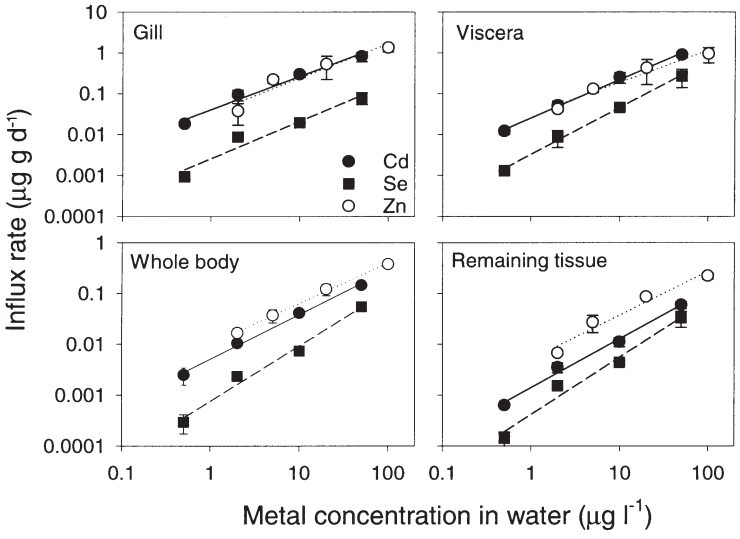

Fig. 4. *Lutjanus argentimaculatus*. Influx rate (I_u, µg g^{-1} d^{-1}) of Cd, Se and Zn in mangrove snappers during 1 d exposure to metals at different dissolved concentrations (C_w, µg l^{-1}). Values are mean ± SD (n = 3). For Cd in gill: $I_u = 0.0410\ [C_w]^{0.81 \pm 0.09}$ ($r^2 = 0.978$); viscera: $I_u = 0.0250\ [C_w]^{0.94 \pm 0.04}$ ($r^2 = 0.996$); remaining tissue: $I_u = 0.0014\ [C_w]^{0.96 \pm 0.06}$ ($r^2 = 0.991$); whole body: $I_u = 0.0051\ [C_w]^{0.87 \pm 0.039}$ ($r^2 = 0.996$). For Se in gill: $I_u = 0.0026\ [C_w]^{0.91 \pm 0.15}$ ($r^2 = 0.950$); viscera: $I_u = 0.0033\ [C_w]^{1.14 \pm 0.05}$ ($r^2 = 0.997$); remaining tissue: $I_u = 0.0004\ [C_w]^{1.12 \pm 0.13}$ ($r^2 = 0.973$); whole body: $I_u = 0.0008\ [C_w]^{1.09 \pm 0.10}$ ($r^2 = 0.983$). For Zn in gill: $I_u = 0.0340\ [C_w]^{0.85 \pm 0.19}$ ($r^2 = 0.913$); viscera: $I_u = 0.0310\ [C_w]^{0.79 \pm 0.11}$ ($r^2 = 0.965$); remaining tissue: $I_u = 0.0050\ [C_w]^{0.87 \pm 0.13}$ ($r^2 = 0.959$); whole body: $I_u = 0.0100\ [C_w]^{0.80 \pm 0.03}$ ($r^2 = 0.997$)

Assimilation of trace metals by the fish

Fig. 6 shows the retention of Cd, Se and Zn by the fish following a pulse feeding on different radiolabeled prey. Retention of the radiotracers by the fish appeared to level off after 1 d of depuration. The metal AEs can thus be calculated as the percentage of metal retained in the fish after 1 d of depuration. The AEs calculated by this method were 6.2 to 9.8% for Cd, 32.1 to 62.7% for Se and 14.5 to 29.8% for Zn (Table 1). Generally, the AEs from different diets were comparable, except for Se and Zn in fish fed clam tissue. For Cd and Zn, there was no significant difference in their AEs by fish feeding on copepods radiolabeled from the aqueous and dietary phases, whereas Se was assimilated at a higher efficiency when it was accumulated by the copepods from the dietary phase rather than from the aqueous phase. Among the 3 metals, Cd had the lowest AE, and Se had the highest AE. The AEs of Zn were intermediate between those of Cd and Se.

The AEs of Cd, Se and Zn in the fish at different ingestion rates (or different copepod densities) are also shown in Table 1. Generally, the metal AEs decreased with increasing rate of ingestion by the fish, especially at the low ingestion rates. At higher ingestion rates (>0.28 g g^{-1} d^{-1}), however, the AEs were relatively constant.

