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RESEARCH ARTICLE

Toxicokinetics of tilapia following high exposure to waterborne and dietary copper and implications for coping mechanisms

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Abstract One of the major challenges in assessing the potential metal stress to aquatic organisms is explicitly predicting the internal dose in target organs. We aimed to understand the main sources of copper (Cu) accumulation in target organs of tilapia (*Oreochromis mossambicus*) and to investigate how the fish alter the process of Cu uptake, depuration, and accumulation (toxicokinetics (TK)) under prolonged conditions. We measured the temporal Cu profiles in selected organs after single and combined exposure to waterborne and dietary Cu for 14 days. Quantitative relations between different sources and levels of Cu, duration of treatment, and organ-specific Cu concentrations were established using TK modeling approaches. We show that water was the main source of Cu in the gills (>94 %), liver (>89 %), and alimentary canal (>86 %); the major source of Cu in the muscle (>51 %) was food. Cu uptake and depuration in tilapia organs were mediated under prolonged exposure conditions. In general, the uptake rate, depuration rate, and net bioaccumulation ability in all selected organs decreased with increasing waterborne Cu levels and duration of exposure. Muscle played a key role in accounting for the rapid Cu accumulation in the first period after exposure. Conversely, the liver acted as a terminal Cu storage site when exposure was extended. The TK processes of Cu in tilapia were highly changed under higher exposure conditions. The commonly used bioaccumulation model might lead to overestimations of the internal metal concentration with the basic assumption of constant TK processes.

Keywords Copper · Coping mechanism · Ecotoxicology · Tilapia · Toxicokinetics

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Introduction

One of the major challenges in assessing the potential heavy metal stress to aquatic organisms is explicitly predicting the internal active dose in specific organs (Escher and Hermens 2004; Green et al. 2010). Aquatic organisms are subject to temporal fluctuations of bulk contaminants as well as waterborne biogeochemical processes that can result in time-variant exposure scenarios. Using the chemical concentration in specific organs or tissues was considered as a surrogate of chemical dose in target site which provides a better linkage between environmental metal bioavailability and the induced toxic effects. For example, the biotic ligand (BLM) model is a tissue-concentration approach that uses the metal level in the gills to predict the metal toxicity (Janssen et al. 2003; Tsai et al. 2009). Although the BLM addresses the effect of water chemistry on metal bioavailability; however, BLM does not consider if the critical body level is independent of exposure condition and durations.

Physiological acclimation and active toxicokinetic (TK) mediation might be developed to overcome potential

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chemical stresses, especially during prolonged exposure to essential metals under sublethal concentrations (Grosell et al. 1997; Adams et al. 2003; Rainbow 2002). Several studies have supported the notion that after previous metal exposure acclimations, fish either decreases (Kamunde et al. 2002a) or remains unchanged (Dang et al. 2009; Grosell et al. 1996). Accordingly, using the TK parameters (i.e., rates of uptake and depuration) estimated from a short-term and single-concentration invariant treatment in laboratories to extrapolate the metal concentration in aquatic organisms might be very different from the real chemical stress encountered by wild populations. Thus, an improved quantitative understanding of the changes in TK processes of chemicals is critical to assess the exposure risk for indigenous species populations (Kraemer et al. 2005).

The tilapia *Oreochromis mossambicus* is a food fish for the people of Taiwan. It is also one of the most abundant invasive species in local freshwater and estuary ecosystems. Copper (Cu) is an essential element in aquatic ecosystems as well as a nondegradable and cumulative pollutant that exerts a wide range of pathologic effects on fish (Iger et al. 1994). Cu sulfate is commonly applied to aquaculture ponds in Taiwan to eradicate filamentous algae as well as blue-green algae. In 2001, Environmental Protection Administration (EPA) Taiwan determined that the total Cu concentration in fish farm water ranged from 63 to 120 $\mu g L^{-1}$, some of which far exceeded the permitted maximum Cu level for aquaculture water (30 $\mu g L^{-1}$) (Environmental Protection Administration, ROC (Taiwan) 2001). A significant increase in Cu levels in pond water could severely affect the health of farmed tilapia and even lead to health problems for the people who consume it.

Metal toxicity to aquatic organisms has previously been found to relate to organ-specific TK, especially in chronic exposure circumstances (Kraemer et al. 2008). However, recent studies have rarely focused on the TK processes leading to the responses observed. While some studies have assessed the histopathological changes upon Cu exposure in juvenile tilapia (Wu et al. 2008), these studies were descriptive, and they lacked quantitative and mechanistic descriptions linking the actual uptake and depuration processes in specific tilapia organs. These are necessary for achieving an integrative interpretation of the chemical toxicity, especially in prolonged exposure scenarios. Farmed fish are exposed to heavy metals through both ambient water and food simultaneously; however, there is a lack of agreement among researchers regarding the relative importance of aqueous and dietary sources contributing to the metal concentration in aquatic organisms because of differences in experimental designs. For example, some studies have used artificial, metal-spiked feeds (Kamunde et al. 2002a; Dang et al. 2012a), while other researchers have assessed the importance of dietary metal by using contaminated live prey (Erickson et al. 2010; Kraemer et al. 2008; Dang et al. 2012b) as the foodborne exposure route. The relative fraction of soluble metal within feed (or prey) might also account for these inconsistencies, because soluble metals tend to be more bioavailable to the consumer than those binding with insoluble fractions (Wallace et al. 1998).

The purpose of this study was threefold: (1) to determine the relative contribution of waterborne and dietary Cu for organ-specific Cu burdens in farmed tilapia under environmentally relevant exposure conditions, (2) to investigate the relationship between the time course of exposure duration, waterborne Cu concentration, and TK of organ-specific Cu, and (3) to implicate the TK coping mechanisms that mediate long-term Cu accumulation.

