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Copper bioaccumulation and biokinetic modeling in marine herbivorous fish *Siganus oramin*

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ABSTRACT

Marine herbivorous fish directly consume macroalgae, which commonly accumulate high levels of trace metals in polluted areas. We proposed that herbivorous fish could be better candidates for biomonitoring marine metal pollution than carnivorous fish. To date, the trophic transfer of Cu from macroalgae to marine herbivorous fish is unclear. In this study, the kinetics of Cu bioaccumulation in a widespread marine herbivorous fish, *Siganus oramin,* were investigated, and biokinetic modeling was applied to estimate the Cu levels in the fish sampled from different sites and seasons. The results showed that Cu accumulation in the fish was linearly correlated to the dietary Cu levels in the different prey species, which were proportional to the waterborne Cu concentrations. The Cu found in the subcellular trophically available metal fraction (TAM) in the prey contributed the largest proportion of accumulated Cu in *S. oramin*. The dietary assimilation efficiencies (AEs) of Cu were 15.56 ± 1.76%, 13.42 ± 2.86%, and 21.36 ± 1.47% for *Ulva lactuca, Gracilaria lemaneiformis* and *Gracilaria gigas,* respectively. The calculated waterborne uptake rate constant (k_u) of Cu was 0.023 ± 0.011 $\mathrm{L\,g^{-1}\,d^{-1}}$, and the efflux rate constant (k_e) was 0.055 ± 0.021 $\mathrm{d^{-1}}$. Dietary Cu accounted for 60%–75% of the body Cu in *S. oramin*, suggesting that dietary uptake could be the primary route for Cu bioaccumulation in herbivorous fish. The biokinetic model demonstrated that the Cu concentrations in the water and fish presented a positive linear relationship, which was in line with our field investigation along the coastal areas of South China. Therefore, we suggested that *S. oramin* could be used as a biomonitoring organism for Cu pollution in the marine environment. However, the heterogeneities between the predicted levels and the measured levels of Cu implied that seasonal changes should be taken into account to improve the accuracy of the model.

1. Introduction

The irresponsible activities of humans have led to elevated concentrations of Cu in aquatic ecosystems, and these levels are a threat to aquatic organisms (Pan and Wang, 2012; Cheng et al., 2013). Therefore, it is important to understand the mechanism of Cu bioaccumulation in aquatic organisms.

Over the past few decades, some studies have been conducted on the biokinetics of Cu bioaccumulation in fish. It was reported that the assimilation efficiency (AE) of Cu was 1–11% in *Oncorhynchus mykiss* and *Acanthopagrus schlegeli* (Julshamn et al., 1988; Handy, 1992; Dang et al., 2009) and lower than the AEs of other essential metals such as Zn (Xu and Wang, 2002; Zhang and Wang, 2006; Wang, 2011). The uptake rate constant (k_u) of Cu (0.004–0.006 $\mathrm{L\,g^{-1}\,d^{-1}}$) determined in carnivorous fish (Dang et al., 2009; Tsai et al., 2013) was 10–1900 times lower than the value (0.74–7.84 $\mathrm{L\,g^{-1}\,d^{-1}}$) determined in invertebrates (Pan and Wang, 2009; Croteau et al., 2014). The efflux rate constant (k_e) of Cu in fish has not frequently been studied except for *A. schlegeli*, where it was 0.091 $\mathrm{d^{-1}}$, which is higher than the k_e of other metals (Zhang and Wang, 2006; Luoma and Rainbow, 2008; Pan and Wang, 2009; Croteau et al., 2014). The characteristics of Cu bioaccumulation in fish could be described as having low assimilation and waterborne uptake rates, and high efflux rates. In addition, it was also reported that Cu bioaccumulation in fish was affected by food selection, salinity, dissolved organic matter (DOM), nutrients, and temperature (Dang et al., 2009; Grosell et al., 2007; Gheorghiu et al., 2010; Silva et al., 2014; Lapointe et al., 2011). However, all these studies focused on carnivorous fish. In contrast, Cu bioaccumulation in marine

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herbivorous fish has scarcely been studied, except for the finding of Guo et al. (2016) that waterborne Cu uptake in the gastrointestinal tract of marine herbivorous fish was mainly influenced by the chyme flow in the rabbitfish *Siganus oramin*. It is interesting to investigate Cu bioaccumulation in marine herbivorous fish since they have much longer digestive tracts than carnivorous fish and consume diets containing high Cu (e.g., macroalgae) (Kramer and Bryant, 1995), both of which probably lead to higher Cu uptake.