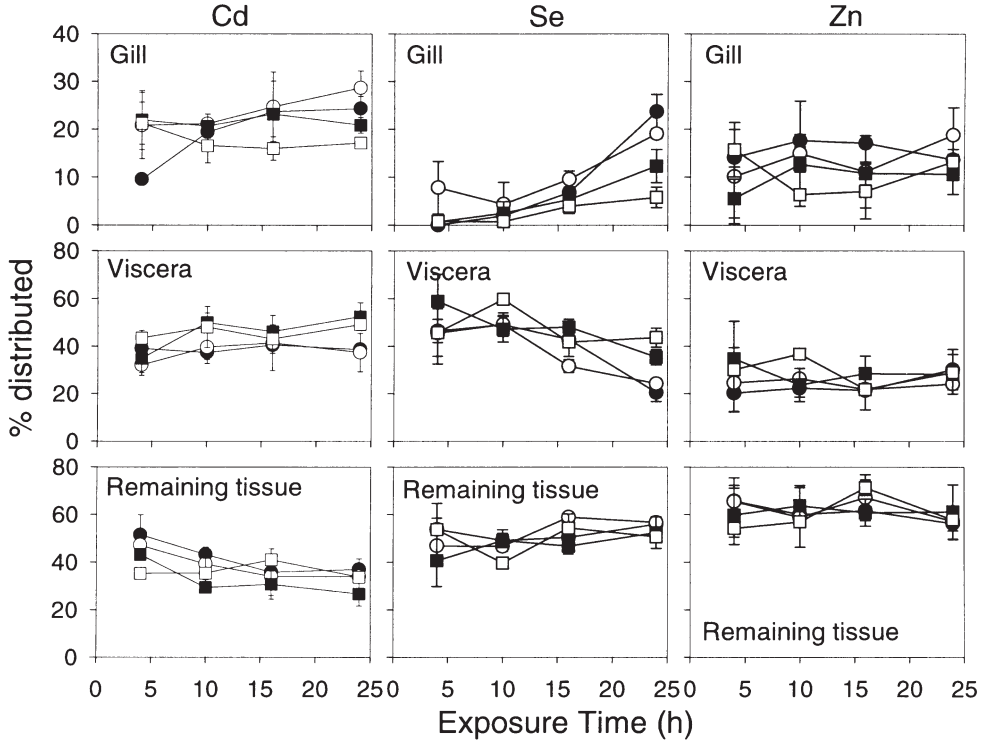

Fig. 5. *Lutjanus argentimaculatus*. Distribution of Cd, Se and Zn in mangrove snappers during 1 d exposure to metals at different dissolved concentrations. (●) 0.5 µg l^{-1} of Cd and Se, 2 µg l^{-1} of Zn; (○) 2 µg l^{-1} of Cd and Se, 5 µg l^{-1} of Zn; (■) 10 µg l^{-1} of Cd and Se, 20 µg l^{-1} of Zn; (□) 50 µg l^{-1} of Cd and Se, 100 µg l^{-1} of Zn. Values are mean ± SD (n = 3)

Table 1. *Lutjanus argentimaculatus*. Assimilation efficiencies (AEs, %) of Cd, Se and Zn by mangrove snappers feeding on different prey or at different ingestion rates. Values are mean ± standard deviation (SD; n = 7 for different prey, or 4 for different ingestion rates)

	Cd (%)	Se (%)	Zn (%)
Prey			
Acartia spinicauda (water)	6.2 ± 0.8	35.7 ± 7.1	20.3 ± 2.7
Artemia sp.	9.8 ± 5.6	32.1 ± 5.6	14.5 ± 1.6
Ruditapes philippinarum	7.2 ± 1.5	54.6 ± 4.4	19.0 ± 4.7
Clam	8.8 ± 4.4	62.7 ± 13.5	29.8 ± 6.9
Ingestion rate (g g^{-1} d^{-1})			
0.05 ± 0.007	23.9 ± 8.9	68.6 ± 8.4	42.7 ± 1.6
0.07 ± 0.04	17.5 ± 4.8	67.8 ± 8.5	45.9 ± 7.1
0.28 ± 0.07	6.8 ± 1.1	50.3 ± 9.6	16.5 ± 3.2
0.33 ± 0.04	6.0 ± 2.0	53.6 ± 3.7	15.2 ± 2.0
0.57 ± 0.13	7.1 ± 1.9	53.9 ± 3.9	17.2 ± 1.6

