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Feeding reduces waterborne Cu bioaccumulation in a marine rabbitfish *Siganus oramin*

Zhiqiang Guo [a, c], Wei Zhang [a], Sen Du [a, b], Yanyan Zhou [a, b], Na Gao [a, b], Li Zhang [a, *], Iain Green [c]

[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, PR China*
[b] *University of the Chinese Academy of Sciences, Beijing 100049, PR China*
[c] *Department of Life and Environmental Sciences, Faculty of Science and Technology, Bournemouth University, Fern Barrow, Poole, Dorset, BH12 5BB, UK*

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A B S T R A C T

Waterborne metal uptake has been extensively studied and dietary metal assimilation is increasingly recognized in fish, whilst the interaction between the two uptake routes is largely overlooked. This study compared the waterborne Cu bioaccumulation (^{65}Cu as tracer) in a juvenile rabbitfish at different feeding regimes (starvation (SG), feeding normal diet (NDG) or diet supplemented with extra Cu (DCG)) to test the hypothesis that feeding can influence waterborne metal uptake in marine fish. NDG and DCG diet was fed as a single meal and then all fish were exposed to waterborne ^{65}Cu for 48 h, during which the time course sampling was conducted to determine ^{65}Cu bioaccumulation, chyme flow and dietary Cu assimilation. The results revealed that SG fish accumulated the highest ^{65}Cu, followed by NDG (61% of SG), whilst DCG fish accumulated the lowest ^{65}Cu (34% of SG). These results suggested a protective effect of feeding against waterborne Cu bioaccumulation. This effect was most notable between 10 min and 16 h when there was chyme in gastrointestinal tract (GT). Dietary Cu assimilation mainly occurred before 16 h after feeding. Waterborne ^{65}Cu influx rate in the GT was positively correlated with ^{65}Cu contents of chyme in NDG, whereas it was largely negatively correlated with ^{65}Cu contents of chyme in DCG. The waterborne Cu uptake in the GT was mainly influenced by the chyme flow and dietary Cu assimilation. Overall, our findings suggested that feeding has an important effect on waterborne metal uptake and that both the feeding status of the fish and the relative metal exposure through water and food should be considered in prediction of the metal bioaccumulation and biomonitoring programs.

1. Introduction

Copper (Cu), as an essential micronutrient for all living organisms, is highly toxic when an excessive intake occurs. Cu concentrations in natural environments have been widely elevated by Cu emissions from multiple anthropogenic activities (e.g. mining operations, industries waste discharges, domestic sewages, agricultural pesticides and fertilizers, Sharma et al., 2008; Zhang et al., 2009). Thus, there are serious concerns over Cu contaminations over the word and it is listed as "the metals of major interest in bioavailability and bioaccumulation studies by the US Environmental Protection Agency". Extensive studies have addressed the water quality criteria for Cu (USEPA, 2007) and toxicity of Cu to aquatic animals (e.g. Gomiero and Viarengo, 2014; Côte et al., 2015). The lack of suitable radioactive tracers of Cu, to a certain extent, used to impede the study of Cu uptake and bioaccumulation. Recently, gamma radiotracers (e.g. ^{64}Cu and ^{67}Cu) and stable isotopes tracers (e.g. ^{65}Cu) have been widely used to quantify Cu bioaccumulation in aquatic animals such as cladocerans (e.g. Fan et al., 2011), and bivalves (e.g. Croteau et al., 2004). For fish species, however, the Cu uptake characteristics have only been thoroughly studied in marine black sea bream *Acanthopagrus schlegeli* (Dang et al., 2009), and part of Cu biokinetic parameters have been determined in freshwater rainbow trout *Oncorhynchus mykiss* (Grosell et al., 1997; Kamunde et al., 2002a, 2002b) and

* Corresponding author.
E-mail address: zhangli@scsio.ac.cn (L. Zhang).

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European eel *Anguilla anguilla* (Grosell et al., 1996). Obviously, the quantitative information on Cu bioaccumulation of fish is surprisingly scarce to date.

Aquatic animals are often simultaneously exposed to both waterborne and dietary metals in polluted environments (Luoma and Rainbow, 2005; Wang and Rainbow, 2008). However, a large number of studies investigated the metal bioaccumulation from waterborne and/or dietary routes separately, but paid a little attention to the interaction between the two uptake routes (Rainbow, 2007; Wang and Rainbow, 2008). Moreover, most studies on fish examined the waterborne metal uptake using unfed fish, whereas several studies have found that feeding has a substantial influence on the waterborne metal uptake and toxicity in long-term exposure experiments (Hashemi et al., 2008; Wood et al., 2010), whilst there are a few studies showing that a long-term pre-exposure of dietary ions/metals seems have significant impact the waterborne metal uptake (Baldisserotto et al., 2005; Chowdhury et al., 2008) and toxicity (Chowdhury et al., 2004; Craig et al., 2009) in fish. These results indicated that feeding status (including dietary metal assimilation) is very likely to have important effects on waterborne metal bioaccumulation in fish. Since aquatic animals exposed to waterborne metals are often simultaneously exposed to dietary metals in field situations, information on interactions between waterborne and dietary metals is critical for predicting the metal bioaccumulation and developing biomonitoring programs in field situations. To date, however, there is surprisingly little information describing those interactions.