Materials and methods

Bioaccumulation bioassays

Mature male tilapia (*O. mossambicus*) aged 4–5 months (mean body length=13.9±1.7 cm (mean±SD) and mean weight=20.2±7.3 g wet weight (WW)) were hatched in our laboratory. The fish were allowed to acclimate in tap water at least 14 days before the beginning of the exposure tests. Two exposure settings were assigned to investigate the Cu accumulation kinetics in fish organs from dietary routes only and with combined exposure of tilapia to dietary and waterborne Cu simultaneously. Cu stock solutions were prepared by dissolving a calculated amount of $CuSO_4 \cdot H_2O$ (Shimada Chemical Works, Japan) in double-deionized water. The stock solutions were diluted to the nominal concentrations with local tap water. Tap water conditions were 60.09±5.45 $\mu g mL^{-1}$ of Ca^{2+}, 15.36±0.6 $\mu g mL^{-1}$ of Mg^{2+}, 10.28±0.62 $\mu g mL^{-1}$ of Na^{+}, 3.45±0.55 $\mu g mL^{-1}$ of K^{+}, and 10.09±6.56 $\mu g L^{-1}$ of Fe^{2+}, pH=7.3±1.6, mean±SD, n=6). Tap water was aerated to drive off chlorine for 2 days before use. All experiments were carried out in a 63-L indoor rectangular glass tank with circulated, aerated tap water at 26–28 °C with a photoperiod of 12 h and an intensity of 1,400±100 lux.

To investigate the Cu accumulation kinetics form the dietary source only, fish were reared in uncontaminated tap water for 14 days and were fed with Cu-spiked commercial fish food at a rate of 4 % (dry weight (DW)/WW) of total fish biomass every day. The Cu concentration of commercial fish diet (Tong-Bau Co., Taiwan) was 13.5±0.51 $\mu g g^{-1}$ DW. A 50 % experimental water was replaced every 1–2 days, and uneaten food and feces were siphoned from the aquaria twice daily to avoid the degression of ambient water quality and to avoid potential Cu leaching from contaminated food and feces. The whole water was replaced weekly in each tank. In a second series of bioassays, tilapia were exposed to Cu concentrations of 0, 100, 200, 300, and 600 $\mu g L^{-1}$ for 14 days. Every 2 days,

50 % of the experimental water was replaced to minimize the metal loss in the exposure mediums and regression of water quality. The entire Cu solution was replaced weekly in each tank. The average background waterborne Cu level in the control group was 0.011±0.004 $\mu g mL^{-1}$, and the mean measured Cu concentrations were 100.3±2.3, 197.5±4.6, 306.4±8.3, and 588±13.7 $\mu g L^{-1}$ (n=6). The variance in Cu concentration was less than 5 % within 48 h. All the bioassays were repeated two times, and each concentration was assigned to two replicate tanks. For each dose of Cu, ten tilapia were exposed.

Fish were fed by following the protocol of dietary exposure assays. Both the food consumption rate (in percent) and body weight (in grams) of fish in different treatments were recorded on a daily basis. Three fish were sequentially harvested from solutions after 0, 0.5, 1, 3, 7, 10, and 14 days of exposure in these two types of bioassays. The fish were rinsed with deionized water and then anesthetized in pH-neutralized tricaine methanesulfonate (MS-222; Sigma Chemical Co., St. Louis, MO) solution. The fish were weighed and then killed by spinal dislocation and dissected. The gills, alimentary canal, muscle, and liver were collected for metal analyses. Fish samples were freeze-dried overnight and then ground to a fine powder in a grinder (Tai-Hsiang S36-89, Taiwan). A 500-mg portion of the powder was digested in 10-mL nitric acid (65 % HNO_3, Merck, Germany) overnight at room temperature. The digested solution was redissolved in 0.2 % HNO_3 for quantification of Cu content.

A Perkin-Elmer Model AA-200 atomic absorption spectrophotometer (Perkin-Elmer, Shelton, CT) was used to quantify the total Cu concentration in samples. Analytical quality control was achieved by digesting and analyzing identical amounts of rehydrated (90 % H_2O) standard reference material (Dogfish muscle, DORM-2, NRC-CNRC, Canada). During the experiments, Cu concentrations in each test solution were measured three times weekly in one replicate aquarium. The 10-mL water samples were acidified (pH<1) with 5-mL 1 N HNO_3 and then stored at −4 °C in the dark until they were analyzed. The recovery rate was 94.6±3.6 %, and the levels of detection were 20 $\mu g\ CuL^{-1}$ for water samples and 20 $\mu g\ Cug^{-1}$ for tissue samples.

Data analysis

A prediction model assumes that metals from various routes additively contribute to total internal concentration, which permits a process-based description of the chemical concentrations in the tissues or organs of organisms as a function of exposure time and dosing level (Cooper et al. 2010; Newman and Unger 2003).

$$\frac{dC(t)}{dt} = k_{uw}C_w + k_{uf}C_f - [(k_{ew} + k_{ef})C(t)], \quad (1)$$

where $C(t)$ is the time-dependent Cu concentration in the selected organ (in micrograms per gram DW); k_{uw} and k_{uf} are organ-specific uptake rate constants for Cu uptake from water (in liters per gram per day) and food (in micrograms per gram per day DW), respectively; k_{ew} and k_{ef} are the depuration rate constants (per day) of metal from water and diet sources, respectively; t is the time in days; C_w is the waterborne Cu concentration (in micrograms per liter), and C_f is the Cu concentration in the tilapia food (in micrograms per gram).

