Trade-offs between elimination and detoxification in rainbow trout and common bivalve molluscs exposed to metal stressors

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ABSTRACT

The purpose of this paper was to examine trade-offs between elimination and detoxification in rainbow trout and three common bivalve molluscs (clam, oyster, and scallop) exposed to cadmium (Cd), copper (Cu), and zinc (Zn) based on recent reported experimental data. We incorporated metal influx threshold with subcellular partitioning to estimate rate constants of detoxification (k_d) and elimination (k_2). We found that the relationships between k_2 and k_d were negative for rainbow trout and positive for bivalve molluscs. However, the relationships between k_d and % metal in metabolically detoxified pool were found positive for rainbow trout and negative for bivalve molluscs. Our results also indicated that rainbow trout had higher accumulation ($\sim$60–90%) in metabolically active pool when exposed to essential metals of Cu and Zn and had only 10–50% accumulation in response to non-essential metal of Cd. Based on a cluster analysis, this study indicated that similarity of physiological regulations among study species was found between Cd and Zn. Our study suggested that detoxification can be predicted by an elimination–detoxification scheme with the known elimination rate constant. We concluded that quantification of trade-offs between subcellular partitioning and detoxification provides valuable insights into the ecotoxicology of aquatic organisms and enhances our understanding of the subcellular biology of trace metals.

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1. Introduction

Due to intensive human activities, metals have been found at high levels in some aquatic ecosystems. Hung et al. (2001), Chen (2002), and Yeh et al. (2009) indicated a strong association between bivalve molluscs and fish and waterborne metals of cadmium (Cd), copper (Cu), and zinc (Zn) in coastal areas of Taiwan regions.

Cadmium is a non-essential trace metal that has a tendency to accumulate in aquatic organisms (Wang and Rainbow, 2006; Wang and Wang, 2008). In the potentially metal sensitive sites of cell, Cd can compete effectively with Zn and displace this essential cellular metal from sulfhydryl groups of enzymes, altering their functions and inducing toxic effects (Wang and Rainbow, 2006; Wang and Wang, 2008). Copper is a micronutrient for both plants and animals at low concentrations. Acute Cu toxicity is always associated with inhibition of sites involved in active Na$^+$ uptake at the gills, resulting in death from failure of NaCl homeostasis (Paquin et al., 2002). Zinc is an essential element for all organisms, playing a critical role in a variety of biochemical processes including regulatory, structural, and enzymatic functions. Although Zn is essential, it is toxic at high concentrations (Koh et al., 1996). When the external concentration gets too high, the organism's homeostatic capacity will fail and toxicity effects will occur (Van Assche et al., 1997). In addition, evidence shows that high intracellular free Zn promotes neuronal death by inhibiting cellular energy production (Dineley et al., 2003).

Recent studies on subcellular metal compartmentalization in aquatic organisms have led to conclusions regarding the significance of subcellular fates of metals to potential biological consequences of accumulated metals (Wallace et al., 2003; Steen Redeker et al., 2007; Wang and Wang, 2008; Buchwalter et al., 2008; Gimbert et al., 2008; Dubois and Hare, 2009; Kamunde, 2009). Wang and Rainbow (2006) suggested that subcellular partitioning of metals in the organisms may serve as a suitable toxicity predictor. Subcellular metal compartmentalization can also be referred to as the subcellular partitioning model (SPM). The SPM takes into account the complex binding of metals in different subcellular pools with different metal-binding ligands.

For example, Cd associated with heat labile proteins and organelle reflects greater potential for Cd to interfere with vital physiological processes, whereas Cd associated with metallothionein-like proteins and metal-rich granules can be accounted for a detoxifying ability (Wang and Rainbow, 2006; Buchwalter et al., 2008; Kamunde, 2009). It has been noted that toxicological significance was not found in metal associated with cellular debris (Buchwalter

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et al., 2008). Although, recent comprehensive data based on bioaccumulation and subcellular partitioning experiments of metals in aquatic organisms have facilitated the development of predictive abilities based on the underlying physiological processes of susceptibility that determine species response to water quality (Buchwalter et al., 2008; Pan and Wang, 2008; Sappal et al., 2009; Kamunde, 2009). However, the interactions between physiological traits and emergent properties of metal toxicity are rarely compared within or between either metals or species. Thus, even with increasing information, we still do not know the extent to which these interactions are correlated, how species differ in their metal susceptibility.