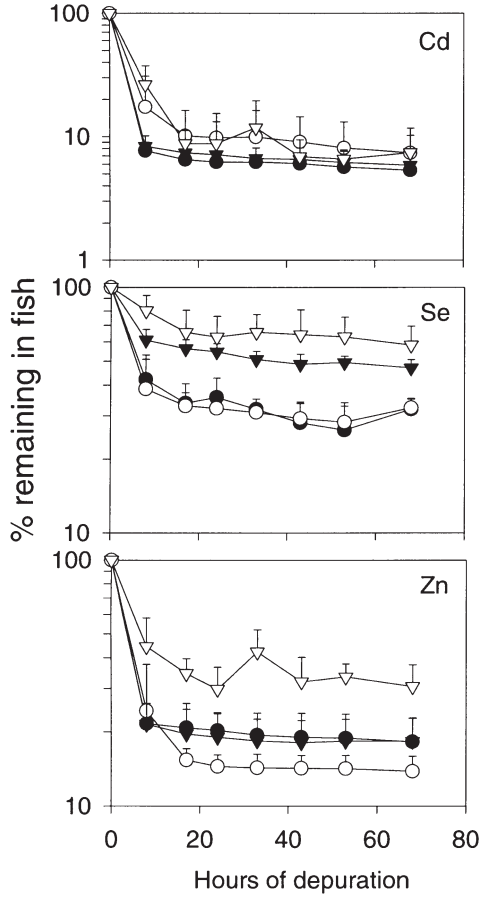

Fig. 6. *Lutjanus argentimaculatus*. Retention of Cd, Se and Zn in mangrove snappers following a pulse ingestion of different radiolabeled prey. (●) Copepod *Acartia spinicauda* radiolabeled with metals in the dissolved phase; (○) *Artemia* sp. larvae radiolabeled with metals in the dissolved phase; (▼) Copepod *Acartia spinicauda* fed radiolabeled diatoms; (▽) clam *Ruditapes philippinarum* fed radiolabeled diatoms. Values are mean ± SD (n = 7)

Efflux of metals from the fish

After the fish were exposed to radiotracers in the aqueous phase or the dietary phase for 7 d, the depuration of metals was approximately 2-compartmental, including an initial rapid loss within the first few days and then a slower loss (Fig. 7). The efflux rate constant was calculated from the slope of the ln% of metals in the fish and the time of exposure between 9 and 31 d. The calculated efflux rate constant and the biological retention half-life of the slow exchangeable compartment are shown in Table 2. The efflux rate constant of Cd was significantly higher ($p < 0.01$, *t*-test) in fish fed radiolabeled copepods (0.047 d^{-1}) than in fish exposed to radiotracers in the dissolved phase (0.025 d^{-1}). There was no statistically significant difference in the efflux of Se and Zn between the 2 routes of exposure. The efflux rate constant of Zn was the lowest (0.015 d^{-1}) among the 3 metals examined.

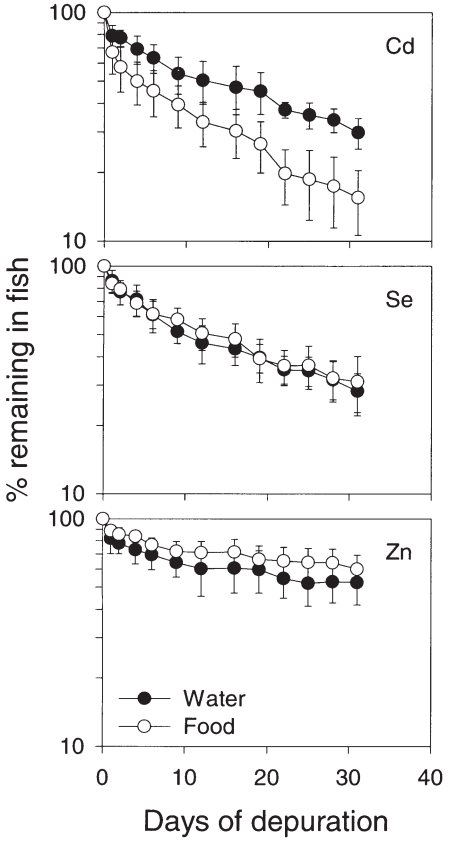

Fig. 7. *Lutjanus argentimaculatus*. Retention of Cd, Se and Zn in mangrove snappers following 7 d exposure to metals in the dissolved phase (water) or 7 d ingestion of radiolabeled copepod *Acartia spinicauda* (food). Values are mean ± SD (n = 5 to 10)

Table 2. *Lutjanus argentimaculatus*. Efflux rate constant and biological half-lives ($t_{1/2}$) of Cd, Se and Zn in mangrove snappers after metal uptake from dissolved and dietary phases. Values are mean ± SD (n = 5 to 8)

	Cd	Se	Zn
Efflux rate constant (d^{-1})			
Dietary exposure	0.047 ± 0.009	0.031 ± 0.01	0.015 ± 0.005
Water exposure	0.025 ± 0.006	0.027 ± 0.007	0.015 ± 0.006
$t_{1/2}$ (d)			
Dietary exposure	15.1 ± 2.9	25.1 ± 8.3	55.1 ± 22.3
Water exposure	29.3 ± 6.3	27.0 ± 6.9	37.9 ± 4.3