TK parameters were quantified by fitting the integrated form of bioaccumulation models (Eq. (1)) to measured organ-specific metal-accumulation profiles by using iterative nonlinear regression (Cooper et al. 2010; Newman and Unger 2003):

$$C(t) = C(0)e^{-(k_{ew}+k_{ef})t} + \left[\frac{(k_{uw}C_w) + (k_{uf}C_f)}{(k_{ew} + k_{ef})}\right] \times \left(1 - e^{-(k_{ew}+k_{ef})t}\right), \quad (2)$$

where $C(0)$ is the initial concentration of the metal in the organ prior to the trial. Generally, for the combined uptake-depuration rate constant-based formulation, TK parameters can be estimated simultaneously from a linear phase (Dang et al. 2009; Kraemer et al. 2008) or from the period that organisms are allowed to accumulate until internal metal concentration approaches the practical steady-state concentration of experiments (i.e., 95 % steady-state concentration or 70 % time period for the steady-state concentration) (Newman and Unger 2003). The TK parameters estimated from linear phase represent the maximum ability of organism to uptake, depurate, and accumulate the chemical without subjected to saturation kinetics at very high concentrations or longer exposure duration. Conversely, those that are estimated from the entire steady-state chemical accumulation curves quantifying the asymptotic process of chemical kinetics at higher concentrations or longer durations.

The dietary TK parameters (k_{uf}, k_{ef}, and bioaccumulation factor (BAF_f)) were firstly determined by fitting Eq. (2) to data collected from dietary exposures by assuming C_w to be 0 $\mu g L^{-1}$ and the rates of metal uptake and depuration from water (i.e., k_{uw} and k_{ew}) could be ignored. Concentration-dependent waterborne TK parameters (k_{uw}, k_{ew}, and bioconcentration factor (BCF)) were estimated from the observed data of the second set of bioassays (i.e., combined exposure to dietary and waterborne Cu) with the input of dietary TK parameters. Organ-specific BCF or BAF_f can be calculated as follows: $\mathrm{BCF\ (or\ BAF_f)} = (k_{uw}\ \mathrm{or}\ k_{uf})/(k_{ew}\ \mathrm{or}\ k_{ef})$, representing the net accumulation ability that is the result of the

competition between uptake, depuration from waterborne or foodborne sources (Muscatello and Liber 2010; Newman and Unger 2003). The relative importance of waterborne and dietary Cu for organ concentrations can be calculated as the ratio of $(k_{uw} \times C_w)/(k_{uw} \times C_w + k_{uf} \times C_f)$ and $(k_{uf} \times C_f)/(k_{uw} \times C_w + k_{uf} \times C_f)$, respectively (Cooper et al. 2010).

We employed a steady-state bioaccumulation model to assess how the tilapia coped with the Cu burdens through inter-organ exchanges. We assumed that all Cu depurated from the gills, alimentary canal, and muscle moved into the circulatory system and finally ended up in the liver. Thus, we regarded the Cu loss from the three organs as inputs to the liver. Accordingly, the predicted steady-state Cu concentration in the liver could be predicted as follows (Kraemer et al. 2008):

$$C_{ss}^{liver}(t) = \frac{\left(k_{ew}^{gill} \times C_{ss}^{gill}\right) + \left(k_{ew}^{AC} \times C_{ss}^{AC}\right) + \left[\left(k_{ef}^{MS} + k_{ew}^{MS}\right) \times C_{ss}^{MS}\right]}{\left[k_{ew}^{gill} + k_{ew}^{AC} + \left(k_{ef}^{MS} + k_{ew}^{MS}\right)\right]}, \quad (3)$$

where C_{ss}^{liver}, C_{ss}^{gill}, C_{ss}^{AC}, and C_{ss}^{MS} are the steady-state Cu concentrations in the liver, gills, alimentary canal, and muscle, respectively. Values of organ-specific steady-state Cu concentrations can be revealed by using a nonlinear regression model developed for corresponding measured data (Kraemer et al. 2008).

A standard analysis of variance test (one- or two-way ANOVA) was used to determine the significance of differences in the Cu accumulation profile datasets between the treatment and control groups. A Student's *t* test was carried out to compare metal concentrations across sampling times (corresponding to day 0 of the bioassay). We employed the nonlinear option of the Statistica® software program (StatSoft, Tulsa, OK) to perform all curve fittings and to calculate the coefficient of determination (r^2) and perform statistical analyses (ANOVA and Student's *t* test). Results were considered statistically significant when *p* values were less than 0.05.

Results

Dietary Cu accumulation and main source of Cu accumulation

For the tilapia exposed to dietary Cu, the highest Cu concentrations were measured in the liver, followed by the alimentary canal, gills, and muscle (Fig. 1). The organ-specific dietary uptake rate constants (k_{uf}) were between 0.07 and 3.36 $\mu g\,g^{-1}\,day^{-1}$. The highest k_{uf} occurred in the liver, followed by the muscle, alimentary canal, and gills. The depuration rate constants (k_{ef}) ranged from 0.12 to 1.75 day^{-1}, revealing that muscle had the best depuration ability, followed by the alimentary canal, liver, and gills. Organ-specific BAF_f values ranged from 0.53 to 19.31, indicating that only the liver and alimentary canal had the potential to accumulate dietary Cu (Table 1). Overall, waterborne Cu was the main source of Cu burdens in the gills (>94 %), alimentary canal (>86 %), and liver (>89 %); the major source of Cu in the muscle (>51 %) was food (Table 2).

