ASSIMILATION AND BIOCONCENTRATION OF Ag AND Cd BY THE MARINE BLACK BREAM AFTER WATERBORNE AND DIETARY METAL EXPOSURE

AIMIN LONG and WEN-XIONG WANG*

Department of Biology, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong

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Abstract—We determined the aqueous uptake and dietary assimilation of Cd and Ag by the marine black bream Acanthopagrus schlegeli following one to four weeks’ exposure (or conditioning) to waterborne or dietary Cd or Ag at different concentrations. The concentrations of metals and metallothioneins (MT) in different tissues were determined. There was a great need to elucidate further the physiological bioaccumulation by fish [1]. Mechanistic understanding of the processes controlling metal assimilation in fish is still limited, and there is a great need to elucidate further the physiological and biochemical controls of metal assimilation in marine fish. Once accumulated in the cells, many metals are known to bind with metal-binding proteins, such as metallothioneins (MTs), a family of low molecular weight proteins having exceptional ability to bind with metals in the I-B and II-B groups of the periodic table (e.g., Ag, Cd, Cu, Hg, Zn) [8]. Metallothionein induction has been shown for a number of metals and has been characterized as a part of metal regulation system [9]. In fish, Cd generally is known to bind to MT [10], and a recent study also has suggested that Ag exposure induced MT in the rainbow trout Oncorhynchus mykiss [11].

INTRODUCTION

Marine fish are exposed to metals in both aqueous and dietary phases of their natural environment. There have been several experimental and modeling attempts to separate the relative importance of various uptake pathways (i.e., food versus water) in marine fish [1,2]. Metal assimilation and bioconcentration are critical steps in determining metal exposure from the dietary and aqueous phases. Bioconcentration is the uptake of a chemical by the organisms directly from the abiotic environment (e.g., surrounding water), whereas assimilation is the uptake via biotic environment (e.g., food intake) [3]. Metal accumulation from the diet phase is directly proportional to the assimilation efficiency (AE), defined as the fraction of ingested metals remaining in the tissues after the animals empty their undigested materials from the gut [4]. A few recent studies have quantified metal AEs in fish [1,5–7]. Despite the fact that metal AE typically is lower in fish than in many marine invertebrates, low dissolved uptake rates often result in a predominance of dietary exposure in the overall metal bioaccumulation by fish [1]. Mechanistic understanding of the processes controlling metal assimilation in fish is still limited, and there is a great need to elucidate further the physiological and biochemical controls of metal assimilation in marine fish.

Once accumulated in the cells, many metals are known to bind with metal-binding proteins, such as metallothioneins (MTs), a family of low molecular weight proteins having exceptional ability to bind with metals in the I-B and II-B groups of the periodic table (e.g., Ag, Cd, Cu, Hg, Zn) [8]. Metallothionein induction has been shown for a number of metals and has been characterized as a part of metal regulation system [9]. In fish, Cd generally is known to bind to MT [10], and a
measured concurrently. We separated the dietary and waterborne exposure in our experiments such that each uptake pathway could be manipulated to evaluate the effects of exposure on metal accumulation in fish.

MATERIALS AND METHODS

Fish and metals

The marine black bream A. schlegeli (Bleeker, 1854, 2.0–4.0 cm long) were purchased from a local fish farm in Hong Kong. The fish were maintained in aerated natural seawater (23°C, salinity of 30) and fed frozen shrimp (obtained from a local supermarket) daily. All experiments were carried out in filtered natural seawater (0.2 μm) at the temperature and salinity mentioned above. The metals used in the exposure were Cd (as CdCl₂) and Ag (as AgNO₃), and the biokinetics of Cd and Ag were studied using their respective radiotracer: ¹⁰⁹Cd (t₁/₂ = 462 d, in 0.1 N HCl, from New England Nuclear, Wilmington, DE, USA) and ¹¹⁰mAg (t₁/₂ = 249 d, in 0.1 N HNO₃, from Riso National Laboratory, Roskilde, Denmark). Radioactivity was measured using a Wallac 1480 NaI (T1) gamma spectrometer from Riso National Laboratory, Roskilde, Denmark. Radioactivity was determined at 658 keV and 88 keV, respectively. Counting times were adjusted to yield a propagated counting error <5%.