By the end of radioactive exposure, a much higher fraction of Cd (81%) was distributed in the viscera of fish exposed to radiolabeled food, compared with 33% in fish exposed to metals in the aqueous phase (Fig. 8). For Se and Zn, 57 to 70% was distributed in the remaining tissue, and there was no significant difference in their distribution between these 2 treatments. After 31 d of depuration, a higher fraction of Se and Zn was distributed in the remaining tissues in both treatments (68 to 78% for Se and 76 to 86% for Zn). For Cd, a higher fraction (92%) was distributed in the viscera in the dietary exposure treatment. Only a small percentage of metals (<23%, and in most cases <10%) was in the gills after 31 d of depuration.

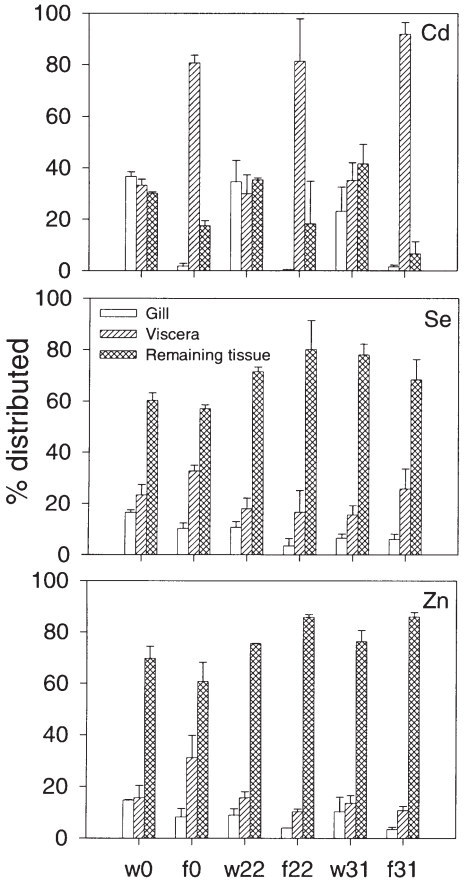

Fig. 8. *Lutjanus argentimaculatus*. Distribution of Cd, Se and Zn in mangrove snappers following 7 d of exposure to metals in the dissolved phase (w0) or 7 d ingestion of radiolabeled copepod *Acartia spinicauda* (f0), following 22 d depuration in non-radioactive waters (w22 and f22) and following 31 d depuration (w31 and f31). Values are mean ± SD (n = 2 for Day 0 and Day 22, and 8 for Day 31)

Modeling the exposure and TTF of metals in fish

Eqs. (2) & (3) identify a few parameters required in the kinetic modeling of exposure and food chain transfer of metals in fish. These values are shown in Table 3. A range of ingestion rate (IR of 1 to 10% of body weight d^{-1}) was used in the modeling calculation, largely because of the uncertainty of this parameter in the field. Within this range of ingestion rate, an AE value of 20% was used for Cd, 65% for Se and 40% for Zn (taken from the food density experiment). For the uptake rate constant (k_u), a mean value of 0.0051 l g^{-1} d^{-1} was used for Cd, 0.0008 l g^{-1} d^{-1} for Se and 0.010 l g^{-1} d^{-1} for Zn, for the whole individual fish. The efflux rate constants for Cd and Zn following dietary and aqueous exposure were taken from Table 2. Two diets were considered in the calculation, namely copepods and planktivorous fish, because they are the dominant preys for the mangrove snapper. The BCFs for Cd, Se and Zn vary considerably (by several orders of magnitude) between the copepods and the planktivorous fish, which also necessitates the separation of these 2 diets in the calculation. A range of BCF for each diet was used in the kinetic modeling (IAEA 2000). Thus, the influences of BCF and IR on the exposure of metals to fish were analyzed. Other parameters were assumed to be constant in the analysis. Calculations indicated that the relative importance of dietary uptake was greatly dependent on the BCFs of Cd and Zn in the prey and the type of prey, whereas over 97% of Se was taken up from the diet regardless of the variation of BCF and the type of prey (Fig. 9). When copepods are the fish's prey, >95% of Zn in the fish may have been derived from the dietary phase, regardless of the variation in BCF. The accumulation of Cd was also dominated by the dietary uptake at different BCFs, and even at the lowest BCF for copepods (10 000 l kg^{-1}), >68% of the Cd in the fish was predicted to have been derived from the dietary uptake. In contrast, when planktivorous fish are the prey organisms, the relative importance of dietary uptake is greatly dependent on the ingestion rate of the fish and the BCF in the prey

