Environmental Pollution 223 (2017) 31–41

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

The simultaneous uptake of dietary and waterborne Cd in gastrointestinal tracts of marine yellowstripe goby *Mugilogobius chulae*☆

Zhiqiang Guo [a, 1], Na Gao [a, b, 1], Yun Wu [a, c], Li Zhang [a, *]

[a] Key Laboratory of Tropical Marine Bio-resources and Ecology, Guangdong Provincial Key Laboratory of Applied Marine Biology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China
[b] University of the Chinese Academy of Sciences, Beijing 100049, China
[c] Collaborative Innovation Center of Atmospheric Environment and Equipment Technology (CIC-AEET), School of Environmental Science and Engineering, Nanjing University of Information Science & Technology (NUIST), Nanjing 210044, China

ARTICLE INFO

Article history:
Received 3 October 2016
Accepted 4 December 2016
Available online 1 February 2017

Keywords:
Heavy metals
Chyme
Intestine
Stomach
Metal uptake routes
Dual stable isotope tracer

ABSTRACT

Aquatic animals under waterborne metal exposure are also very likely exposed to elevated dietary metals. This study quantified the simultaneous uptake of dietary and waterborne Cd in gastrointestinal tracts (GT) of marine yellowstripe goby using a dual stable isotope tracer method. The Cd spiked diet (10–100 μg g^{-1}, ^{111}Cd as tracers) were fed to the fish as a single meal, and then the fish were exposed to waterborne Cd (0–500 μg L^{-1}, ^{113}Cd as tracers) for 48 h, during which the time-course uptake of Cd in the stomach and intestine was determined. The findings revealed that the dietary Cd uptake mainly occurred within 12 h after feeding. The fish exposed to 500 μg L^{-1} waterborne Cd showed significantly lower Cd assimilation efficiency (2.07%) than the control group (3.48%) at the dietary Cd of 100 μg g^{-1}. Moreover, during 4–12 h when there was chyme in the GT, the waterborne Cd uptake in the intestine was lowest but the stomach showed the highest waterborne Cd uptake rate. The uptake of dietary and waterborne Cd, and the relative importance of dietary *vs* waterborne Cd was positively correlated with the Cd concentration in the chyme. Overall, this research demonstrated that there was interaction between dietary and waterborne Cd uptake in the GT of marine fish. The simultaneous uptake of metal from two routes is far more complex than the situation of a single route of metal uptake, which should be evaluated in determining metal bioaccumulation and toxicity in both laboratory and field metal exposure scenario.

1. Introduction

In the realistic environmental conditions, it is very likely that aquatic animals under waterborne metal exposure are also exposed to elevated dietary metals (Niyogi and Wood, 2003; Wood et al., 2012). Extensive studies have focused the waterborne uptake of metals previously, and dietary metal uptake has been increasingly recognized as the dominant route of metal bioaccumulation in most aquatic animals (Rainbow, 2007; Wang and Rainbow, 2008), while the simultaneous uptake and interaction of metals from the two uptake routes has not been well derived yet. For instance, recent studies have casted important lights on effects of feeding status on waterborne metal uptake in fish (e.g. feeding frequency (Guo et al., 2015), feeding ratio (Hashemi et al., 2008; Guo et al., 2015), feeding *vs* starvation (Wood et al., 2010; Guo et al., 2016a)), which often gets ignored previously. Moreover, the pre-exposure of dietary ions/metals has also been found to have significance on toxicity of waterborne metals (Kamunde et al., 2002a, 2003; Hashemi et al., 2008). Regarding effects of waterborne metals on dietary metal uptake, however, only did Chowdhury et al. (2008) report that a long-term pre-exposure to waterborne nickel down-regulated the gastrointestinal nickel uptake in rainbow trout (*Oncorhynchus mykiss*), and Kamunde et al. (2002a) demonstrated that Cu pre-exposed juvenile rainbow trout decreased uptake rates of waterborne Cu *via* the gills but not of dietary Cu uptake *via*

☆ This paper has been recommended for acceptance by Klaus Kummerer.
* Corresponding author.
E-mail address: zhangli@scsio.ac.cn (L. Zhang).
[1] Both authors contributed equally to this work.

http://dx.doi.org/10.1016/j.envpol.2016.12.007
0269-7491/

gastrointestinal tracts (GT). Those findings obviously yield a limited picture of the simultaneous uptake of metals from both dietary and waterborne in fish.