The model predictions can be achieved by using the essential experiments where direct determinations of toxicokinetic rate constants are obtained based on certain environmental conditions. Well-performed metal exposure experiments associated with toxicokinetic model and subcellular partitioning could capture the essence of dynamics of several model systems. The mechanistic linking of toxicokinetics and subcellular partitioning can incorporate the organ- and cell-scale exposure experimental data to investigate the mechanisms of ecophysiological response for aquatic organisms exposed to metal stressors (Cain et al., 2004; Vijver et al., 2006; Gimbert et al., 2008). It would be of interest to investigate similar parameters related to detoxification rate constants via dietary exposure routes (Croteau and Luoma, 2009), providing additional perspectives on coping mechanisms in response to metal stressors.

To address this complexity of ecophysiological mechanisms underlying the metal exposure, we quantified two key parameters that are determined to link bioaccumulation and subcellular metal distribution: the ability to eliminate metal (elimination rate constant) and the ability to detoxify metal (detoxification rate constant). Here, we provided a comprehensive study of bioaccumulation and subcellular partitioning correlations among metal stressors and species, based on data generated by recent published experiments, to investigate the potential trade-offs between the abilities of elimination and detoxification across species.

The objective of this study was twofold: (1) to evaluate physiological traits related to Cd, Cu, and Zn bioaccumulation, compartmentalization, and susceptibility in fish and bivalve molluscs and (2) to construct a prediction scheme by which detoxification capacity can be predicted by the elimination–detoxification relationships.

2. Materials and methods

2.1. Study data

A systematic search was conducted to collect recent publications that focused on physiological studies related to rainbow trout and bivalve molluscs exposed to metals to understand the mechanisms underlying linkage between bioaccumulation and subcellular partitioning. "Systematic search" means to collect all available data that published in the peer-reviewed journals, literature, or on the web. We conducted a search with hand-searching of appropriate journals, reference lists, database, and used ISI Wed of Knowledge (Thomson Reuters) and PubMed (National Library of Medicine; http://www.ncbi.nlm.nih.gov/sites/entrez) to find all related articles that focused on studies about metals, body burden (bioaccumulation), subcellular (intracellular) partitioning, and toxicokinetics.

Of seven articles were selected in this study. The adopted published data contained the valuable information allowing us to examine the potential trade-offs between bioaccumulation and coping mechanism in rainbow trout and bivalve molluscs exposed to metals. The study data included (i) rainbow trout (*Oncorhynchus mykiss*) exposed to Cu, Zn, and Cd (Kamunde and MacPhail, 2008; Ng and Wood, 2008; Kamunde, 2009; Sappal et al., 2009) and (ii) oyster (*Crassostrea virginica*) exposed to Cu (Blanchard et al., 2009), scallop (*Chlamys nobilis*) exposed to Zn and Cd (Pan and Wang, 2008), and clam (*Ruditapes decussatus*) exposed to Cd (Serafim and Bebianno, 2007).

Table 1 summarizes the experimental characterizations of selected published studies including exposure organisms, metal concentration, bioaccumulation experiment, and classification of subcellular fractionation. Physiological traits such as uptake, elimination, and detoxification could be determined by fitting the proposed toxicokinetic model and metal influx threshold theory to the experimental data.

2.2. Subcellular metal fractionation

Due to the different protocols used in the differential centrifugation procedures in the adopted publications, we separated various intracellular compartments into five fractions: (i) cellular debris and membrane, (ii) metal-rich granules, (iii) organelles including nucleus, mitochondria, and microsomes–lysosomes, (iv) heat labile (sensitive) proteins (HLP), and (v) metallothionein-like proteins (e.g., heat stable proteins, HSP), designating as MTLP (Wallace et al., 2003; Vijver et al., 2004; Wang and Rainbow, 2006). Here, we grouped above five fractions into metabolically active pool (MAP) and metabolically detoxified pool (MDP). The MAP comprises of organelle and HLP fractions, whereas the MDP comprises metal associated with granule and MTLP fractions. Moreover, we used accumulation of metal in MAP to characterize the potential for causing the toxicity to species.

2.3. Mechanistic models

A first-order biokinetic model was fitted to accumulation data of Cd, Cu, and Zn as,

$$C_b(t) = C_{b0}e^{-k_2 t} + \frac{k_1}{k_2}C_e(1 - e^{-k_2 t}), \tag{1}$$

where $C_b(t)$ is the metal burden in tissue/organ varied with time t ($\mu g\,g^{-1}$ wet wt), C_{b0} is initial concentration of metal in target tissue/organ ($\mu g\,g^{-1}$ wet wt), k_1 is the uptake rate constant ($mL\,g^{-1}\,d^{-1}$), k_2 is the elimination rate constant (d^{-1}), and C_e is the exposure metal concentration in water ($\mu g\,L^{-1}$) or in food ($\mu g\,g^{-1}$ wet wt). During the fitting procedure we incorporated different exposed concentrations (C_e) and time-varying tissue/organ concentrations (C_b) into the model to estimate concentration-, tissue-, and subcellular fraction-specific toxicokinetic parameters of k_1 and k_2. Uptake rate and elimination rate constants in metabolically active pool (MAP) ($k_{1,MAP}$ and $k_{2,MAP}$) of subcellular partitioning can also be estimated by fitting the proposed bioaccumulation model to corresponding data.