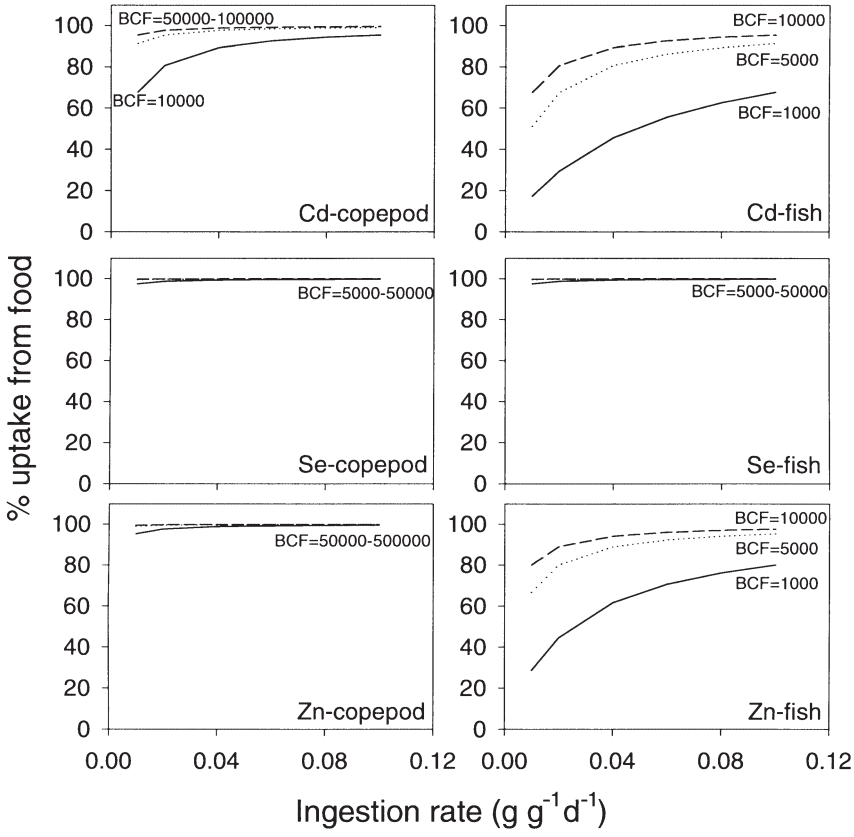

Fig. 9. *Lutjanus argentimaculatus*. The model predicted percentage of Cd, Se and Zn taken up from food phase as a function of the ingestion rate of mangrove snappers. BCF: Bioconcentration factor of Cd and Zn in prey organisms (l kg^{-1}). Both copepod (left panel) and fish prey (right panel) are modeled

fish. At the lowest BCFs, up to 83% of Cd and 70% of Zn may derive from the aqueous uptake. At the highest BCF, dietary uptake still dominates Cd and Zn accumulation in the fish. The calculated TTF is <0.5 for Cd within the normal range of a fish's ingestion rate (Fig. 10). For Se and Zn, the TTF is <1 at the low end of the AE (30% for Se and 15% for Zn). At a higher AE, the TTF is >1 when the ingestion rate is at the medium level. The highest predicted TTFs for Se and Zn were about 2.2 to 3.0, indicating that these metals may potentially be biomagnified when the AE and the ingestion rate of the fish are high.

DISCUSSION

An approximately linear uptake over the exposure time was found for Cd and Zn within the 2 d exposure period, whereas Se uptake was characterized by a 2-compartmental pattern, especially at low Se concentrations. A similar linear pattern of metal uptake has been found in juvenile rainbow trout for Cd (Szebedinszky et al. 2001) and in plaice for Zn during 15 d of exposure (Pentreath 1973). The CFs of metals decreased with increasing metal concentration in the ambient environment after 1 d exposure. In this exper-

Table 3. *Lutjanus argentimaculatus*. Parameters used in modeling metal exposure in mangrove snappers

	Cd	Se	Zn	References
Assimilation efficiency	0.2	0.65	0.40	This study
Ingestion rate (g g^{-1} d^{-1})	0.01 to 0.1	0.01 to 0.1	0.01 to 0.1	Garnier-Laplace et al. (2000)
Concentration factor of metals in copepods (l kg^{-1})	10 000 to 100 000	5000 to 50 000	50 000 to 500 000	IAEA (2000)
Concentration factor of metals in plantivorous fish (l kg^{-1})	1000 to 10 000	5000 to 50 000	1000 to 10 000	IAEA (2000)
Uptake rate constant (l g^{-1} d^{-1})	0.0051	0.0008	0.0100	This study
Efflux rate constant from water (d^{-1})	0.025	0.027	0.015	This study
Efflux rate constant from food (d^{-1})	0.047	0.031	0.015	This study