Metal exposure (conditioning) treatments

Four independent experiments were performed by exposing groups of fish separately to either Ag or Cd through either the waterborne or dietary phase. The exposure treatments and regimes are shown in Table 1. In the waterborne-exposure treatments, the fish were exposed to metal-spiked seawater (100 L) and fed with the unspiked frozen shrimp. In the dietary-exposure experiments, the fish were maintained in clean seawater and fed with metal-enriched Aquarian fish flakes (Walther Aquacentre, Chalfont, PA, USA), which had been soaked in metal-enriched seawater (10 and 100 μg L⁻¹ for Ag, and 100 and 1,000 μg L⁻¹ for Cd) for 1 d. Metal concentrations in the fish flakes, measured by inductively coupled plasma-mass spectroscopy (Perkin-Elmer, Elan 6000, Norwalk, CT, USA) following acid digestion, are shown in Table 1. In the control treatments, fish were maintained in clean seawater and fed with the untreated food. Approximately 10% of the exposure water for all groups was replaced daily, and the feces were siphoned from the tank bottom every 2 d to keep the nominal metal concentrations relatively constant and the water quality in good condition. In all experiments, the assimilation efficiency, dissolve uptake of metals, metal concentrations in different fish tissues, and MTs in fish were measured following the exposure. Due to experimental constraints, there was only one tank for each experimental exposure, and replicated measurements were conducted for different individual fish within each tank. Thus, we only analyzed the trend of metal uptake as a function of metal exposure and MT induction by performing regression analysis.

Metal assimilation in fish after conditioning

Metal AE was determined using an established pulse-chase feeding technique [4]. Prey copepods (Acartia erythrea) were collected from the Clearwater Bay, Hong Kong, and were labeled (500 individuals in 1-L, filtered seawater) with the radioisotopes ¹⁰⁹mAg (111 kBq L⁻¹) and ¹⁰⁹Cd (148 kBq L⁻¹) overnight. After the radiolabeling, the copepods were collected by a mesh, rinsed with seawater, and then fed to the fish for 30 min. Radioactivity in the prey copepods was not quantified before they were fed to the fish. Five individual fishes were in each treatment and the copepods were added every 10 min to maintain a constant prey density. After the radioactive feeding, the radioactivity of each fish was measured immediately. No radioactive feces was produced during the 30-min radioactive feeding period, thus the radioactivity measured in the fish represented the total amount of metals ingested. The fish subsequently were placed in nonradioactive water and allowed to depurate any ingested metals for 48 h, with seawater being changed at 24 h of the depuration period. Any feces produced during the depuration period were removed every 8 h in order to minimize the possibility of radioisotope recycling from the feces into the ambient water. The radioactivity retained in fish was measured over the depuration period at 3- to 12-h intervals. The AE was calculated as the percentage of initial radioactivity (Aₒ) retained in the fish after 48 h of depuration (A₄₈h)

\[
AE = A₄₈h/Aₒ \times 100
\]

Metal uptake from the dissolved phase after conditioning

Metal uptake by the fish was quantified over a relatively short period of exposure (acute uptake). The radioisotopes (17.5 kBq L⁻¹ for ¹¹⁰mAg, corresponding to 1.6 nM, and 5.1 kBq L⁻¹¹⁰⁹Cd, corresponding to 1.0 nM) were spiked into the 0.2-μm filtered seawater for 12 h before the uptake measurements. At time intervals from 2 to 12 h, three or four fish were removed from the radioactive medium and the radioactivity was counted after the fish were rinsed twice (transferred from one beaker to another) with filtered, nonradioactive seawater. The radioactivity in the seawater also was measured at the beginning and at each time interval when the fish were radioassayed. After each radioactivity measurement, each fish was dissected into three fractions: Gills, viscera, and remaining