Organ-specific waterborne Cu TK

The maximum level of Cu concentrations from combined exposure was observed in the liver, followed by the alimentary canal, gills, and muscle (Fig. 2). Cu concentrations quickly built up during the first few days of exposure and then reached steady-state concentrations or showed a decreasing trend thereafter until the end of the bioassays (Fig. 2). This showed that the tilapia were able to decrease Cu burdens when the accumulated Cu exceeded a certain level in the gills, alimentary canal, and muscle. Substantial differences were apparent in TK profiles of Cu in the liver, which increased predictably with time at each concentration, and no reductions in Cu content were observed throughout the experiments (Fig. 2m–p). This indicated that the TK mediation for Cu in the liver was relatively inactive compared with that in the muscle, gills, and alimentary canal.

Figure 2 also reveals the comparison between the fitting curves of the bioaccumulation model to the linear phases of the Cu profiles and their predictions for the entire exposure

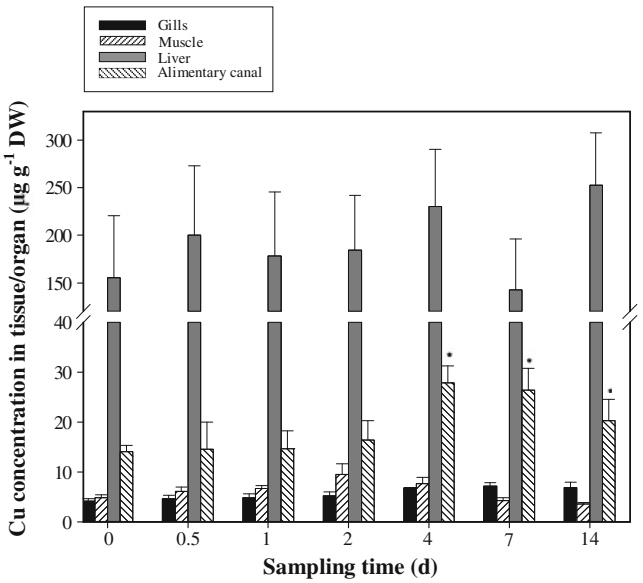

Fig. 1 Time series of Cu concentrations (mean±SD, n=6) observed in tilapia organs during the 14-day dietary Cu exposures. *Asterisks* indicate significant difference of Cu concentrations in organs relative to the concentration at the beginning of bioassays

Table 1 Estimated values (mean±SE) of dietary Cu uptake rate (k_{uf}), elimination rate (k_{ef}), and bioaccumulation factor (BAF_f) in selected tissues or organs of tilapia exposed to Cu-spiked food (13.5±0.51 μg g^{-1}) for 14 days

Organ	k_{uf} ($\mu g\,g^{-1}\,day^{-1}$)	K_{ef} (day^{-1})	BAF_f ($\mu g\,g^{-1}$)[a]	r^2*
Gills	0.07 ± 0.04	0.12± 0.08	0.58	0.92*
Muscle	0.94± 1.25	1.75± 2.41	0.53	0.81
Liver	3.36± 2.41	0.17± 0.13	19.31	0.76
Alimentary canal	0.46± 0.48	0.25± 0.29	1.84	0.65

*$p<0.05$

[a] Organ-specific BAF_f from food can be calculated as $BAF_f = k_{uf}/k_{ef}$

duration in the selected tissues/organs. For each organ, the model fit the linear phases of the Cu profiles well (r^2=0.67 to 0.99) (Table 3). On the contrary, despite the fact that the model fit the entire liver data well, the model predictions deviated from the experimental data when Cu concentrations exceeded a certain level in the gills, alimentary canal, and muscle (Fig. 2a–l). From the linear uptake phases, values of organ-specific k_{uw} ranged between 0.0009 and 7.123 $L\,g^{-1}\,day^{-1}$. The highest k_{uw} occurred in the liver, followed by the alimentary canal, gills, and muscle tissue (Table 3).

The BCF values of the target organs were all above 1 (ranging from 13 to 7,385) (Table 3), showing their potential to accumulate waterborne Cu. The liver characterized with the highest BCF value (ranging from 1,520 to 7,385). The muscle had the lowest ability to accumulate waterborne Cu (BCF=13–152). Results showed that the k_{uw}, k_{ew}, and BCF of fish tissues/organs were dependent upon the exposure concentration and exposure duration. In general, values of k_{uw}, k_{ew}, and BCF decreased with increases in waterborne metal concentration (Table 3). The stronger decrease k_{uw} than reduction of k_{ew} might account for the decrease in BCF at higher exposure conditions. Otherwise, changes in the Cu TK process were observed with respect to the extension of exposure durations. The increase in Cu depuration ability might be responsible for the decrease in their ability to accumulate waterborne Cu (BCF) with time. No general pattern was observed in the change in k_{uw} of organs.

The relatively higher r^2 values of model fits in the liver also suggested that the liver Cu concentrations were only slightly mediated by changing TK processes at near sublethal levels of exposure (e.g., 600 $\mu g L^{-1}$) (Table 3; Fig. 2m–p). This indicated that the uptake rate and depurate rate constants for the liver were relatively insusceptible to changes in liver Cu concentration. The results revealed that the assumption of constant TK processes could lead to incorrect predictions by overestimating the Cu concentrations in most tissues/organs of tilapia under prolonged exposure scenarios.