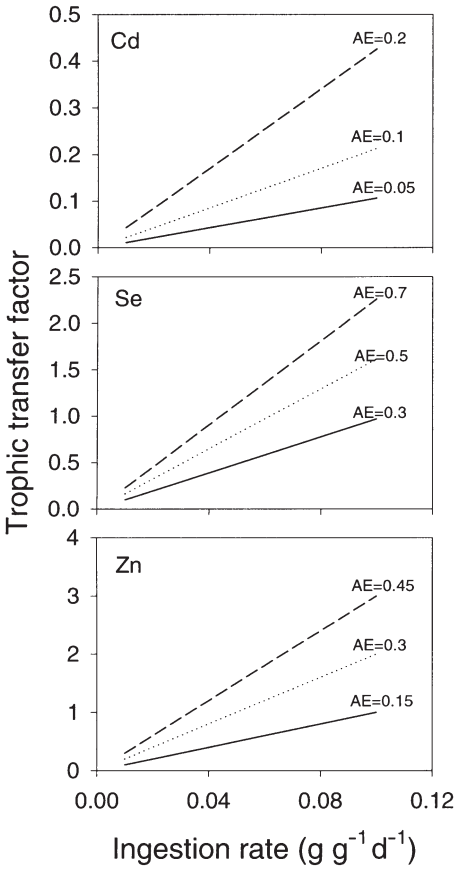

Fig. 10. *Lutjanus argentimaculatus*. The model predicted food chain transfer factor (TTF) for Cd, Se and Zn as a function of ingestion rate in mangrove snappers. AE: assimilation efficiency

iment, at the highest Zn concentration (100 µg l^{-1}), uptake in the remaining tissue appeared to level off between 16 and 24 h of exposure, whereas the gill and viscera still had a significant uptake of Zn, indicating some degree of Zn regulation by the fish muscle. Because muscle contributed the most mass to the whole body of the fish, the majority of Zn was found in the muscle. The regulation of Zn has also been found in freshwater fish, in which metal uptake was either from the water or from the food (Dallinger & Kautzky 1985, Memmert 1987, Spry et al. 1988). Zn is an essential metal and can be regulated internally to maintain homeostasis (Spry et al. 1988, Hogstrand & Haux 1991). For Cd, there is no evidence of its accumulation being regulated by the fish.

When the tissue-specific uptake was considered, the highest CFs for Cd, Se and Zn were found in the gills, indicating that gill may be the primary site for aqueous uptake. However, the distribution of metals in the fish was rather metal-specific. Cd was equally distributed in the viscera and the remaining tissue, with a smaller percentage in the gills, whereas higher percentages of Se and Zn were found in the remaining tissues. The uptake pathways (aqueous vs dietary) may also account for the notable difference in Cd distribution in the snappers after 1 wk exposure to metals. Over 80% of Cd was distributed in the viscera in fish exposed to Cd in their food, compared with 33% distribution in the viscera of fish exposed to Cd in water. Similarly, McGeer et al. (2000) found that the major organ for Cd accumulation from the aqueous phase in the rainbow trout was the kidney, whereas Zn accumulation was significant only in the gills. Pentreath (1973) also found that the main site for Zn uptake in the plaice was the gill as a result of adsorption of Zn onto the mucus of the gill and the opercular cavity.

Fish can take up metals by drinking seawater and through passage of seawater over their gills (Pentreath 1977). A higher percentage of Cd and Se was found in the viscera at high metal concentrations, presumably because the liver and the kidney are the main sequestration and detoxification organs in the fish at high metal concentrations. Metallothionein is an important chelating molecule for Cd (Roesijadi 1992). Significant correlation has been found between metallothionein concentrations and Cd concentrations in the liver and interrenal tissue of yellow perch collected from the Abitibi lakes (Laflamme et al. 2000). A similar correlation was found in the liver and kidney of Arctic char and trout (Marr et al. 1995, Dallinger et al. 1997). Moreover, more Cd was found in the kidneys of rainbow trout exposed to dietary Cd than in those exposed to aqueous Cd, because the uptake of dietary Cd across the gut resulted in substantial accumulation of Cd in internal organs such as the gastrointestinal tract, kidney and liver (Szebedinszky et al. 2001). Therefore, the physiological responses may be metal-specific. The kidney and liver may act as sites for Cd sequestration and storage to protect fish from an accumulation of Cd to toxic levels from food, resulting in its accumulation in the viscera (Szebedinszky et al. 2001). Zn, on the other hand, was actively regulated and eliminated by the fish (Baskin 2000).