Table 1. Silver and Cd concentrations used in different exposure (conditioning) experiments. The food (fish flakes) was soaked previously in metal-enriched seawater (10 and 100 μg L⁻¹ for Ag, and 100 and 1,000 μg L⁻¹ for Cd) for 1 d

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Exposure duration</th>
<th>Metal</th>
<th>Dissolved (nominal, μg L⁻¹)</th>
<th>Food (μg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 week</td>
<td>Ag</td>
<td>Control (0.007), 0.2, 2, 10, 20</td>
<td>Control (0.05), 1, 10, 50, 100</td>
</tr>
<tr>
<td>2</td>
<td>1 week</td>
<td>Cd</td>
<td>Control (0.007), 0.2, 2</td>
<td>Control (0.05), 1, 10</td>
</tr>
<tr>
<td>3</td>
<td>4 weeks</td>
<td>Ag</td>
<td>Control (0.007), 0.2, 2</td>
<td>Control (0.05)</td>
</tr>
<tr>
<td>4</td>
<td>1 week</td>
<td>Cd</td>
<td>Control (0.05), 0.3, 5.2</td>
<td>Control (0.5), 3.2, 10.5</td>
</tr>
</tbody>
</table>
tissues. The radioactivity of each fraction subsequently was counted. Finally, the dry weight of each fraction was measured after drying the tissues at 80°C for 1 d. The dry weight concentration factor (DCF, L kg⁻¹) of metals was calculated as the ratio of the radioactivity in the whole fish or in each tissue fraction (cpm kg⁻¹) to the radioactivity in the water (cpm L⁻¹), calculated as the geometric mean before and after the exposure at each time interval. The uptake rate constant for the whole individual fish was calculated as the slope of the linear regression between the DCF and the time of exposure.

**Stable metal tissue concentrations**

Fish from each treatment were dissected, and the gills, viscera, and remaining tissues were dried at 80°C to a constant weight and then digested in concentrated nitric acid (HNO₃, Aristar grade BDH, Poole, UK). These digests were diluted with Nanopure water to make the Ag and Cd concentration in an appropriate range for analysis by inductively coupled plasma-mass spectroscopy. Standard reference material (1566A Oyster tissue, National Institute of Standards and Technology, Gaitherburg, MD, USA) was used throughout the analysis for quality control and assurance. Agreement generally was within 10%. The stable Ag and Cd tissue concentrations were expressed as μg g⁻¹ dry weight.

**MT quantification**

The metallothionein concentrations in the gills, viscera, and remaining tissues of fish were measured using a modified silver saturation method [24]. After the determination of wet weight, each tissue (gills, viscera, and remaining tissues) was homogenized in cold 0.25-M sucrose, and the homogenates were further ultrasonicated. The homogenates were centrifuged at 16,000 g for 20 min to obtain the supernatants, which were incubated with 0.5-M glycine buffer, 20-μg Ag ml⁻¹, and 3.7-kBq ml⁻¹ ¹¹⁰Ag at room temperature for 10 min. After this saturation stage, the excess Ag was removed by rabbit blood cell haemolysate by heating (5 min at 100°C) and centrifugation (5 min at 5,400 g). The supernatant was centrifuged again at 19,000 g for 20 min and the ¹¹⁰Ag left in the final supernatant was radioassayed. The MT concentrations were calculated as 3.55 × the Ag concentrations, and expressed as μg g⁻¹ wet weight.

**RESULTS**

**Experiment 1: Ag conditioning for one week**

After exposure to waterborne Ag at different concentrations for one week, the Ag concentrations in different parts of fish (gills, viscera, and remaining tissues) were higher than the controls (Fig. 1). Similar trends also were observed for the induced MT concentrations in different fish tissues (Fig. 1). For example, the Ag and MT concentrations in the gills at 20 μg Ag L⁻¹ were 43.4 μg g⁻¹ and 4.8 μg g⁻¹, respectively, as compared to 2.6 μg g⁻¹ and 2.4 μg g⁻¹, respectively, of the controls. Among the three different body parts, the viscera had the highest Ag and MT concentrations, and the remaining tissues had the lowest concentrations.