The biokinetic model has been a useful tool to help understand the kinetic change of metal bioaccumulation (Thomann, 1981). Over the past few decades, the biokinetic model was not only used to understand the accumulation mechanisms of metals (Wang and Rainbow, 2008; Wood et al., 2011b) but also employed to predict metal accumulation in aquatic organisms (Pan and Wang, 2009; Guo et al., 2016). However, to date, for only few fish species has the metal accumulation as a function of the metal concentration in the environment been monitored. The reason was probably because previous studies focused on carnivorous fish, and carnivorous fish are not the ideal organism to model as their complex food sources confound the relationship between the metal levels in fish and the environment (Handy, 1992; Xu and Wang, 2002; Dang et al., 2009; Zhang and Wang, 2006; Wang, 2011). Compared to marine carnivorous fish, marine herbivorous fish feed on different kinds of macroalgae as their main food source. Marine macroalgae are known to have high capacities to accumulate metals, and metal uptake in macroalgae has positive linear relationships with metal levels in the surrounding environment (Kumar et al., 2006; Wang et al., 2014). Therefore, both the waterborne and dietary metal sources for herbivorous fish are dependent on the environmental waterborne metal levels. In addition, waterborne and dietary metal uptake in fish has been known to be linearly correlated to the metal concentration in the water/diet (following first-order kinetics, Zhang and Wang, 2007), indicating there should be a strong relationship between metal levels in the water and those in herbivorous fish.

Therefore, we hypothesize that Cu accumulation in herbivorous fish is probably high and has a strong relationship with the surrounding waterborne Cu level, and we propose that herbivorous fish could be good organisms for monitoring the changes in Cu levels in the marine environment. In the present study, the rabbitfish *S. oramin* was used to explore Cu bioaccumulation in marine herbivorous fish. *S. oramin* is a widely distributed species in eastern and southeastern Asia and is a common and economically viable species in the South China Sea (Chan et al., 2003; Hoey et al., 2013). *S. oramin* is abundant with a wide geographical distribution but a restricted home range, and it is thus representative of local environmental conditions (Fang et al., 2009). It has been reported that *S. oramin* could be used as a model fish species for monitoring PAHs and PCBs because of the finding that the spatiotemporal patterns and potential human health risks of PAHs and PCBs in fish muscle align with those established in the sediments and/or mussels in Victoria Harbour (Fang et al., 2009). However, few studies have used *S. oramin* to monitor metal pollution. In this study, we first determined the assimilation efficiencies (AEs) of Cu in different natural prey species, as well as the uptake rate constant (k_u) and efflux rate constant (k_e) in *S. oramin*. Then, the accuracy of the dynamic model was verified by a comparison between the measured values in the field and predicted values from the different locations and seasons. The overall objectives of our study were to understand the bioaccumulation mechanism of Cu and determine whether *S. oramin* could be used as a model organism to accurately predict the concentration of Cu in the environment.

2. Materials and methods

2.1. Organisms

The green alga *Ulva lactuca* and the red algae *Gracilaria lemaneiformis* and *Gracilaria gigas* are three common, natural prey species of *S. oramin* in the coastal area of South China. *U. lactuca* was collected from Daya Bay, in Shenzhen, China (114°31′E, 22°33′N), and the red algae *G. lemaneiformis* and *G. gigas* were collected from Nan'ao Island, in Shantou, China (117°15′E, 23°29′N). The macroalgae were maintained in a laboratory aquarium at 23 °C ± 1 °C and 33 psu for one week before the experiment began. The seawater was changed every day without the addition of nutrients.