This study has demonstrated that the influx rate of metals increases linearly (in a log-log relationship) with an increase of metal concentration in the ambient seawater. Gill had the highest influx rate for Cd and Zn, whereas for Se the viscera had the highest influx rate. For Cd and Se, the power coefficients describing the relationship between influx rate and metal concentration were close to 1, whereas for Zn, they were lower than 1, further indicating the regulation of Zn body burden by the fish. There is very limited literature with which to compare the uptake rate constants of metals in fish. Alsop & Wood (2000) report that Zn loading rates in juvenile rainbow trout gill increased

with an increase in metal concentration. According to the data they presented, an uptake rate constant of 0.038 l g^{-1} d^{-1} without Zn acclimation and 0.022 l g^{-1} d^{-1} with acclimation can be calculated. These are similar to the present measurements in snapper gills (0.034 l g^{-1} d^{-1}). However, the uptake rate constants for metals in fish are 1 to 2 orders of magnitude lower than those quantified in suspension-feeding invertebrates. For example, the uptake rate constants for metals in mussels are 0.180 to 0.182, 0.019 to 0.031 and 0.350 to 0.483 l g^{-1} d^{-1} for Cd, Se and Zn, respectively (Wang 2001). This higher uptake rate constant in bivalves may primarily be due to the high clearance rate and the large permeable area of the gills and mantle. The low uptake rate in fish may result in a dominance of dietary uptake for metals in marine fish.

The metal AEs were comparable in the fish feeding on the different diets, except for Se and Zn in fish feeding on clam tissue. The AEs for Cd (6 to 10%) were lower than a few measurements in previous studies, e.g. 22% by the juvenile striped bass (Fisher & Baines et al. 2001), and 10 to 30% by the mudskipper and glassy fish (Ni et al. 2000), but were higher than other measurements (e.g. 2.7% by the silversides: Reinfelder & Fisher 1994; and 0.5 to 5.4% by the rainbow trout: Kumada et al. 1980). For Se, these results (32 to 63%) were somewhat higher than the previous measurements in silversides and striped bass (29 to 36%, Reinfelder & Fisher 1994, Fisher & Baines 2001), but the AEs of Se were highly dependent on the diets used. The AEs of Zn ranged between 15 and 30% in the snappers and are comparable to those of other fish such as the rainbow trout (16%: Hardy et al. 1987), juvenile striped bass (24%: Fisher & Baines 2001) and the mudskipper (10 to 30%: Ni et al. 2000). Reinfelder & Fisher (1994) have suggested that metal AE in fish may be controlled by the distribution of the metals in the soft tissue of zooplankton prey, in which metals bound with the chitinous exoskeleton may not be bioavailable. Such a relationship was not examined in this study, although Ni et al. (2000) did not find a significant relationship between the AEs of Cd and Zn and the metal distribution in copepods' soft tissue.

In invertebrates such as marine mussels and the copepods, metal AEs are influenced by the ingestion rate of the animals (Wang & Fisher 1996, Xu & Wang 2001). The ingestion rate has usually been ignored when the metal AEs in fish are quantified. Our study demonstrates for the first time that the metal AEs are dependent on the ingestion rate of the fish. The dramatic increase in AE at low ingestion rates indicates that the fish can increase metal assimilation when food is not abundant to maintain metal levels in the body. One of the mechanisms underlying the increase in metal AE at lower food abundance in marine invertebrates is the increase in gut passage time (Wang & Fisher 1996, Xu & Wang 2001), allowing the digestive enzymes to act upon the metals. Whether the gut passage time plays an important role in regulating metal AE remains unclear from this study.

The measured efflux rate constants (k_e) for Cd (0.025 to 0.047 d^{-1}) and Zn (0.015 d^{-1}) were similar to those measured in juvenile striped bass (0.05 d^{-1} for Cd and 0.01 d^{-1} for Zn: Fisher & Baines 2001), and for Se (0.027 to 0.031 d^{-1}) were similar to those in fathead minnow larvae (0.025 d^{-1}: Bennett et al. 1986) but lower than those measured in juvenile striped bass (0.09 d^{-1}: Fisher & Baines 2001). The efflux rate for Cd varied with the route of exposure, and higher k_e was found in fish after dietary exposure. Such differences in efflux rates may be largely attributed to the difference in the metal tissue distributions after exposure. Most Cd was distributed in the viscera following uptake from the dietary phase, whereas Cd was evenly distributed in the gills, viscera and other tissues after the fish were exposed to Cd in the aqueous phase. Similarly, Harrison & Klaverkamp (1989) have reported that Cd taken up by rainbow trout from food was eliminated faster than that taken up from water, whereas in the lake whitefish the efflux rates were comparable between the 2 routes of exposure. Between the aqueous and dietary exposure there was no difference in the elimination rate of Se and Zn, and the distribution of these metals in different body tissues was comparable. In addition to defecation, the gills may play a major role in the excretion of dietary Zn (Hardy et al. 1987).