The uptake of Ag and Cd from the aqueous phase by the whole individual fish and different body parts of fish (gills, viscera, and remaining tissues) was approximately linear between 4 and 24 h of exposure (Fig. 2). The calculated DCFs of Ag generally were higher than those of Cd in either the whole fish or different body parts. The DCF increased with increasing Ag conditioning concentration. Among the different body parts, the viscera had the highest DCF and the remaining tissues had the lowest DCF. The uptake rate constant (k_u) for the whole individual fish was calculated as the slope of the linear regression between the DCF and the time of exposure (Table 2). The uptake was quantified for different individual
found in the viscera, and remaining tissues had the lowest values.

Similar trends of Ag and Cd retention in fish following ingestion of radiolabeled copepods were found in this experiment (data not shown). The calculated AEs increased with increasing Cd exposure concentrations, and were higher for Ag than for Cd (Table 2). The AEs of Ag and Cd were 30.1% and 21.4%, respectively, at 100 μg L⁻¹, as compared to 15.0% and 9.9%, respectively, of the controls. In this experiment, the uptake of Ag and Cd from the dissolved phase was quantified over a period of 12 h. The calculated DCFs in fish similarly exhibited a linear pattern over exposure period, and the resulting k_u for each experimental treatment is shown in Table 2. The k_u of both metals increased with increasing Cd exposure concentration. At the highest Cd concentration, the k_u of Ag and Cd was 31.7 L kg⁻¹ d⁻¹ and 7.1 L kg⁻¹ d⁻¹, respectively, as compared to 14.3 L kg⁻¹ d⁻¹ and 2.2 L kg⁻¹ d⁻¹ of the controls. The k_u measured in this experiment was somewhat higher for Ag than that determined in experiment 1 (Ag exposure), whereas the results were comparable for Cd between these two experiments.

**Experiment 3: Cd or Ag conditioning for four weeks**

After exposure to waterborne Cd and Ag at different concentrations for four weeks, their respective concentrations in different tissues (gills, viscera, and remaining tissues) were higher than those in the controls, with a few exceptions noted (Fig. 1). The highest MT and stable metal concentrations were found in the viscera among different fish tissues for all treatments. The AEs of Cd and Ag were quantified in the fish similarly exhibited a linear pattern over exposure period, and the resulting k_u for each experimental treatment is shown in Table 2. The k_u of both metals increased with increasing Cd exposure concentration. At the highest Cd concentration, the k_u of Ag and Cd was 31.7 L kg⁻¹ d⁻¹ and 7.1 L kg⁻¹ d⁻¹, respectively, as compared to 14.3 L kg⁻¹ d⁻¹ and 2.2 L kg⁻¹ d⁻¹ of the controls. The k_u measured in this experiment was somewhat higher for Ag than that determined in experiment 1 (Ag exposure), whereas the results were comparable for Cd between these two experiments.

**Experiment 2: Cd conditioning for one week**

The measured Cd and MT concentrations in different tissue parts, and the calculated AE and k_u, are shown in Figure 1 and Table 2. All the values of these parameters increased with increasing Cd exposure concentrations. Cadmium concentrations in the viscera were most responsive to increasing Cd exposure, and its concentration was 103 μg g⁻¹ at 100 μg L⁻¹ as compared to 1.2 μg g⁻¹ in the controls. Cadmium and MT concentrations in the remaining tissues showed the least increase with Cd exposure concentration among the three different body parts. The highest Cd and MT concentrations were higher generally than those of Ag at each Cd exposure concentration. The AEs of Ag also were higher generally than those of Cd respectively, as compared to 14.8% and 7.5% of the controls.