Detoxification rate (k_d, d^{-1}) can be estimated followed the metal influx threshold (MIT) concept introduced by Croteau and Luoma (2009). Croteau and Luoma (2009) proposed that MIT occurs when metal influx $\geqslant$ the combined rates of metal loss and detoxification. Thus, the external metal uptake in MAP of aquatic organisms exceeds the MAP influx threshold, the metal will be lost and detoxification will be triggered. Based on the MIT perspective, k_d can be calculated as

$$k_d = \frac{k_1 C_e}{C_{b0}} - k_{2,MAP}, \tag{2}$$

where k_d is the detoxification rate constant (d^{-1}) and $k_{2,MAP}$ is the metal elimination rate constant in MAP (d^{-1}).

Table 1
Qualitative summary of experimental characterization used in this analysis.

Species	Body weight/ length	Exposure metal	Bioaccumulation	Subcellular partitioning	Reference
Rainbow trout (O. mykiss)	20 g	$CuSO_4 \cdot 5H_2O$; Cu: 40 µg L^{-1}	21-d exposure	Nuclei, cellular debris, NaOH-resistant granules, mitochondria, microsomes–lysosomes, heat-labile proteins, heat-stable proteins	Kamunde and MacPhail (2008)
			Sampling time: 0, 7, 14, and 21 d		
	14 g	$ZnSO_4 \cdot 7H_2O$; Zn: 150, 600 µg L^{-1}	40-d exposure	Nuclei, cellular debris, NaOH-resistant granules, mitochondria, microsomes–lysosomes, heat-labile proteins, heat-stable proteins	Sappal et al. (2009)
		Zn (dietborne): 1500, 4500 µg g^{-1}	Sampling time: 0, 14, 28, and 40 d		
	13.28 ± 0.25 g[a]	$Cd(NO_3)_2 \cdot 4H_2O$; Cd: 5, 25, 50 µg L^{-1}	96-h exposure	Nuclei, cellular debris, NaOH-resistant granules, mitochondria, microsomes–lysosomes, heat-labile proteins, heat-stable proteins	Kamunde (2009)
			Sampling time: 0, 24, 48, and 96 h		
	10–12 g	$Cd(NO_3)_2$; Cd (dietborne): 0.6, 2.2, 30.3 µg g^{-1}	28-d exposure	Cellular debris, metal-rich granules, organelles, heat-denaturable proteins, metallothionein-like proteins	Ng and Wood (2008)
			Sampling time: 0, 7, 14, 21, and 28 d		
Oyster (C. virginica)	NA	Cu: 4, 5, 11 µg L^{-1}	96-d exposure	Cellular debris, metal-rich granules, organelles, enzymes, metallothionein-like proteins	Blanchard et al. (2009)
			Sampling time: 0, 60, and 96 d		
Scallop (C. nobilis)	25–30 mm	$ZnCl_2$; Zn: 0.5, 10, 100 µg L^{-1} $CdCl_2$; Cd: 2, 50, 200 µg L^{-1}	22-h exposure	Cellular debris, metal-rich granules, organelles, heat-sensitive proteins, metallothionein-like proteins	Pan and Wang (2008)
			Sampling time: 2, 6, 12, and 22 h		
Clam (R. decussatus)	30–35 mm	$CdCl_2 \cdot H_2O$; Cd: 4, 40 µg L^{-1}	40-d exposure	Insoluble fraction, high-molecular-weight protein fraction, low-molecular-weight protein fraction	Serafim and Bebianno (2007)
			Sampling time: 0, 7, 14, 21, 30, and 40 d		

[a] Mean ± standard error.

2.4. Data analysis

TableCurve 2D (Version 5.0, AISN Software Inc., Mapleton, OR, USA) and Statistica® software (Version 6.0, StatSoft, Tulsa, OK, USA) were used to perform the model fittings. Coefficient of determination (r^2) was used to describe the quality of the fit. To determine the uncertainty in all parameters, the distributions of parameters were generated by performing the Monte Carlo technique using Crystal Ball® software (Version 2000.2, Decisionerring, Inc., Denver, Colorado, USA). The results showed that 10 000 iterations were sufficient to ensure the stability of results. A simple linear regression analysis was used to perform correlations between estimated parameters (i.e., uptake rate, elimination rate, detoxification rate constants, and % metal in MDP). We applied cluster analysis to classify selected reference data into groups on the basis of similarities within estimated parameters. Cluster analysis was performed on the $\log_2$-transformed parameter set using Euclidean distances as a distance measure of similarity and Ward's method linkage to acquire dendrograms by using Statistica® software 6.0 for Windows.