A kinetic model was used to quantitatively evaluate the relative contribution of dietary and aqueous metals to metal bioaccumulation in fish. Because the ingestion rate can greatly influence the AE and the bioaccumulation of metals in fish, a range of feeding rates was used in the modeling calculation (Garnier-Laplace et al. 2000). The relative importance of different exposure pathways depends on the BCF in the prey organism, the type of prey organism and the ingestion rate of the predatory fish. Using a medium ingestion rate and a medium BCF in the prey organism, the model predicts that >98% of Cd and Zn in the fish are derived from the dietary source when copepods are the prey organisms. Similarly, Willis & Sunda (1984) compared Zn bioaccumulation between a model food chain, algae → zooplankton → fish, and waterborne Zn. They demonstrated that food accounted for 78 to 82% of the total accumulation of Zn by the fish. Milner (1982) also demonstrated that in the plaice *Pleuronectes platessa*, up to 50% of Zn accumulation may be contributed by the aqueous phase. A few studies also demonstrated that dietary intake of Cd and Zn is important for metal accumulation in fish (Spry et al. 1988, Harrison & Klaverkamp

1989, Langston & Spence 1995, Besser et al. 2001). Dietary uptake is important in maintaining the metal tissue levels, especially for essential metals such as Zn. However, the uptake of metals from the dissolved phase cannot be ignored under some conditions. For example, Spry et al. (1988) suggested that aqueous uptake was significant at elevated metal concentration because the fish can drink seawater equivalent to 4 to 13% of their body weight daily. Furthermore, Hardy & Eddy (1990) found that the contribution of Zn from the aqueous phase increased to maintain body burdens if the accumulation of Zn from food was removed.

The TTF is useful in evaluating the magnitude of metal biotransfer in a clearly defined food chain, as well as the contribution of dietary metals to total metal bioaccumulation (Besser et al. 2001). The type of prey was also incorporated in the model of this study because it influences the metal AE, the ingestion rate, the metal concentration in food (reflected in a different BCF) and the trophic position of the fish in the food chain (Willis & Sunda 1984). Trace metals can be considered to be biomagnified when TTF is >1 (or when the metal concentration in the predator is higher than that in the prey: Reinfelder et al. 1998, Wang & Fisher 1999). The biomagnification of CH_3Hg and Cs in aquatic food webs is well recognized in both freshwater and marine ecosystems (Cabana et al. 1994, Hill et al. 1996, Rowan et al. 1998, Zhao et al. 2001). Other metals, such as Cd and Zn, are less likely to be biomagnified because the concentrations of these metals are regulated to low levels in fish. However, our model calculations indicated a potential biomagnification of both Se and Zn in the mangrove snappers from zooplankton or other fish preys, which is probably related to their somewhat high AE and low efflux rate constant. Cd was biodiminished regardless of the ingestion rate and AE. Suedel et al. (1994) have reviewed the trophic transfer of contaminants in aquatic ecosystems and found little evidence of Cd biomagnification, e.g. the trophic transfer coefficients (TTC) in fish generally ranged from 0.1 to 0.9, which is similar to our calculations (TTF is <0.4 for Cd).

The calculated TTC for Se in fish ranges from 0.7 to 1.4 (Suedel et al. 1994), which does not indicate a biomagnification in fish. These values are comparable to our predictions, especially when the ingestion rate is in the medium range (3 to 8% of body dry weight d^{-1}). However, we predict a potential Se biomagnification at a high ingestion rate or when the AE is high. Because Se is primarily accumulated from food, any regulation of the trophic transfer of Se may be manifested in its assimilation process (e.g. different Se contents in food). In contrast to our prediction, the TTC for Zn in marine fish ranges form 0.26 to 0.68 and in freshwater fish from 0.6 to 1.4, indicating that biomagnification in aquatic food chains is unlikely for Zn. One likely reason for the discrepancy between our model calculations and the data from field studies is that fish can regulate Zn body burdens through the induction of elimination pathways, in which the efflux rate would increase with metal concentration (Reinfelder et al. 1998). Our calculation assumes a single and constant value of Zn efflux. Nevertheless, in a recent study Chen et al. (2000) indicated that, in the lake food chain they investigated, Zn in fish is also biomagnified in the top predatory fish, and that Zn in fish is positively correlated with the length of the food chain and the metal level in their prey organisms. In addition, another field study also found a higher Zn concentration in the tissue of rainbow trout than in the gut contents (Dallinger & Kautzky 1985), implying a potential for biomagnification through the absorption of metals from prey in the alimentary tract.

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