In contrast to branchial route, the metal uptake from gastrointestinal route is not very well characterized for aquatic animals. In general, the gastrointestinal tracts (GT) uptake metals *via* metal-specific carriers, substitution on nutrient ion transporters and simple diffusion, which are similar with the gills (Shahsavarani et al., 2006; Wood et al., 2012). However, the assimilation of metals in the GT shows some distinct characteristics that are not same as those in the gills. For instance, the metal-specific transporters in the GT usually function at much higher substrate levels and have much lower affinities in relation to the gills (values of the affinity constant are generally in the mmol L^{-1} in the GT rather than μmol L^{-1} range in the gills, Ojo and Wood, 2008). Metals (e.g. Cu, and Zn) in the GT can sometimes bind to amino acids and then can be "piggy-back" transported *via* amino acid transporters as amino acid complexes (e.g. L-histidine, L-cysteine, Glover and Hogstrand, 2002a; Glover and Wood, 2008). Moreover, some essential ion transporters in the GT may function differentially with those in the gills (e.g. the elevated Na^+ in chyme was found to increase Cu uptake in the GT (Nadella et al., 2007), while it inhibited Cu uptake of gills (Grosell and Wood, 2002)). In addition, the gills can often greatly regulate uptake rates at times of dietary metal deficiency or excess, and the gills serve as a dynamic fine-tuning mechanism for metal homeostasis in fish (Kamunde et al., 2002a; b), while metal uptake rates in the GT seem relatively irrelevant to waterborne metal deficiency or excess, though there is very few (if any) direct evidences for the irrelevance.

It is now well acknowledged that ingestion rate (IR) and gut passage time (GPT) of diets are the two important factors determining dietary metal assimilation efficiency (AEs) in aquatic organisms. Consequently, the factors that influence the IR and GPT may have a substantial impact on metal AEs (e.g. ambient temperature (Baines and Fisher, 2008), feeding ratio or frequency (Guo et al., 2015)). Moreover, a growing body of studies has also shown that AEs may also vary with food quantity (e.g. food density/concentration (Zhao et al., 2009)), food quality (e.g. food type/composition (Wang and Wong, 2003), metal concentrations (Guan and Wang, 2004) or subcellular metal distribution in diets (Zhang and Wang, 2006)), animals' body sizes and/or life-history stages (Guo et al., 2016b). There are a few evidences that AEs of metals could be modulated by pre-exposure of dietary metals (e.g. Chowdhury et al., 2004a; Nadella et al., 2007). However, little is currently known about the influences of waterborne metals on AEs of dietary metals.

In seawater fish, the GT is very critical for the uptake of waterborne metals because they requires obligatory drinking of seawater for osmoregulation, and fish GT is thus directly exposed to waterborne metals, in distinct contrast to freshwater fish, which exhibit very little drinking and the gills are the dominant route of waterborne metals uptake (Zhang and Wang, 2007; Guo et al., 2015). Due to the continuous exposure of waterborne metal by drinking in marine fish, the waterborne and dietary metals simultaneously occur in the GT and are highly potential to interact each other in the GT. For instance, the chyme flow and dietary metal assimilation shows a protective effect against waterborne metal uptake in the GT (Wood et al., 2010; Guo et al., 2016a). Several studies have also provided the evidence of significant effects of dietary metals on waterborne metal uptake (e.g. Kamunde et al., 2003; Baldisserotto et al., 2005; Guo et al., 2016a). Moreover, when waterborne metals present in the GT *via* drinking, the digestion and absorption dietary metal would be far more complex than the situation in the fish without water derived metals in the GT (e.g. the pre-exposure to elevated dietary Cd increased Cd uptake rate in the guts (Chowdhury et al., 2004a)). To date, nevertheless, it is uncertain about the presence and magnitude of interaction between the dietary and waterborne metals uptake in marine fish when they are exposed to two routes of metals simultaneously.