TK coping mechanisms

The total Cu accumulation in the entire lumped fish sample increased as the waterborne Cu concentration increased (Fig. 3). In terms of Cu burden, at the end of the 14-day combined-exposure bioassays, over 46.2–56.8 % of the total Cu in tilapia was recorded in the liver, followed by the alimentary canal (13.4–32.0 %) and muscle (14.6–26.7 %) (Fig. 3). The amount of Cu continued to increase in the liver; however, the time trend of Cu burdens decreased in the muscle and fluctuated in the alimentary canal and muscle with the progress of duration, suggesting that the liver played an important role in harvesting internal Cu with the increase of exposure time. However, the importance of muscle decreased with time. Results indicated that the muscle tissues played a key role in depositing the rapid Cu accumulation during the very first period after exposure; alternatively, the liver acted as a long-term Cu storage site under chronic exposure. Although the proportions of Cu fluctuated in the gills and alimentary canal, this variation does not seem related to the treatment levels or exposure durations, which suggests that these two organs acted as metal transporters during the TK process of Cu.

We assessed the capability of the liver to accommodate Cu from other organs when exposed for 14 days to high levels of Cu (600 $\mu g L^{-1}$). With the input of the steady-state Cu concentration in the gills (C_{ss}^{gill}, 41.1 $\mu g g^{-1}$) (Fig. 2d), alimentary canal (C_{ss}^{AC}, 116.8 $\mu g g^{-1}$) (Fig. 2l), muscle (C_{ss}^{MS}, 4.3 $\mu g g^{-1}$) (Fig. 2h), and organ-specific depuration rates (k_e) estimated from the 14-day exposures (Table 3) into

Table 2 Relative contribution (in percent) of water and food as sources of Cu residues for selected organs of tilapia exposed to various waterborne Cu concentrations for 14 days

Organ	100 $\mu g L^{-1}$		200 $\mu g L^{-1}$		300 $\mu g L^{-1}$		600 $\mu g L^{-1}$	
	Water	Food[a]	Water	Food[a]	Water	Food[a]	Water	Food[a]
Gills	95	5	97	3	94	6	98	2
Muscle	49	51	80	20	45	55	40	60
Liver	40	60	89	11	97	3	94	6
Alimentary canal	95	5	94	6	86	14	98	2

[a]Cu concentration in food is 13.5±0.51 $\mu g g^{-1}$ DW

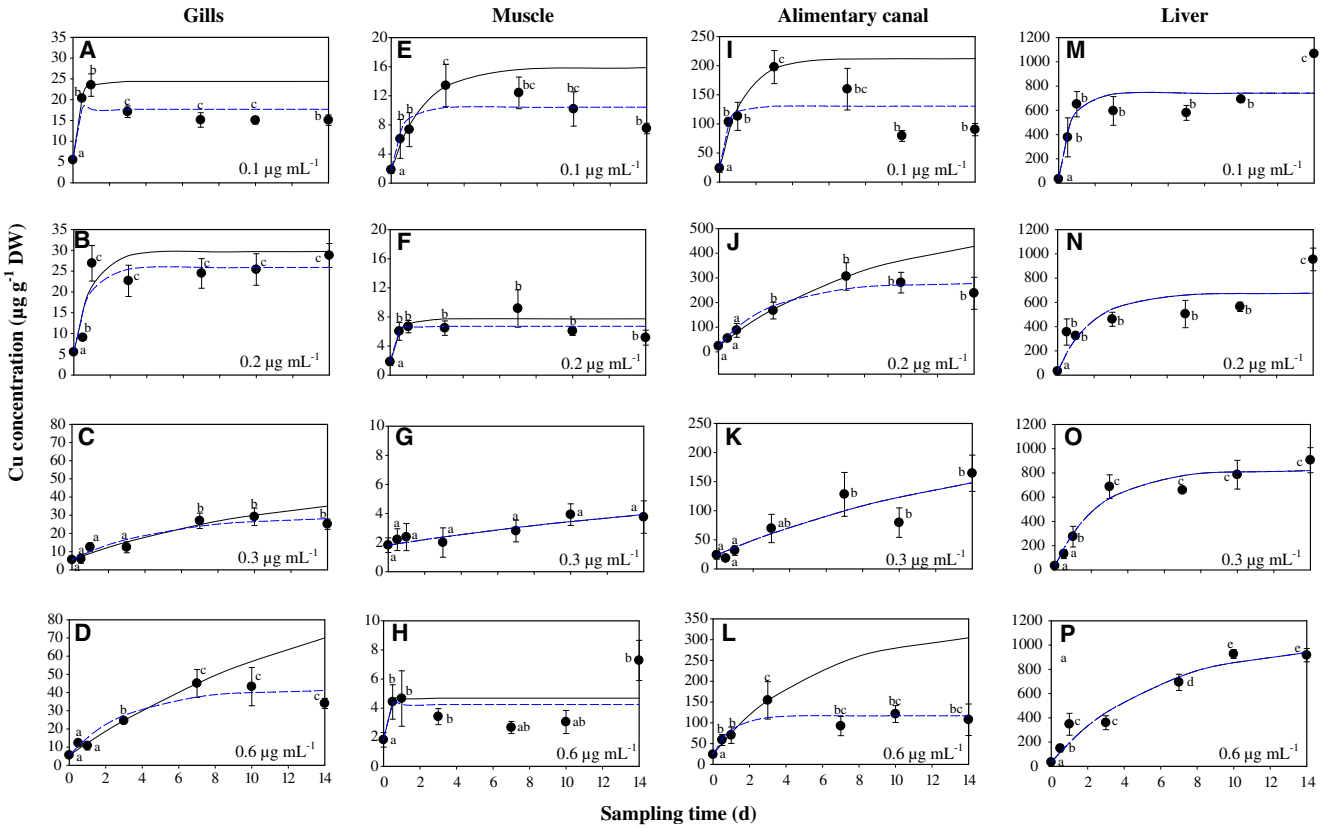

Fig. 2 Time series of Cu accumulation (mean±SD, n=6) in the gills (**a**–**d**), muscle (**e**–**h**), alimentary canal (**i**–**l**), and liver (**m**–**p**) of tilapia exposed to selected waterborne Cu concentrations of 100, 200, 300, and 600 μgL^{-1} for 14 days. *Fitted lines* show the comparison between the TK model predictions for the linear phase (*solid lines*) and for the entire exposure duration (*dashed lines*). Results with different letters are statistically different from each other (ANOVA; Tukey's HSD, p< 0.05)