**Table 2. Calculated uptake rate constants (k_u) and assimilation efficiency (AE) of Ag and Cd in the black breams Acanthopagrus schlegeli following exposure (conditioning) to Ag and Cd in different experiments.**

<table>
<thead>
<tr>
<th></th>
<th>Ag (L kg⁻¹ d⁻¹)</th>
<th>Cd (L kg⁻¹ d⁻¹)</th>
<th>AE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong> (1 week Ag)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.0</td>
<td>2.6</td>
<td>14.8 ± 4.4</td>
</tr>
<tr>
<td>Ag 0.2</td>
<td>10.9</td>
<td>4.2</td>
<td>15.2 ± 1.5</td>
</tr>
<tr>
<td>Ag 2</td>
<td>14.8</td>
<td>4.5</td>
<td>17.1 ± 0.9</td>
</tr>
<tr>
<td>Ag 10</td>
<td>16.8</td>
<td>5.7</td>
<td>26.8 ± 1.6</td>
</tr>
<tr>
<td>Ag 20</td>
<td>22.4</td>
<td>7.5</td>
<td>28.9 ± 2.7</td>
</tr>
<tr>
<td><strong>Experiment 2</strong> (1 week Cd)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14.3</td>
<td>2.2</td>
<td>15.0 ± 1.8</td>
</tr>
<tr>
<td>Cd 1</td>
<td>19.4</td>
<td>3.1</td>
<td>17.9 ± 1.4</td>
</tr>
<tr>
<td>Cd 10</td>
<td>25.6</td>
<td>5.0</td>
<td>20.1 ± 1.4</td>
</tr>
<tr>
<td>Cd 50</td>
<td>28.9</td>
<td>5.8</td>
<td>25.3 ± 2.5</td>
</tr>
<tr>
<td>Cd 100</td>
<td>31.7</td>
<td>7.1</td>
<td>30.1 ± 3.4</td>
</tr>
<tr>
<td><strong>Experiment 3</strong> (4 weeks Ag/Cd)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.3</td>
<td>2.6</td>
<td>20.1 ± 1.4</td>
</tr>
<tr>
<td>Ag 0.2</td>
<td>14.8</td>
<td>4.0</td>
<td>20.5 ± 1.6</td>
</tr>
<tr>
<td>Ag 2</td>
<td>24.4</td>
<td>5.5</td>
<td>21.2 ± 0.7</td>
</tr>
<tr>
<td>Cd 1</td>
<td>12.2</td>
<td>3.7</td>
<td>21.1 ± 1.2</td>
</tr>
<tr>
<td>Cd 10</td>
<td>18.3</td>
<td>5.5</td>
<td>27.8 ± 2.6</td>
</tr>
<tr>
<td><strong>Experiment 4</strong> (1 week diet Ag/Cd)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12.6</td>
<td>2.4</td>
<td>15.4 ± 1.9</td>
</tr>
<tr>
<td>Ag 0.3</td>
<td>15.7</td>
<td>4.4</td>
<td>18.4 ± 6.0</td>
</tr>
<tr>
<td>Ag 5.2</td>
<td>19.4</td>
<td>4.8</td>
<td>22.4 ± 2.0</td>
</tr>
<tr>
<td>Cd 3.2</td>
<td>13.7</td>
<td>2.4</td>
<td>18.3 ± 2.6</td>
</tr>
<tr>
<td>Cd 10.5</td>
<td>16.7</td>
<td>2.5</td>
<td>22.1 ± 7.6</td>
</tr>
</tbody>
</table>

fishes at different time intervals. It was evident that the k_u for both Ag and Cd increased with increasing Ag conditioning concentration. The degrees to which k_u was increased were comparable between Ag and Cd at each Ag exposure concentration.

The retention of both Ag and Cd in the exposed fish after a pulse ingestion of radiolabeled copepods is shown in Figure 3. Following the initial intense digestion, metal depuration leveled off after 48 h, indicating that assimilation was completed within this period. The AEs, therefore, were calculated as the percentage of initial ingested metals remaining in the fish after 48 h (Table 2). An increase was notable in metal AEs for the exposed fish as compared to the controls. The AEs were 28.9% and 23.8% for Ag and Cd at 20 μg Ag L⁻¹, respectively, as compared to 14.8% and 7.5% of the controls. The AEs of Ag also were higher generally than those of Cd in all treatments (Table 2).