In the present study, therefore, we used Cd (a highly toxic and commonly found elevated metal in nature (USEPA, 2001)) and marine yellowstripe goby *M. chulae* (a potential marine model fish (Cai et al., 2015)) as a model system to determine the simultaneous uptake of dietary and waterborne Cd in the GT of marine fish. To differentiate the diet or water derived Cd uptake in the fish tissues, we used a dual stable isotope tracer method (i.e. using ^{111}Cd to trace diet and ^{113}Cd to trace water derived Cd). Specifically, we first determined the time-course flow of chyme, AEs and influx rate of dietary Cd (^{111}Cd as tracers) in the fish with the challenge of a gradient of waterborne Cd exposure (^{113}Cd as tracers) to test the simultaneous uptake of dietary uptake in the GT. Moreover, the time-course waterborne Cd uptake in the fish GT was determined, with the aim of characterizing the simultaneous uptake of waterborne Cd in the GT of the fish after a pulse feeding. The time-course relative importance of Cd from waterborne *vs* dietary sources was also investigated in the fish GT.

2. Materials and methods

2.1. Experimental animals and metals

The juvenile marine yellowstripe goby were purchased from Guangdong Laboratory Animals Monitoring Institute (Guangzhou, China). The fish were cultured in our laboratory 3 weeks for acclimation, during which they were fed an artificial extruded diet (Fujian Tianma Science & Technology, Co., Ltd., Fuzhou, China). The Cd content of the diet was 0.30 ± 0.11 μg g^{-1} in dry matter. In the laboratory culture, water temperature was 21–24 °C. The seawater was aerated to maintain dissolved oxygen >5.5 mg L^{-1}. The concentration of Cd in the seawater was 0.56 ± 0.07 μg L^{-1}. The experiment was subjected to the photoperiod of 12 h light:12 h dark. Fish of a uniform size were then selected for the experiment (mean body weight of 1.45 ± 0.10 g $fish^{-1}$ in wet weight, mean ± SD; SFig. 1A). The initial Cd concentration of the fish was 0.14 ± 0.01 μg g^{-1} in dry weight.

The stable isotope ^{111}Cd and ^{113}Cd (99.8%, International Atomic Energy Agency Office at USA, New York) were used as the tracers for dietary Cd and waterborne Cd, respectively.

2.2. Dietary ^{111}Cd exposure and waterborne ^{113}Cd exposure

The nominal ^{111}Cd concentration in the diet was 10, 30 and 100 μg g^{-1} (a range of Cd concentration widely used in laboratory studies, and also frequently occurs in ambient environments (Spry and Wiener, 1991; McGeer et al., 2003)). The diet was spiked with ^{111}Cd solution (the ratio of the diet to the solution was 1:3 g ml^{-1}). The diet was immersed in the solution for 1 h and then was dried for 48 h at 60 °C. The measured ^{111}Cd concentration in the spiked diet was 9.32 ± 1.05, 27.08 ± 1.84, 95.46 ± 8.91 μg g^{-1}, respectively (STable 1).

Fish were maintained individually in a 100 ml beaker 10 days for acclimation before the Cd exposure. All fish were then deprived food for 48 h and fed with the ^{111}Cd supplemented diet for 1 h. After feeding, the fish immediately transferred to another 100 ml beaker that contained 50 ml ^{113}Cd spiked seawater for waterborne Cd exposure (the seawater was filtered with 0.22 μm polycarbonate membranes, Whatman). The uneaten feed were collected, dried and re-weighed to calculate the ingestion rate, which was 0.032 ± 0.005 g g^{-1} and did not show significant difference among groups (one-way ANOVA, $p > 0.05$).