Eq. (3), the estimated steady-state liver Cu concentration was calculated as 14.9 μgg^{-1} DW, which is around 53 times lower than the measured value (938.6 μgg^{-1} DW). This result implies that the increase in liver Cu burden could account for all of the Cu lost from the gills, alimentary canal, and muscle.

Discussion

Changes in TK rates during chronic exposures

Our study revealed that the TK process of Cu was highly concentration and time dependent, suggesting that Cu burdens were under physiological controls. As the experimental Cu-spiked solutions were renewed regularly to maintain the assigned Cu levels, the decrease in Cu concentrations in fish organs could be attributed to physiological homeostasis processes rather than a decrease in the waterborne Cu level or the degrading of bioavailable fractions of free Cu ions in the water.

Although there is currently no comparable study addressing how tilapia adjust to Cu accumulation in circumstances analogous to those in our study, studies investigated Cu regulation in tissues or organs of freshwater teleosts that exposed to similar Cu levels with this study supports our findings. Kamunde et al. (2002b) showed that Cu-pre-exposure juvenile rainbow trout displayed significantly lower uptake rates of waterborne Cu than the controls after 14 days exposure to environmentally realistic waterborne Cu concentrations (i.e., 22 μgL^{-1}). Carriquiriborde and Ronco (2008) indicated that the value of BCF in the gills and liver of pejerrey (*Odontesthes bonariensis*) showed an inverse relationship with waterborne Cu concentration when the fish were exposed to 50 and 100 μgL^{-1} Cu for 16 days. Similar results were also observed in selected organs of European eel (Grosell et al. 1996). However, quantitative models to depict the relationship between the rates of uptake and depuration, exposure concentration, and exposure duration were absent in these studies.

We found that two interface organs (i.e., the gills and alimentary canal) and muscle tissue eliminated the internal Cu compared with the metal storage organ (i.e., the liver). Our findings were similar to those of Carriquiriborde and Ronco (2008) and Wu et al. (2008), which revealed a rapid loading of Cu into these two organs, followed by a partial clearance and

Table 3 Comparison between estimated values (mean±SE) of waterborne Cu uptake rate (k_{uw}), depuration rate (k_{ew}), and bioconcentration factor (BCF) in selected organs of tilapia in various waterborne treatment levels for the entire 14-day exposure duration and all linear accumulation phases

Organ/treatment ($\mu g L^{-1}$)	Linear phase[c]					Entire exposure			
	Fitted days	k_{uw} ($L g^{-1} day^{-1}$)	k_{ew} (day^{-1})	BCF[a] ($L g^{-1}$)	r^2*, **, ***	k_{uw} ($L g^{-1} day^{-1}$)	k_{ew} (day^{-1})	BCF[a] ($L g^{-1}$)	r^2*, **, ***
Gill									
100	0–1	0.746±0.0007	0.0031±0.0003	241	0.99***	0.177±4.998	0.0271±3.681	7	0.67
200	0–1	0.158±0.0087	0.0011±0.0007	144	0.80*	0.168±0.078	0.0013±0.0006	129	0.80
300	0–7	0.014±0.0054	0.0001±0.0008	140	0.94*	0.021±0.007	0.0002±0.0001	105	0.91**
600	0–7	0.012±0.0025	0.00006±0.00005	200	0.99**	0.022±0.007	0.0002±0.0001	110	0.91**
Muscle									
100	0–3	0.091±0.015	0.0006±0.0001	152	0.99*	0.133±0.066	0.0012±0.0006	111	0.76
200	0–1	0.264±0.272	0.0039±0.0042	67	0.86	0.083±0.040	0.0022±0.0012	38	0.69
300	0–1	0.0009±0.0008	0.00004±0.1	23	0.84	0.0009±0.0008	0.00004±0.00009	23	0.84
600	0–1	0.004±0.0029	0.0003±0.0002	13	0.97**	0.0373±0.0007	0.0048±0.00001	8	0.49
Liver									
100	0–14	7.123±0.843	0.0013±0.0009	5,479	0.73**	7.123±0.843	0.0013±0.0009	5,479	0.73**
200	0–14	2.954±0.447	0.0004±0.0003	7,385	0.67*	2.954±0.447	0.0004±0.0003	7,385	0.67*
300	0–14	1.124±0.257	0.0004±0.0001	2,810	0.95**	1.124±0.257	0.0004±0.0001	2,810	0.95**
600	0–14	0.304±0.071	0.0002±0.00006	1,520	0.95**	0.304±0.071	0.0002±0.00006	1,520	0.95**
Alimentary canal									
100	0–3	2.091±0.204	0.0012±0.0004	1,742	0.92*	1.279±0.204	0.0021±0.00076	609	0.49
200	0–7	0.309±0.021	0.0001±0.00002	3,090	0.99**	0.473±0.128	0.0003±0.00001	1,573	0.94**
300	0–14	0.050±0.033	0.00006±0.0001	833	0.83	0.050±0.033	0.0001±0.00006	836	0.83
600	0–3	0.101±0.021	0.0002±0.0001	505	0.99*	0.185±0.019	0.0008±0.0005	231	0.73**