S. oramin (wet weight 10.2 ± 2.0 g fish^{-1}) was collected from Daya Bay, in Shenzhen, China (114°31′ E, 22°33′ N). The fish were divided into three groups and acclimated in 500 L fibreglass cylinders for one week. Each group was kept at 23 ± 1 °C under a 12 h light/12 h dark schedule and fed approximately 4% of their body weight (wet weight) of *U. lactuca*, *G. lemaneiformis*, or *G. gigas,* with half of the water changed every day. The background Cu concentration of the rabbitfish was 2.1 ± 0.5 μg g^{-1} in dry weight. All procedures were approved by the Animal Research Ethics Board of the Chinese Academy of Sciences and were in accordance with the Guidelines of the Chinese Council on Laboratory Animal Care (State Scientific and Technological Commission, 1988).

The cationic composition and chloride ion concentration of seawater were analyzed by ion chromatography (Dionex ICS5000, Sunnyvale, CA, USA). The background dissolved Cu concentration in seawater was measured by ICP-MS (7700X, Agilent Technologies Inc., California, USA). The 0.45 μm filtered seawater had 9.05 ± 0.01 g L^{-1} Na^+, 0.34 ± 0.01 g L^{-1} K^+, 0.39 ± 0.01 g L^{-1} Ca^{2+}, 1.41 ± 0.01 g L^{-1} Mg^{2+}, 17.31 ± 0.02 g L^{-1} Cl^-, and 2.6 ± 0.1 μg L^{-1} Cu.

2.2. Cu and ^{65}Cu labeling of prey

As the Cu levels in macroalgae were reported to be above 0.5 mg g^{-1} in the field (Ho, 1990; Melville and Pulkownik, 2007), the Cu levels in macroalgae (0.30, 0.60, and 1.20 mg g^{-1}) for this study were set to a level to assess the ability of *S. oramin* to bioaccumulate Cu on the condition of Cu contamination. The macroalgae were maintained in 0.45 μm filtered seawater for one day before exposure. Then, they were collected, and their surfaces were cleaned using a cotton swab. To obtain prey macroalgae with the nominal Cu concentrations (control, 0.30, 0.60, and 1.20 mg g^{-1}), *G. lemaneiformis* and *G. gigas* were exposed for 9 days, and *U. lactuca* was exposed for 4 days at a control, 0.15, 0.30, and 0.60 mg L^{-1} Cu in 90 L 0.45 μm filtered seawater according to the preliminary experiments (see supplemental material Table. S1–S2). The seawater was completely replaced daily, and an appropriate volume of Cu stock solution (1 g L^{-1}, $CuSO_4{\cdot}5H_2O$, 99.5%, Guangzhou Chemical Reagent Factory, Guangzhou, China) was added to maintain a constant nominal Cu concentration. At the end of the exposure period, the macroalgae were washed with 0.03 g L^{-1} EDTA and ultrapure water (Millipore) to remove the surface adsorbed Cu and then stored at 4 °C as prey for the fish.

The macroalgae were also directly labeled with waterborne 0.15 mg L^{-1} ^{65}Cu (^{65}Cu: ISOFLEX, Isotopes for Science, Medicine and Industry, USA, San Francisco) as described above for the assimilation assay.

The concentrations of Cu in the prey were quantified by ICP-MS (7700X, Agilent Technologies Inc., California, USA). The concentrations of Cu and ^{65}Cu in the macroalgae are shown in Table 1.

2.3. Dietary Cu exposure in S. oramin

The *S. oramin* were divided into twelve groups to feed on the three prey species (*U. lactuca*, *G. lemaneiformis* and *G. gigas*) with four Cu concentrations. In each group, forty fish were maintained and fed two respective Cu-exposed fresh prey macroalgae for 4 weeks. Fish were fed 2% wet weight twice a day. In this study, fish could eat all the prey in 30 mins. Faeces were collected every two hours, and half of the water was changed every day. At the end of the exposure period, fish were