**Experiment 2: Cd conditioning for one week**

The measured Cd and MT concentrations in different tissue parts, and the calculated AE and k_u, are shown in Figure 1 and Table 2. All the values of these parameters increased with increasing exposure Cd concentrations. Cadmium concentrations in the viscera were most responsive to increasing Cd exposure, and its concentration was 103 μg g⁻¹ at 100 μg L⁻¹ as compared to 1.2 μg g⁻¹ in the controls. Cadmium and MT concentrations in the remaining tissues showed the least increase with Cd exposure concentration among the three different body parts. The highest Cd and MT concentrations were
Metal uptake by marine fish after exposure

Experiment 4: Dietary Ag or Cd conditioning for one week

Generally, the resulting Ag and Cd concentrations in different tissue parts of the fish increased with increasing Ag and Cd dietary exposure for one week (Fig. 4). In contrast to the other experiments in which the fish were exposed to the waterborne metals, the metal and MT levels in the remaining tissues of fish were most affected. For example, the Ag and MT concentrations in the remaining tissues at 5.2 µg g⁻¹ were 4.0 µg g⁻¹ and 7.1 µg g⁻¹, respectively, as compared to 0.67 µg g⁻¹ and 3.4 µg g⁻¹, respectively, for the controls. In contrast, the increases in the gills and viscera were much smaller. However, the highest metal and MT concentrations also were found in the viscera among the different fish parts.

The quantified AEs of Cd and Ag in exposed fish generally were higher with increasing dietary metal levels (Table 2). The DCFs of both metals were quantified over a period of 8 h (data not shown). The calculated DCFs for both Ag and Cd had a similar trend as AEs, i.e., increasing uptake with increasing exposure concentration (Table 2). However, the $k_u$ for Cd was similar among the different treatments.

Relationships between metal uptake and body concentration and MT concentration

The relationships of the $k_u$ and AE of Ag and Cd with the metal body concentration and the MT concentration in all four independent experiments are indicated in Figures 5 through 7. The metal and MT concentrations in the whole fish body were calculated based on the tissue concentration and MT concentration in each fish tissue (gills, viscera, and remaining tissues). When all four experiments were considered together, the $k_u$...
and AE for both Ag and Cd increased significantly with increasing MT and metal body concentration in the fish. The increases of $k_u$ and AE with increasing Ag body concentrations were linear, whereas both parameters reach a maximum when the Cd body concentrations were $>$5 μg g$^{-1}$.

**DISCUSSION**

The AEs of Cd measured in this study (6–24%) generally were comparable to previous measurements [1,5,6,25,26]. To our knowledge, there has been no report of Ag AEs in marine fish to date. The quantified Ag AEs (15–30%) were higher than the Cd AEs, but were somewhat comparable to Zn AEs measured in previous studies [1]. The low $k_u$ measured in this study (2.2–7.5 L kg$^{-1}$ d$^{-1}$ for Cd and 8.0–32 L kg$^{-1}$ d$^{-1}$ for Ag) presumably was due to the low ventilation rate of the fish. They were, however, comparable to the $k_u$ measured in the mangrove snappers [1]. Xu and Wang [1] also found that $k_u$ was relatively independent of the metal concentration in the exposure medium.

Metal concentrations in fish increased with increasing ambient metal concentration and duration of metal exposure. The quantified metal concentrations in different tissue parts were higher or comparable to those found in field-collected fish. For example, Wood et al. [27] measured the hepatic Ag levels in farmed fingerlings and larger rainbow trout (250–450 g) at approximately 4 to 10 μg g$^{-1}$. Hollis et al. [28] also detected Cd concentrations in the field-collected rainbow trout gall-bladder of approximately 1.4 μg g$^{-1}$. Our study showed a notable difference in the relative distribution of metals in different body parts following waterborne and dietary metal exposure. When exposed to waterborne Cd or Ag (experiments 1–3), the viscera had the highest Cd and Ag concentrations and the remaining tissues the lowest. However, when the fish were fed with metal-enriched food (experiment 4), the concentration in the remaining tissues was higher than that in the gills, and the viscera still had the highest metal concentration.