3. Results

3.1. Parameters estimation for rainbow trout

Fitted toxicokinetic parameters (k_1 and k_2) and estimated detoxification rate constant (k_d) of target organs and MAP for rainbow trout exposed to metals are summarized in Table S1 (see Supplementary material). Our results indicated that (i) k_1 decreased with increasing Cd concentrations in gill (3.46–11.85 mL g^{-1} d^{-1}),

(ii) no obvious change of k_1 was found in liver (nearly 1 mL g^{-1} d^{-1}), and (iii) k_1 increased with increasing dietborne concentrations in gut (0.018–0.028 d^{-1}). Gill and gut had similar k_2 values ranging from 0.075 to 0.46 d^{-1}. However, liver exhibited a very low elimination rate with order of magnitudes 10^{-6}–10^{-5} d^{-1}, indicating a major organ of accumulation for Cd.

The toxicokinetic parameters of MAP in gill and liver were similar to that in the entire organs of gill and liver with $k_{1,MAP}$ of 4.56–12.97 and 0.56–0.69 mL g^{-1} d^{-1} and $k_{2,MAP}$ of 0.62 and 2.52×10^{-6} d^{-1} in gill and liver, respectively. Moreover, MAP of gut experienced a relative lower uptake rate (~0.005 d^{-1}), whereas elimination rate constant (0.17 d^{-1}) was close to that of in organs. However, k_d estimates increased with increasing exposure concentrations, with 0.45–1.72 d^{-1} for gill and 0.26–2.24 d^{-1} for liver exposed to waterborne Cd of 5–50 µg L^{-1} and 2.24–87.42 d^{-1} for gut exposed to dietborne Cd of 0.6–30.3 µg g^{-1} wet wt.

Results showed that rainbow trout had higher uptake rate constants of 23.78–4029 mL g^{-1} d^{-1} in organs exposed to Cu and Zn. Not surprisingly, gill had low metal uptake capacity with k_1 of 0.004–0.016 g g^{-1} d^{-1} when exposed to dietborne Zn. In contrast, k_2 estimates were low with 0.033–0.128 d^{-1} exposed to waterborne and dietborne metals. We also found that high k_1 was observed when rainbow trout exposed to low exposure concentration.

On the other hand, the k_1 estimates of Cu and Zn in MAP were estimated to be 27.4–1902 and 11.93–90 mL g^{-1} d^{-1}, respectively. The values of k_2 in MAP were close to that in organ (0.049–1.502 d^{-1}), except in liver with value of 4.5×10^{-5} d^{-1} at high Cu exposure. Generally, k_d estimates ranged from 0.076 to 6.65 d^{-1} for rainbow trout exposed to Cu and Zn. However, some estimated k_ds had negative values, which indicating that the detoxification capacity was not activated.

3.2. Parameters estimation for common bivalve molluscs

The fitted toxicokinetic parameters (k_1 and k_2) and estimated detoxification rate constant (k_d) for target organs and MAP in three bivalve molluscs exposed to metals are summarized in Table S2 (see Supplementary material). The k_1 estimates of clam ranged from 65.9–248, 122–175, and 16.2–20.9 mL g^{-1} d^{-1} in gill, digestive gland, and remaining tissues, respectively, exposed to waterborne Cd of 4–40 µg L^{-1}. In scallop, there were no clear patterns of k_1 estimates appeared across the exposed Cd concentrations with mean value of 239.9 mL g^{-1} d^{-1}. For k_2, we found different elimination capacities across target organs with mean values of 0.030, 7.8×10^{-7}, 0.003, and 1.87 d^{-1} for gill, digestive gland, remaining tissues, and whole body, respectively (Table S2).

The $k_{1,MAP}$ estimates were less than k_1s in organs, except for remaining tissues, whereas $k_{2,MAP}$s were higher than that in entire organs (k_2) with mean values of 0.075, 0.060, and 2.44 d^{-1} for gill, remaining tissues, and whole body, respectively. These results indicated that Cd was not stored in MAP fraction and could only be eliminated in above mentioned organs. Only $k_{2,MAP}$ in digestive gland was slightly less than k_2 in whole organ with an average of 2.6×10^{-7} d^{-1}. The k_ds of Cd in bivalve molluscs were apparently increased with increasing of waterborne Cd (Table S2).

