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Developmental patterns of copper bioaccumulation in a marine fish model *Oryzias melastigma*

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ABSTRACT

Allometry is known to be an important factor influencing metal bioaccumulation in animals. However, it is not clear whether effects are due to body size *per se* or changes in physiological traits during the animals' development. We therefore investigated the biokinetics of copper (Cu) and predicted Cu bioaccumulation during the development of a fish model, the marine medaka. The results revealed that the waterborne Cu uptake rate constant decreased and dietary Cu assimilation efficiency increased during development from larvae to adults. Thus, the allometric dependency of the biokinetic parameters in juveniles and adults can not be simply extrapolated to the whole life cycle. The body Cu concentration in the fish was predicted by the biokinetic model, which showed a rapid increase in the larval stage, followed by a slight increase from juveniles to adults, and then a relatively stable plateau in the post-adult stage. Dietary Cu uptake became more important as fish developed from larvae to juveniles, but became less important from juveniles to adults. These findings suggested that the developmental patterns of metal bioaccumulation are driven by an integrated biological/physiological shift through animals' ontogeny rather than a simple allometric dependent change. The developmental changes of metal uptake should be considered in ecological bioassessment and biomonitoring programs.

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1. Introduction

The life of an organism usually consists of a sequence of ontogenetic steps, each with a significant shift in biological and physiological traits. The variations of these traits may have great significance in determining the bioaccumulation (Hare, 1992; Luoma and Rainbow, 2005) and toxicity of contaminants (Grosell et al., 2007; Weltje et al., 2013). In aquatic animals, for instance, the bioaccumulation of metals is often highly complicated by ontogeny, including size changes (Zhong et al., 2013; Poteat and Buchwalter, 2014), growth (Zhang and Wang, 2007; Ward et al., 2010), reproduction (Conley et al., 2014), diet quantity (Guo et al., 2015a,b) or quality (Kraemer et al., 2012; Ponton and Hare, 2015), other physiological factors (e.g. pigment contents in insects (Hare, 1992) and condition factor (Mubiana et al., 2006; McIntyre and Beauchamp, 2007)).

The body size/weight of animals, for instance, often changes greatly during the ontogeny, and it is the most frequent biological factor taken into account when investigating metal uptake in aquatic taxa (e.g. insects (Hare, 1992), bivalves (Wang and Fisher, 1997a,b; Mubiana et al., 2006), and fish (McIntyre and Beauchamp, 2007; Zhang and Wang, 2007)). There is often a negative correlation between waterborne metal uptake rate and body size in aquatic animals such as insects (Hare, 1992), amphipods (Wang and Zauke, 2004), bivalves (Wang and Fisher, 1997a,b) as well as fish (Newman and Mitz, 1988; Zhang and Wang, 2007; Dang and Wang, 2012; Zhong et al., 2013). Dietary metal assimilation (AEs) is also found to be size-related in some cases. For example, Zhang and Wang (2007) found AEs of the essential metals Se and Zn were positively correlated with fish body size, but the AEs of non-essential metal Cd was found to be independent of body size in their study. Body size might also influence the metal efflux rate (e.g. a decrease in metal efflux rate with increasing body size; Newman and Mitz, 1988; Zhao et al., 2009), although metal efflux was suggested to be relatively stable within species (Wang and Fisher, 1997a,b). Moreover, numerous attempts have been made to illustrate the age- and size-specific patterns of heavy metal bioaccumulation in natural environments,

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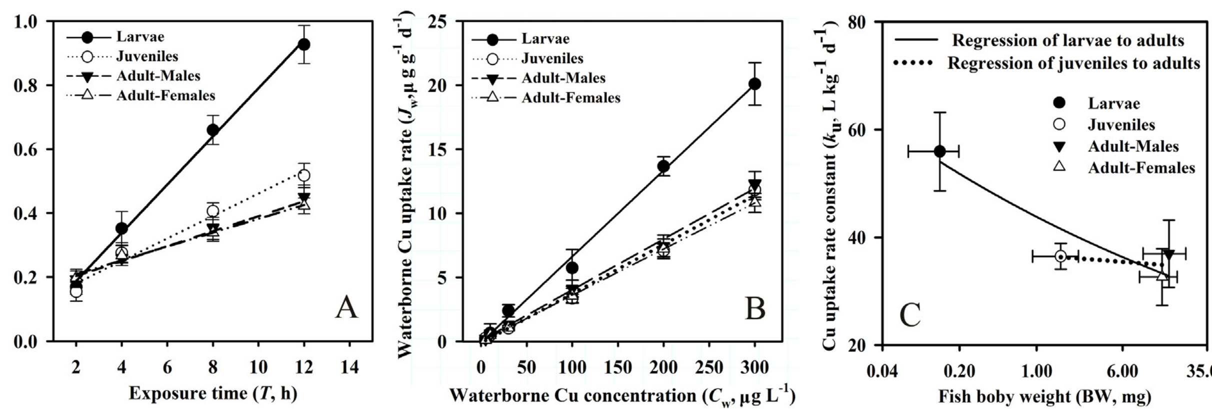

Fig. 1. The regression of the newly bioaccumulated Cu concentrations (C) with exposure time (T, panel A), the waterborne Cu uptake rate (J_w) with waterborne Cu concentration (C_w, panel B), and the uptake rate constant (k_u) with the fish body weight (BW, panel C) in the marine medaka. Values of each point are means ± standard deviations ($n = 6$ in the panel A and panel B, and $n = 24$ in the panel C). In panel C, $k_u = 43.80\text{BW}^{-0.10}$ from the larva to adult stage ($r^2 = 0.88$, $p = 0.03$), and $k_u = 36.69\text{BW}^{-0.02}$ from the juvenile to adult stage ($r^2 = 0.13$, $p = 0.76$).

and the findings generally suggest that species- and metal-specific size-related metal bioaccumulation are due to the variations in field situation (Mubiana et al., 2006; McIntyre and Beauchamp, 2007; Ward et al., 2010).