It is well acknowledged that the two main sites for metal uptake in fish are gills and gastrointestinal tract (GT). The branchial uptake route of dissolved metals has been fairly well characterized as it is directly associated with acute metal toxicity (Campbell, 1995; Di Toro et al., 2001). The bioavailability and bioaccumulation of waterborne metals is strongly dependent on water chemistry such as competing cations, inorganic and/or organic ligands (Niyogi and Wood, 2003; Rainbow, 2007). Recently, the significance of dietary metal exposure has been increasingly recognized and evidence is growing that dietary cations/metals can also substantially influence waterborne metal uptake and toxicity in fish (e.g. dietary sodium, Kamunde et al., 2003; Pyle et al., 2003; dietary iron, Cooper et al., 2006; Craig et al., 2009; dietary calcium, Baldisserotto et al., 2004; Baldisserotto et al., 2005). For instance, Kamunde et al. (2001) reported that rainbow trout *O. mykiss* exposed to dietary Cu (4.68 and 16 μmol g^{-1}) for 28 d showed a significant reduction in branchial Cu uptake compared with those on control diet. In addition, the tolerance to waterborne Cu in rainbow trout was found to increase greatly after long-term dietary Cu exposure (Miller et al., 1993). These studies mainly examined the effects of long-term pre-exposure of dietary ions/metals on waterborne metal uptake (Niyogi and Wood, 2003) and few studies exactly detail the effects of dietary metal assimilation on waterborne metal uptake when fish are exposed to metals from the two routes simultaneously.

Osmoregulation in marine teleost fish requires seawater ingestion and intestinal fluid absorption. Thus the gastrointestinal tract (GT) is extremely important not only for food ingestion and digestion, but also for osmoregulation in hypertonic marine environment (Grosell, 2006, 2011). Due to the continuous exposure of fish to waterborne metal by drinking seawater, the GT of marine fish plays a much more important role in dissolved metal uptake compared with freshwater fish (Zhang and Wang, 2007; Wood et al., 2010). For example, after 5 weeks exposure to waterborne Cd, the GT of juvenile black sea bream (*A. schlegeli*) showed significantly higher Cd bioaccumulation than in the gills of fasted fish, while Cd uptake rate in the GT significantly decreased and showed comparable values with the gill when fish were fed (Guo et al., 2015). In most previous studies, unfed animals were used to examine waterborne metal uptake in marine fish (Zhang and Wang, 2007; Bucking et al., 2011), but when food is present in the GT, metal uptake is far more complex than the situation in the fasted fish, especially via the GT. Moreover, food ingestion and digestion in fishes is often directly associated with ion load in the food (Taylor and Grosell, 2006), changes in sea water drinking rate (Secor, 2009; Wood et al., 2010), and dietary metal exposure via the GT (Taylor and Grosell, 2006; Bucking et al., 2011). There have been number of studies showing that feeding also alters the pH and acid–base balance (Taylor et al., 2007; Bucking et al., 2009) or minor ion concentration (Bucking and Wood, 2009; Bucking et al., 2011) along the GT of marine fish. Consequently, understanding metal uptake in an unnatural fasting state is insufficient to predict the metal toxicity and bioaccumulation in fish dwelling in the natural environment.

Siganids (rabbitfish) are widely distributed in the Indo-Pacific region and *Siganus oramin* (Park) is resident along the coast of the South China Sea (Chan, 1981). Several studies have examined the bioaccumulation of xenobiotics (Fang et al., 2010) and heavy metals (Chan, 1995; Lai et al., 1999) in wild population of *S. oramin*, suggesting that it has great potential as biomonitoring organism for local environmental conditions and thus can be used for marine pollution monitoring (Fang et al., 2009a, 2009b). Using Cu as a model metal, the present study compared the waterborne Cu bioaccumulation of *S. oramin* at different feeding regimes (*i.e.* starvation, feeding normal diet or feeding diet supplemented with extra Cu) to test the general hypothesis that feeding can influence waterborne metal uptake in marine fish. Specifically, we firstly hypothesized that feeding could reduce waterborne Cu bioaccumulation in the examined fish species compared with starvation. Thus, the first objective of the present study was to determine the time course of waterborne Cu influx rate in specific tissues under different feeding regimes. Moreover, it was hypothesized that the Cu uptake in GT is mainly influenced by food digestion and dietary Cu assimilation. To test this, the time course flow of chyme and Cu contents in chyme and the time course of dietary Cu assimilation in GT were determined, with the aim of investigating the potential influence of food digestion and dietary Cu assimilation on waterborne metal uptake in the GT.

2. Materials and methods

2.1. Experimental animals and feeding regimes

In early April 2014, wild juvenile *S. oramin* were collected from a bay in Dongyu Village, Shenzhen, South China (114°31′ E, 22°33′ N). The fish were housed in indoor aquaria for 1 month for acclimation, during which they were fed an artificial extruded diet (Fujian Tianma Science and Technology, Co., Ltd., Fuzhou, China). The proximate composition is shown in Table S1. Fish of a uniform size (body weight 12.5 ± 2.1 g fish^{-1}, mean ± SD; Fig. S1A) were then selected for the experiment. The initial Cu content of the fish was 2.1 ± 0.5 μg g^{-1} in dry matter.

All fish were deprived food for 3 d and then subjected to 3 different feeding regimes (*i.e.* starvation (Starvation Group, SG), feeding normal diet (Normal Diet Group, NDG) or diet supplemented with extra Cu (Diet with Cu Group, DCG)) before waterborne ^{65}Cu exposure (^{65}Cu as tracer). Fifty four fish were randomly selected for each group. In NDG and DCG, the rabbitfish were fed for 1 h to apparent satiety in 200 L aquaria with the water renewal rate of three times per hour. The uneaten feed were collected, dried and re-weighed. The voluntary feed intake was 1.35% body weight for NDG fish and 0.94% body weight for DCG. The SD fish was not fed.