On the other hand, oyster had a high uptake and a relative lower elimination rates exposed to waterborne Cu concentrations ranging from 4–11 µg L^{-1}. Notably, the lowest Cu concentration of 4 µg L^{-1} exposure resulted in a relative higher k_1 of 28 734 mL g^{-1} d^{-1}. For Zn exposure, however, k_1 (207.8–359.3 mL g^{-1} d^{-1}) and k_2 (1.63–4.34 d^{-1}) estimates did not vary with exposure concentrations. The same accumulative pattern of Cu was observed in MAP but with a lower k_2 of 4.7×10^{-4} d^{-1}. For Zn in MAP, we found that a similar elimination level with whole body but with a lower $k_{1,MAP}$. Hence, Cu may be preferred to be accumulated in MAP fraction, whereas Zn was accumulated in other fractions. The k_d estimates of oyster exposed to Cu did not change significantly under different exposure concentrations. Scallop had a relative higher detoxified capacity with k_d of 1233 d^{-1} exposed to a high Zn concentration of 200 µg L^{-1}.

3.3. Relationships between toxicokinetics and coping mechanism

A comparative scheme describing the relationships between (i) k_2–% in MDP, (ii) k_d–% in MDP, (iii) k_2–k_d, and (iv) BCF–k_d was constructed to understand the potential linkage between toxicokinetics and coping mechanism for rainbow trout (Fig. 1) and bivalve molluscs (Fig. 2) exposed to Cu, Zn, and Cd. The slopes of the linear regression defined the relationships between the parameters considered. Our results showed a negative relationship between k_2 and % metal in MDP for rainbow trout and bivalve molluscs exposed to Cu, Zn, and Cd (Fig. 1A, E, 2A and E).

We also found that species-specific differences existed in the relationship between k_d and % metal in MDP with a positive correlation for rainbow trout and a negative correlation for bivalve molluscs (Fig. 1B, F, 2B and F). Species-specific differences were also found in relations between k_2 and k_d, indicating that rainbow trout had a negative and bivalve molluscs had a positive relationships (Fig. 1C, G, 2C, and G). Notably, the relationships between logBCF and k_d revealed a negative trend for rainbow trout and bivalve molluscs exposed to Cu, Zn, and Cd, except a positive trend for rainbow trout exposed to Cu and Zn (Fig. 1D, H, 2D, and H).