*$p<0.05$; **$p<0.01$; ***$p<0.001$

[a] Organ-specific bioconcentration factor (BCF) can be calculated as $BCF = k_{uw}/k_{ew}(L\ g^{-1})$

[b] The linear phase is defined as the Cu profile from the beginning of exposure (day 0) to the highest data point when the accumulative Cu is statistically judged as steady-state status

the Cu concentration reaching lower steady-state levels. This suggested that fish might be able to accelerate Cu depuration from the gills and alimentary canal either by transferring Cu to other organs or by excreting Cu to the external environment when Cu accumulation exceeds a critical level, resulting in fluctuating trajectories of Cu concentrations.

We found that the alimentary canal accumulated appreciable amounts of Cu from aqueous exposure (Table 2) and accounted for a large portion of the Cu deposition in the whole body (from 13.4 to 32.0 % of the total accumulated Cu) (Fig. 3). Because freshwater fish do not drink a great deal and the contribution of the diet uptake route was minor in this study (Table 2), the Cu concentrations in the alimentary canal should mainly have been derived from an aqueous source via gill respirations and/or ion exchanges and internal circulation. A similar, albeit less distinct, pattern was also observed when tilapia were exposed to 100 $\mu g L^{-1}$ Cu (Pelgrom et al. 1995). High Cu accumulation in the alimentary canal might be a specific mechanism for tilapia, because, in general, about 95 % of the whole-body Cu was allocated to the liver for other exposed fish species (Pelgrom et al. 1995). The sufficient accumulation and high depuration or transportation rate via the alimentary canal might explain the higher tolerance of tilapia to metal toxicity compared with other species.

Transition of Cu between organs

We illustrated the temporal trend of Cu distribution between organs in different treatments in order to understand how the tilapia mediated the Cu burdens. The total Cu burden increased with the extension of the exposure duration. The relative amount of total Cu persistently increased in the liver. In contrast, Cu in the muscle, gills, and alimentary canal tended to decrease or plateau, suggesting that the change in TK processes in tilapia included accelerating the movement of the gill and muscle Cu into the liver rather than just suppressing the Cu uptake or accelerating the depuration across the apical epithelial membrane into the external environment.

Overall, the organ-specific Cu distribution profiles revealed two main strategies of Cu control in tilapia. Firstly, although the Cu concentration in muscle tissue is relative low compared with other organs (about 10 to 30 times lower than the alimentary canal and liver, respectively), around 50 % of the total Cu burden accumulated in the muscle in the initial