Kraal et al. [29] also found that the Cd accumulation in the carp *Cyprinus carpio* fed Cd-enriched food was, in decreasing order, gut $>$ muscle $>$ gills, while the order was gut $>$ gill $>$ muscle in fish exposed to metals in water. Similar results have been demonstrated for other fish species such as trout and lake whitefish [2].

Hogstrand and Wood [30] found that the viscera rather than the gills of fish were the primary uptake sites for several waterborne metals, including Cd and Ag. Because marine fish normally drink considerable quantities of water to prevent dehydration by the hyperosmotic environment, the excess Na$^+$ and Cl$^-$ are excluded from the body across the gills. The viscera, especially the intestinal tracts, are important uptake routes for marine fish. This situation is in contrast with that of freshwater fish, which take up metals mainly across the gills [27,31]. However, gill uptake by marine fish still may be an important site for waterborne metals, only second to the viscera. The remaining tissues (mainly muscles and bones) had the lowest concentration, probably due to the fact that they represent the largest fraction of fish body and the metal concentrations in these tissues were correspondingly low.

In our study, MTs were induced significantly by the exposure of both metals from the waterborne and dietary phases, similar to many previous studies [10,11,13]. Exposure to waterborne or dietary metal causes a build-up of metals in the tissues, the increasing intracellular capacity to sequester metals, and replacement of destroyed enzymes [32]. Thus, the exposure of fish to metals brought not only metal accumulation but also physiological effects to the fish itself. Induction of MT may be considered as an adaptive mechanism in defending the metal exposure. In our study, MT concentration increased with increasing metal concentrations in different body parts of fish (gills, viscera, and remaining tissues). It appears that Ag was a more effective inducer of MT than Cd. For example, MT concentration in the viscera was 22.1 μg g$^{-1}$ exposed to 10 μg L$^{-1}$ Ag (experiment 1), as compared to 7.9 μg g$^{-1}$ exposed at the same waterborne Cd concentration (experiment 2).

Metallothioneins can bind the metals accumulated in cells at a relatively stable ratio. For example, 1 mol of MT can bind with 7 mol of Cd normally [13], and 15.4 mol of Ag [24]. In our experiments, when the black breams were exposed to Cd, the highest MT concentration was 42 μg g$^{-1}$ wet weight, and the corresponding tissue Cd concentration was 6.1 μg g$^{-1}$ dry weight (experiment 2). Thus, Cd may have been bound mostly with the induced MT in the fish. When the black breams were exposed to Ag either via food or seawater (experiments 1, 3, and 4), the ratios of MT to Ag were higher than 1:15.4, implying that the induced MT also may bind with the majority of Ag built up in the fish tissues. However, other studies have suggested that Ag also can bind with the sulfide [33].

Corresponding to the metal-distribution pattern among the different tissues of fish, a similar trend in tissue-specific distribution was observed for the MT in our study. Unlike the obvious induction in all body parts when the fish were exposed to waterborne Ag and Cd (experiments 1–3), MT induction was only significant in viscera and remaining tissues when they were exposed to dietary Ag or Cd (experiment 4). When the fish were exposed to Ag-spiked food, the highest MT concentrations in different tissues were 1.2 to 2.0 times of the control fish. Similar relative concentrations of MT and metals in different tissues also verified the important physiological function of MT in metal accumulation and detoxification in marine fish.