3.4. Cluster analysis

Here a functional dendrogram was used to describe the functional relationships among adopted experimental data (Fig. 3). The dendrogram with 25 groups of adopted experimental data
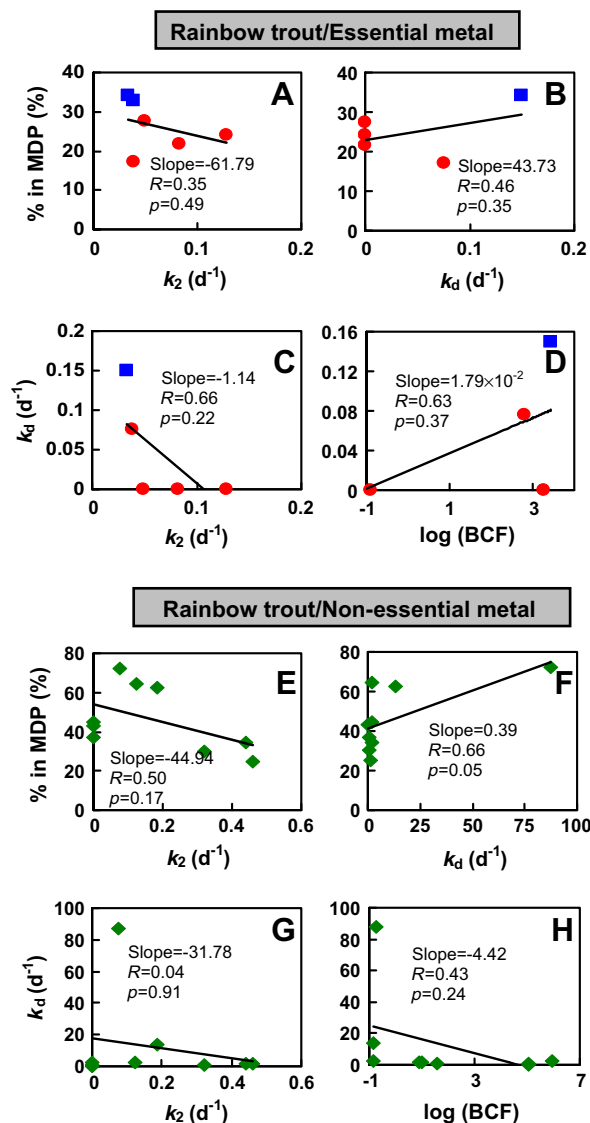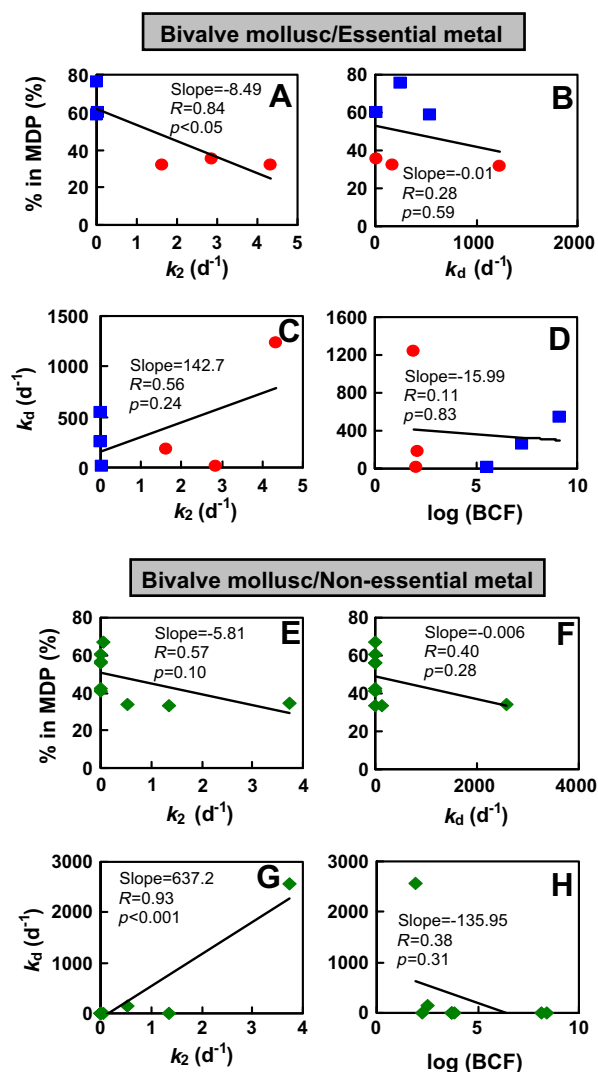

Fig. 1. Overall display relationship among % metal in MDP, elimination rate (k_2), detoxification rate (k_d), and bioconcentration factor (BCF) in rainbow trout exposure to (A–D) essential metals of copper (■) and zinc (●) and (E–H) non-essential metal of cadmium (♦).

was divided into two main clusters branched by metals (Fig. 3). This plot highlights that a number of data sets were functionally very similar. The first cluster included Cd and Zn was functionally very similar and appeared to be as one cluster. The second cluster included Cd and Cu could also be clearly grouped into two subclusters for Cd and Cu, respectively. This present cluster analysis indicated that physiological regulations of rainbow trout and bivalve molluscs could be approximately classified by exposure metals. We also found that the similarity of physiological regulations in rainbow trout and bivalve molluscs occurred between exposure to Cd and Zn, whereas the functional similarities were hardly occurred between Zn and Cu.

3.5. Metal-specific bioregulation comparison

A systematic comparison between metal-specific toxicokinetic and detoxification parameters was performed. In this study, we analyzed the relationships between (i) k_1 and k_2, (ii) $k_{1,MAP}$ and $k_{2,MAP}$, (iii) k_2 and k_d, and (iv) k_2 and % metal in MDP, to examine the physiological variations between bioaccumulation and coping

Fig. 2. Overall display relationship among% metal in MDP, elimination rate (k_2), detoxification rate (k_d), and bioconcentration factor (BCF) in bivalve mollusc exposure to (A–D) essential metals of copper (■) and zinc (●) and (E–H) non-essential metal of cadimum (♦).

mechanism in bivalve molluscs and rainbow trout exposed to Cd, Cu, and Zn (Figs. 4–6). The fitted normal distributions with mean and standard deviation values of log-transformed physiological parameters (k_1, k_2, $k_{1,MAP}$, $k_{2,MAP}$, and k_d) with % metal in MDP were summarized in Table S3 (see Supplementary material).