However, in previous studies the effects of body size (or body weight) on metal bioaccumulation were often treated as a "black box". Consequently, it was not clear whether the effects were due to body size *per se*, or due to shifts in other biological/physiological traits during the animals' growth and development. The key biological/physiological shifts, however, are often saltatory during development (e.g. the changes from endogenous feeding to exogenous feeding, becoming mature and spawning activities (Belanger et al., 2010)), and cannot be fully interpreted by allometry. Given this fact, the allometric dependency of metal bioaccumulation may be valid within a given ontogenetic stage (e.g. juveniles; Zhang and Wang, 2007; Dang and Wang, 2012) when animals' biological/physiological status is relatively stable, and this yields a limited picture of metal bioaccumulation for an animals' whole life cycle. For instance, it is uncertain whether the allometric dependency from a single ontogenetic stage could be extrapolated to the whole life cycle, and could fully describe the developmental changes in metal bioaccumulation through the animals' life cycle. Therefore, further understanding of the developmental dynamics of metal uptake is critical in predicting the bioaccumulation and toxicity of trace metals in aquatic organisms.

Many fish species have been used as models in ecotoxicology research, environmental risk assessment and bioassay (Van der Oost et al., 2003). A single surrogate development stage is pervasively used to represent the entire life history of the tested fish species (Van der Oost et al., 2003; Belanger et al., 2010). Fish, however, often display a wide range of developmental ontogeny and have distinctive ontogenetic stages (egg, embryo, larva, juvenile, adult, and senescence) in their life cycles. The range of body sizes between conspecific individuals can usually span several orders of magnitude during the development (Van der Oost et al., 2003; Belanger et al., 2010). Consequently, fish size/weight is a key factor influencing metal bioavailability and bioaccumulation in both laboratory (Zhang and Wang, 2007; Dang and Wang, 2012), and field studies (Ward et al., 2010; Kraemer et al., 2012; Ponton and Hare, 2015). Nevertheless, the size-related bioaccumulation of trace metals is often species-, metal- and/or site- specific, suggesting weight/size is not a good predictor for metal bioaccumulation during fish development (Ponton and Hare, 2015).

Biokinetic modelling has been applied to explore the mechanisms underlying the observed size-specific bioaccumulation of metals. It has shown that body size could drive the differences in metal biokinetic parameters, including dissolved metal uptake (e.g. Zhong et al., 2013; Wang and Fisher, 1997a,b), dietary metal assimilation (e.g. Zhang and Wang, 2007; Dang and Wang, 2012), and metal efflux rate (e.g. Newman and Mitz, 1988; Zhang and Wang, 2007). For fish species, however, few studies have focused on the allometric dependence of metal biokinetic parameters in the juvenile stage (Zhang and Wang, 2007; Zhong et al., 2013). These studies, at least, imply that the biokinetic model is a useful tool in predicting metal bioaccumulation during fish development (Wang and Rainbow, 2008).

Recently, the marine medaka (*Oryzias melastigma*) has been strongly proposed as a new model fish for marine and estuarine ecotoxicology studies (Kong et al., 2008; Bo et al., 2011), yet a few studies have investigated the ecotoxicology of trace metals on this species (Wang et al., 2013). In the present study, therefore, the stable isotope ^{65}Cu was used as a tracer (Croteau et al., 2004) to quantify Cu uptake biokinetics in this species at three distinctive developmental stages (larvae, juveniles and adults) with the general aim to explore the developmental patterns of trace metal bioaccumulation in fish. Specifically, the waterborne Cu uptake, dietary Cu assimilation and body Cu efflux were quantified and compared among the three developmental stages. Furthermore, we evaluated the extrapolation of the allometric dependency in juveniles and adults to the whole life cycle since most previous study focused on the allometric dependency of metal bioaccumulation at juvenile and/or adult stage of fish. Finally, the developmental changes in Cu bioaccumulation, and the relative importance of Cu from waterborne vs dietary sources were predicted. Such information contributes to the understanding of the developmental changes in trace metal bioaccumulation and toxicity in fish, and has significance in environmental risk assessment and bioassay of trace metal contamination.

2. Methods and materials

2.1. Experimental fish and metals

The marine medaka has been cultured in our laboratory for more than 2 years, which represents 4 generations. The fish used in this study were at 3 development stages, including larvae (10 days post-hatching (dph)), juveniles (40 dph), and adults (120 dph). The individual body weight (BW) was 0.15 ± 0.01, 1.34 ± 0.17, 13.32 ± 2.3 and 14.74 ± 2.01 mg (mean ± SD) in dry weight (dw) for the larvae, juveniles, adults males and adult females, respectively.

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