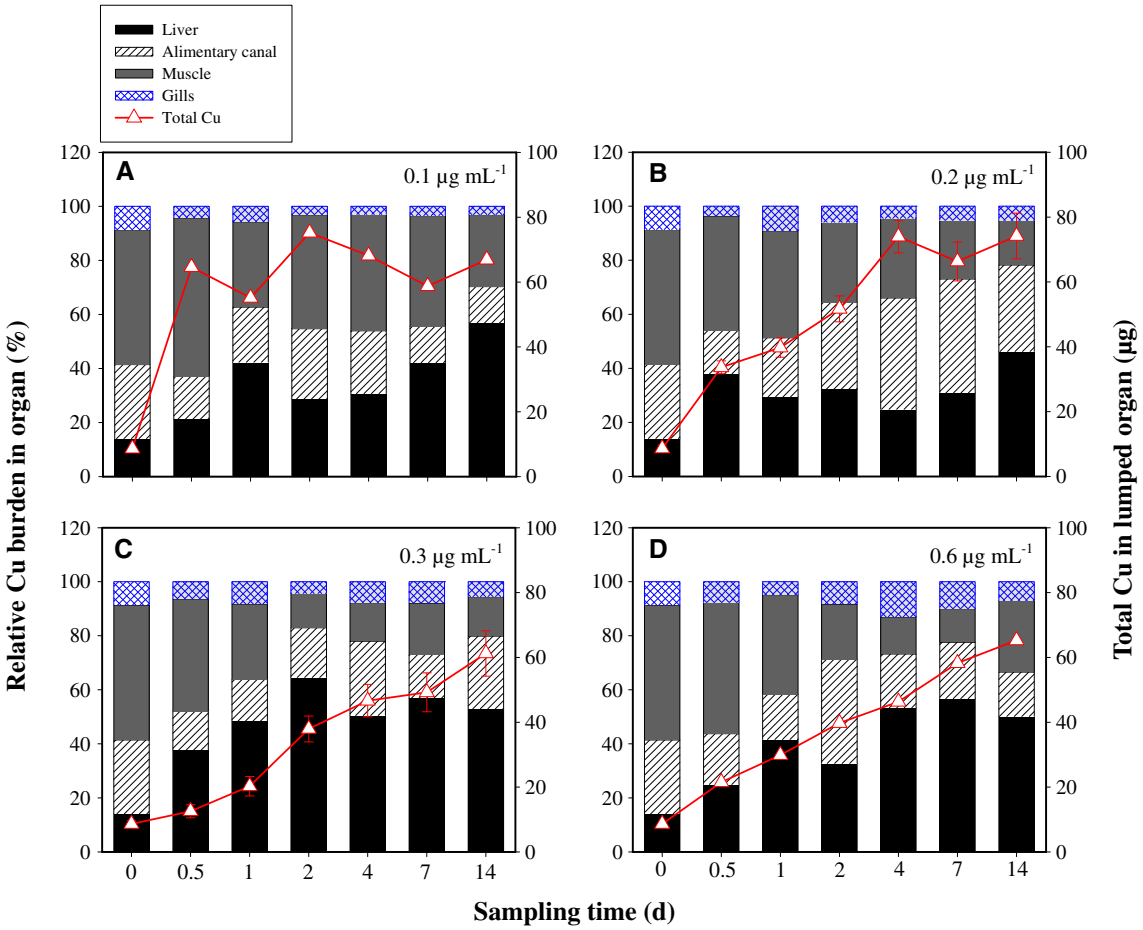

Fig. 3 Temporal trends of relative Cu burdens (in percent) observed in organs of tilapia and the corresponding total Cu in all lumped organs during the 14-day exposure periods in various waterborne Cu treatments of **a** 100, **b** 200, **c** 300, and **d** 600 $\mu g L^{-1}$

period of exposure, because it accounted for over 80 % of the total fish biomass. The proportion of Cu in muscle rapidly decreased with the extension of the duration in each treatment. This suggested that muscle tissue plays a key role in short-term Cu storage. In other words, muscle tissue serves as a buffer or surge protector. Secondly, unlike muscle, which works as a short-term metal buffer, the liver prevents Cu toxicity under extended exposure.

To our knowledge, this is the first study addressing the internal transition of Cu across the time course and treatments between tilapia organs. Our data showed a trend similar to that found in a study on Ag accumulation in two freshwater teleosts (rainbow trout and European eel) through water (Hogstrand et al. 2003), and Cu accumulation in a marine fish (blackhead seabream) after waterborne and dietary exposures (Dang et al. 2012a). The contribution of the liver Ag increased from 25 % after 1 day to 70–80 % after 67 days of exposure, because fish have more time to redistribute newly accumulated Cu from muscle tissues to the liver or alimentary canal for excretion to minimize toxic effects (Hogstrand et al. 2003). Cu contents in seabream liver and alimentary canal accounted for more than 70 % of total Cu burden in fish after 4 weeks of exposure, which indicates the more important role of liver than the gills and muscle during Cu accumulation irrespective of exposure routes (Dang et al. 2012a).

Model application to organ-specific bioaccumulation

Our results showed that the TK model tended to overestimate the Cu concentrations in the gills, muscle, and alimentary canals—most notably under extended exposure periods. Because the TK model does not currently consider possible TK regulations of fish (i.e., changes in the values of k_u, k_e, and BCF). Poor modeling for metal accumulation in freshwater fish was also observed in other studies; for example, obvious changes in Cd accumulation processes in wild yellow perch (*Perca flavescens*) (Kraemer et al. 2008). Yellow perch reduced the Cd concentration in the gills and guts once the metal concentrations reached a certain level in these organs. Similar to our case, the good fit between the modeled and observed data was only observed in liver metal concentrations. Bioaccumulation models generally

overestimated steady-state Cd concentrations, because *P. flavescens* were able to reduce metal accumulation by altering their ability to take up and/or depurate through the gills and guts. These studies support the notion that it is questionable whether the classic TK model explicitly illustrates metal concentrations, especially under prolonged exposure.

The extrapolation of metal concentrations between different treatments with a constant TK rate estimated from a single treatment is also questionable, because we found that the uptake and elimination rate constants showed dependence not only on exposure duration, but also on waterborne metal concentration. We found that the uptake rate constants for waterborne Cu (k_{uw}) and the BCF decreased with increases in waterborne metal concentration (Table 3). McGeer et al. (2003) found that BCF data for Cu, Zn, Cd, Pb, and other metals show inverse relationship between BCF and aqueous exposure by comprehensively reviewing theoretical and experimental data. Luoma and Rainbow (2005) suggested that the assumption of a constant uptake rate might only be acceptable in natural conditions or up to concentrations that are an order of magnitude higher than those seen in nature. The uptake rate could be subjected to saturation kinetics at very high concentrations because most of the metal ions pass through the gill membranes via channels or other facilitated diffusion processes (Green et al. 2010; Newman and Unger 2003). In our experiments, Cu concentrations were set up to 1.5 to 5 times higher than those in field ecosystems. Thus, a reduction of the uptake rate constant might have occurred, which might explain why the k_u decreased with the time course of exposure and waterborne Cu concentration.

A first-order bioaccumulation model was developed to account for the change in bioaccumulation of organic contaminate over the time course of exposures as $C_i(t) = C_i(0)e^{-k_{ew}t} + \frac{k_{uw} \times C_w}{k_{ew} - \lambda} \times \left(e^{-\lambda t} - e^{-k_{ew}t}\right)$, where λ refers to the rate constant (in days) that allows for decreasing chemical levels over the course of the exposure (Higgins et al. 2009). This model attributes the change in chemical accumulation to the difference in bioavailability and/or the potential transformation of chemicals within organisms (Higgins et al. 2009). Although this model has not been validated for metal accumulations, the latter process might be a possibility, because subcellular Cu partitions and metallothionein concentrations in fish tissues have been shown to play a primary role in Cu regulation during long-term exposure in marine and freshwater fish (Dang et al. 2009; De Boeck et al. 2010).

Conclusions

This study indicated that the bioaccumulative processes of Cu in tilapia were highly dependent on exposure durations and concentrations. It is necessary to consider changes in metal bioaccumulation behavior by TK coping mechanisms when modeling Cu accumulation, especially for chronic risk predictions. It is suggested that future models integrate organ-specific TK mediation with the knowledge of the subcellular participation of metal might be promising to predict the active Cu dose in specific organs.

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