The relationships between k_1 and k_2 in rainbow trout and bivalve molluscs exposure to Cd were positively related (Fig. 4A). Fig. 4B reveals a positive correlation between $k_{1,MAP}$ and $k_{2,MAP}$ for rainbow trout and bivalve molluscs exposed to Cd, that were similar to the relationship between k_1 and k_2. The relationship between k_2 and k_d revealed a positive trend for bivalve molluscs exposed to Cd, whereas an opposite pattern was found for that of rainbow trout (Fig. 4C). Fig. 4D shows a strong negative relationship between k_2 and % Cd in MDP for rainbow trout, whereas an insignificant relationships was found for bivalve molluscs. These results indicated that the potential trade-offs between the ability to eliminate Cd and the ability to detoxify it existed in rainbow trout, but not in bivalve molluscs.

The relationship between k_1 and k_2 for essential metals of Cu and Zn was found to be a positive trend for bivalve molluscs and rainbow trout, except for Cu bioaccumulation relationship in bivalve molluscs (Fig. 5A and 6A). The similar trends occurred in

the association between $k_{1,MAP}$ and $k_{2,MAP}$ (Fig. 5B and 6B). The coping capacities (k_2 and k_d) in response to essential metals were shown a negative interaction to Cu and a positive interaction to Zn in bivalve molluscs, whereas a positive association to Cu in rainbow trout was observed (Fig. 5C and 6C).

Notably, Zn detoxifying mechanism did not trigger in rainbow trout at waterborne 150 µg L^{-1} and dietborne 1500–4500 µg g^{-1} levels. Furthermore, the relationship between k_2 and % in MDP in rainbow trout had an opposite pattern for Cu and Zn with a negative for Cu and a positive for Zn; whereas a negative relationship was found for bivalve molluscs exposed to Cu and Zn (Fig. 5D and 6D). These results indicated that bivalve molluscs performed a more effective ability to accumulate, eliminate, and detoxify Cd and Zn than that of rainbow trout. Yet bivalve molluscs had a relative lower capacity to eliminate Zn compared with that of rainbow trout.

4. Discussion

4.1. Elimination and detoxification capacities

In this study, we have presented a systematic comparison between elimination rate constant and detoxification rate constant at different levels of exposure setting based on a mechanistic explanation. We integrated toxicokinetics, subcellular partitioning, and metal influx threshold concept for assessing trade-offs between the ability to eliminate and the ability to detoxify the essential/non-essential metals in rainbow trout and three bivalve molluscs. Vijver et al. (2004) indicated that metals can be eliminated from tissue, stored in tissue, or stored as a detoxified form. Therefore, elimination and detoxification capacities are very important strategies for organisms in response to surrounding metal stressors. Despite the estimation of elimination rate constant by bioaccumulation model have been widely used in recent years (Tsai et al., 2009; Liao et al., 2011), the quantification of detoxification rate is virtually non-existent.

There are three critical assumptions in proposed MIT concept. First, threshold exists when metal influx begins to exceed the combined efflux rates (Croteau and Luoma, 2009). Based on current knowledge, we found that MDP could always accumulate metals even aquatic organisms exposed to relative lower metal concentrations. Therefore, we used metal concentration in MAP of the control group as the threshold active concentration. The second assumption is that MAP is the first compartment to accumulate metals during exposures (Rainbow, 2002; Wang and Rainbow, 2006). Consequently, detoxification rate constant can be estimated by metal flow effusing to MDP from MAP. Third, MIT concept was developed based on a dietborne metal exposure experiment. In this study, we assumed that MIT concept was valid for dietborn and waterborne metal exposure data. Recently, Pan and Wang (2008) have investigated subcellular fate of Cd and Zn in the scallop *C. nobilis* and found that most accumulated Cd in *C. nobilis* was associated with the organelles (35–45%) even at low exposure concentration of 0.5 µg L^{-1} Cd. Their findings support the MIT concept. The MIT hypothesis is still in developing, therefore, future experimental research is needed for completing and validating the concept (Wang and Rainbow, 2006; Croteau and Luoma, 2009; Rainbow and Smith, 2010).