Both the AE and $k_u$ of Ag and Cd generally increased following exposure to waterborne or dietary metals. With the accumulation of metals such as Cd and Ag, more metal-binding complexes such as MTs may have been induced, enabling the fish to further accumulate more Ag and Cd from the ambient environments. Ag has a high affinity for Na$^+$/K$^+$ ATPase because of its strong binding to sulphydryl groups, thus resulting in an inhibition of the enzyme. However, Ag binding with MT may abolish its inhibition on the Na$^+$/K$^+$ ATPase, which may explain the apparent absence of toxicity associated with the high internal Ag accumulation. This mechanism also may explain the increased Cd AE and dissolved uptake following Ag exposure. Thus, the internal mobilization of metal-binding proteins such as MT is an important mechanism for the restoration phase through detoxification and storage of metal in tissue [34]. Furthermore, the induced MT in the viscera also resulted in increasing metal accumulation because of seawater drinking.

Similar situations were observed when the black breams were exposed to Cd, which exhibited similar uptake pattern as Ag. When exposed to Ag- and Cd-enriched diets, enhanced metal AE and $k_u$ also may be due to the MT induction in the fish. Therefore, the route of Ag and Cd exposure, via either waterborne or dietary, resulted in difference in Cd and Ag uptake dynamics from the water, and their assimilation from the preys. It also was possible that the increased metal concentration led to the enhanced uptake. Due to the exchange of
metals in the tissues with those in the environments, higher metal tissue concentration may lead to greater uptake.

Besides MT induction, exposure can bring about other physiological effects in fish, which may modify metal uptake from the ambient environments. It has been suggested that gill metal-binding characteristics will undergo significant changes as an adaptive response to sublethal exposure to waterborne or dietary metals [35]. Szebedinszky et al. [36] found an obvious branchial Cd load following waterborne or dietary Cd exposure. Harrison and Klaverkamp [2] also reported Cd accumulation in the gills of rainbow trout after dietary exposure. Both studies showed that Cd-gill binding kinetics changed greatly as a result of chronic sublethal waterborne or dietary exposure, such as a decrease in affinity and an increase in capacity of the gill surface for Cd. Szebedinszky et al. [36] showed a decrease in $\log K_{\text{Cd-gill}}$ from 7.05 (control) to 6.54 in rainbow trout exposed to 2 g L $^{-1}$ of waterborne Cd, whereas the number of binding sites increased from $3.12 \, \text{mmol g}^{-1}$ to $4.80 \, \text{mmol g}^{-1}$ after exposure. Consequently, routes of gill metal-loading, whether dietary or waterborne exposure, resulted in differences in metal uptake dynamics from the water. Once accumulated by the gills, metals are then transferred into blood and distributed via the arterial system throughout the internal organs. This mechanism also may explain the increasing uptake rate and tissue concentration of Cd and Ag following waterborne or dietary exposure observed in our study. However, we did not quantify the gills’ affinity and binding site in the black breams after waterborne or dietary Ag and Cd exposure.

Our study also suggested that the bioaccumulation of Ag was influenced by Cd exposure, and vice versa. Given the degree to which both metals were affected by one metal exposure, it appears that the induced MT by either Cd or Ag exposure also was responsive in binding with the other metals. Thus, the MT may not be specific in binding with metals, but may share a common binding affinity with Cd and Ag. After Cd exposure via either the dissolved or dietary phase, the mole ratio of MT to Cd was higher than 1:7, suggesting that there was still a large amount of MT available for metal binding and bioaccumulation was facilitated. It would be interesting to examine further the isofoms of MT induction by both Cd and Ag exposure in the black breams.

CONCLUSION

In conclusion, this study showed that the black breams accumulated Ag and Cd via both waterborne and dietary pathways. Both the gills and intestine were the main targets for waterborne metal uptake, and the intestine was the main target of dietary metal bioaccumulation. Following exposure to waterborne or dietary Ag and Cd, the assimilation and bioconcentration of these two metals were facilitated, possibly due to MT induction as well as increase in available binding sites in fish gills. A significant linear relationship was found between the AE and $k_0$ of both metals and the Ag and MT body concentrations. Metal uptake also increased with increasing Cd body concentration $>5 \, \mu g \, g^{-1}$. Our study also suggested that the exposure of fish to a specific metal might cause alteration of accumulation of other metals.

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