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Effects of Zn pre-exposure on Cd and Zn bioaccumulation and metallothionein levels in two species of marine fish

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Abstract

Zinc is an essential trace metal but also a potential toxicant to aquatic organisms. In this study, two juvenile marine fish species, the black sea bream *Acanthopagrus schlegeli* and the grunt *Teraponjarbua*, were pre-exposed to Zn either from waterborne (0.74–170 $\mu g\,L^{-1}$) or dietary (39–5926 $\mu g\,g^{-1}$) Zn for 1 or 3 weeks. The concentrations of Zn and metallothionein (MT) in the whole body of the black sea bream and in the gills, viscera and carcass of the grunt were then measured during this pre-exposure. Following the pre-exposure, both fish species were then exposed to ^{109}Cd and ^{65}Zn labeled food or water to quantify the dietary assimilation efficiency (AE) and the uptake rate of dissolved Cd and Zn. Zn concentrations in both fish species were enhanced after pre-exposure, but the increases were much less than the increase of ambient Zn pre-exposure concentration. Following Zn pre-exposure, MT concentrations in the viscera and carcass were significantly elevated, whereas the MT levels were not significantly elevated in the gills. Waterborne and dietary Zn exposure enhanced the uptake rates of dissolved Cd and Zn in both fish. The maximum increases of uptake rate constants of dissolved Cd and Zn were up to 1.9–2.8 and 2.1–2.6 times, respectively, in the seabream and grunt. In contrast, dietary assimilation efficiency of Cd and Zn was not significantly enhanced following Zn pre-exposure. A positive linear relationship was found between the uptake rate constants of dissolved metals and Zn or MT concentrations in the fish. The results suggested that Zn pre-exposure increased the potential of metal uptake from ambient water, but had little effect on dietary metal uptake. Furthermore, the Zn body concentration and metal uptake from the dissolved phase were significantly dependent on the fish body size.

Keywords: Zinc; Marine fish; Biokinetics; Metallothionein; Pre-exposure

1. Introduction

Zinc (Zn) is an important micronutrient for aquatic organisms including fish, but can be toxic by interfering with calcium uptake and metabolism when its environmental concentrations are exceedingly high. In fish, Zn is taken up both from waterborne and dietary phases. To waterborne Zn, gill and intestine are the two major uptake sites. The branchial uptake of waterborne Zn in freshwater fish has been well studied due to its direct involvement in acute Zn toxicity in freshwater (Hogstrand and Wood, 1996; Hogstrand et al., 1996).

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Fewer studies have been carried out on intestinal waterborne Zn uptake. Until recently, several studies tried to examine the physiological processes and mechanisms underlying the intestinal Zn uptake using in vivo perfusion technique (Glover and Hogstrand, 2002; Glover et al., 2003). However, waterborne intestinal uptake is important to marine fish, which drink considerable volumes of seawater for osmotic homeostasis (Evans, 1993), thus a large fraction of dissolved Zn may be taken up via this route. On the other hand, previous studies have suggested that dietary Zn is the predominant route by which marine fish accumulate Zn from the environment. For example, Willis and Sunda (1984) showed that food ingestion represented 78–82% of total Zn accumulation by the two species of marine fish *Cambusia affinis* and *Leiostomus xanthurus* fed with ^{65}Zn labeled natural naupli of *Artemia*. Xu and Wang (2002), using the biokinetic model, similarly showed that the dietary uptake of Zn dominates its accumulation in the mangrove snapper *Lutjanus argentimaculatus* when zooplankton including copepods and *Artemia* were the main prey. In freshwater rainbow trout, dietary intestinal uptake has also been shown to be more important for Zn accumulation than the branchial route (Spry et al., 1988).

In polluted environments, fish are continuously exposed to ambient Zn in both the water and the food. Many recent studies of rainbow trout have indicated that changes in metal uptake and physiological processes occur after Zn exposure (reviewed in Clearwater et al., 2002; Niyogi and Wood, 2003). For example, after chronic waterborne Zn exposure, the gill Zn binding site density increases whereas gill binding affinity decreases (Alsop et al., 1999; Alsop and Wood, 1999). In contrast, after chronic dietary Zn exposure, the maximum rate of branchial Zn uptake increases only slightly, although the transport affinity decreases as well. Moreover, the best known intracellular Zn binding ligands, namely, the metallothioneins (MTs), which are a family of low-molecular weight, cysteine-rich proteins having high affinity for metals, are induced by waterborne Zn exposure in rainbow trout and marine flatfish (Bradley et al., 1985; George et al., 1992).

Despite these previous studies, the effects of Zn exposure on the bioavailability of metals, especially from the dietary phase, are not exactly known. Most previous work has used the rainbow trout as a model (freshwater) fish, with only sporadic studies considering marine fish. The influence of Zn pre-exposure on metal assimilation from dietary source has not yet been studied. In a recent study, Long and Wang (2005a) examined the influence of Cd and Ag pre-exposure on their dietary assimilation and uptake from the dissolved phase by a marine teleost (the black sea bream *Acanthopagrus schlegeli*). Their study indicated that both assimilation and uptake of Cd and Ag increased with higher MT concentration and accumulated metal concentration in the whole body. Whether the response to Zn is similar to that of Cd and Ag remains to be further investigated.

The primary objective of this study was to investigate the effects of Zn pre-exposure on the bioavailability of divalent Cd and Zn in marine fish. The juvenile black sea bream *A. schlegeli* and the grunt *Terapon jarbua* were used as the model species in our study. The two species were exposed to either aqueous (0.74–170 μg L^{-1}) or dietary (39–5926 μg g^{-1}) Zn for one and three week periods. The uptake rate constant (k_u) and the dietary assimilation efficiency (AE) of both Cd and Zn were measured, as well as the accumulated Zn body burden and MT concentration. The k_u quantifies the rate of metal uptake from the water, and may be closely coupled with the ventilation rate of the fish. The AE, defined as the fraction of ingested dietary metals remaining in the body after the fish have empted undigested materials from the gut (Wang and Fisher, 1999), quantifies the absorption of metal from food. The Zn body burden reflects the bioaccumulation of Zn in fish exposed to elevated ambient Zn. The MT concentration reflects the biochemical reaction to the enhanced inner Zn condition.

2. Materials and methods

2.1. Fish and metals

Two common species of marine fish, the black sea bream *A. schlegeli* (1–3 cm in length) and the grunt *T. jarbua* (4–5 cm in length) were cultured in a fish farm at Yung Shu Au, Hong Kong after catching from nature coastal environment. After being transported to the laboratory, the fish were acclimated in aerated natural seawater (20 °C, 30 psu) and fed minced shrimp (from a local supermarket) at about 5% of their body weight daily in a 14 h light:10 h dark photoperiod