There are three key advantages on linking subcellular partitioning with MIT concept. First, it is useful to explain quantitatively the detoxification capacity of metal exposure. Specifically, subcellular partitioning allows us to consider the internal metal sequestration and to explore the actually effective bioaccumulation for estimating detoxification rate constant. Second, we can only use a simple equation with toxicokinetic parameters to estimate detoxification
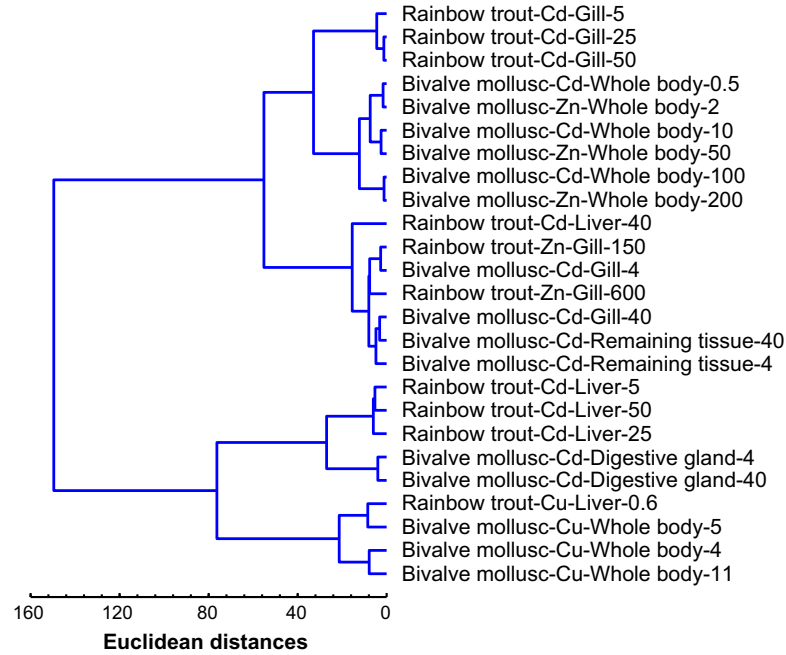
Fig. 3. Dendrogram showing clustering analysis of aquatic organism exposure to metal according to Ward's method using Euclidean distance. Each group represents "organism-exposed metal-organ-exposed concentration ($\mu g \, L^{-1}$)".
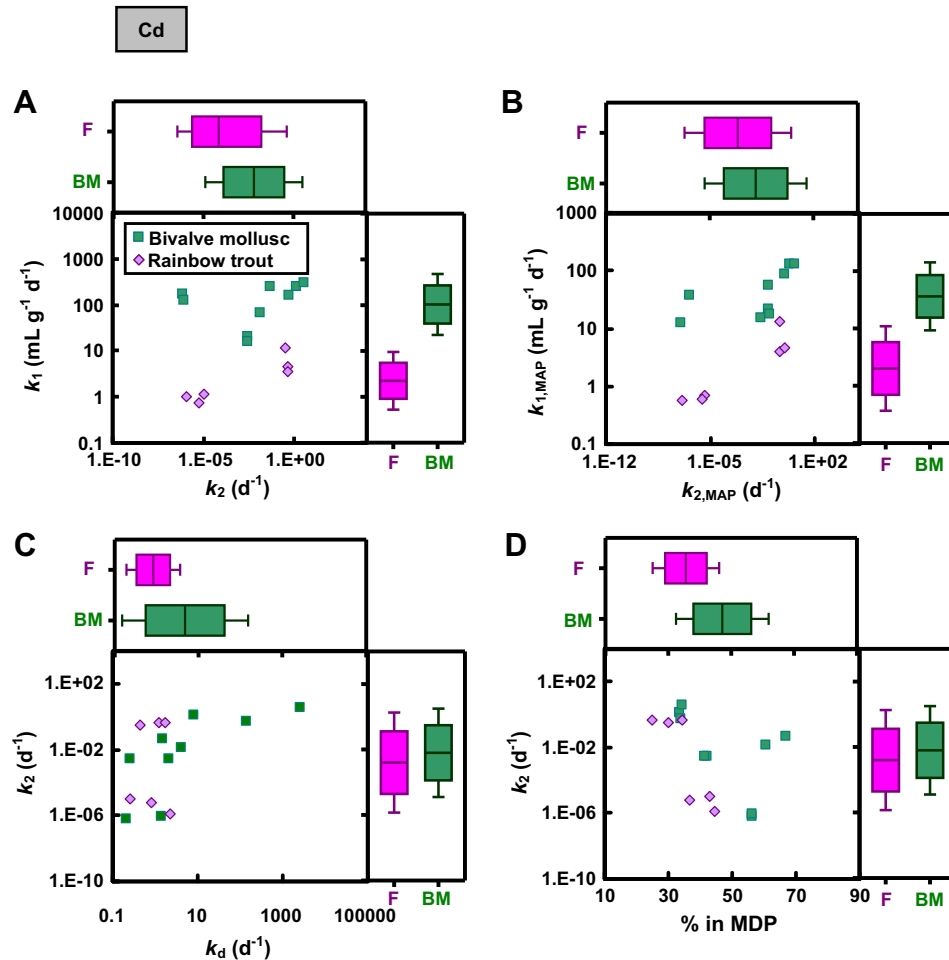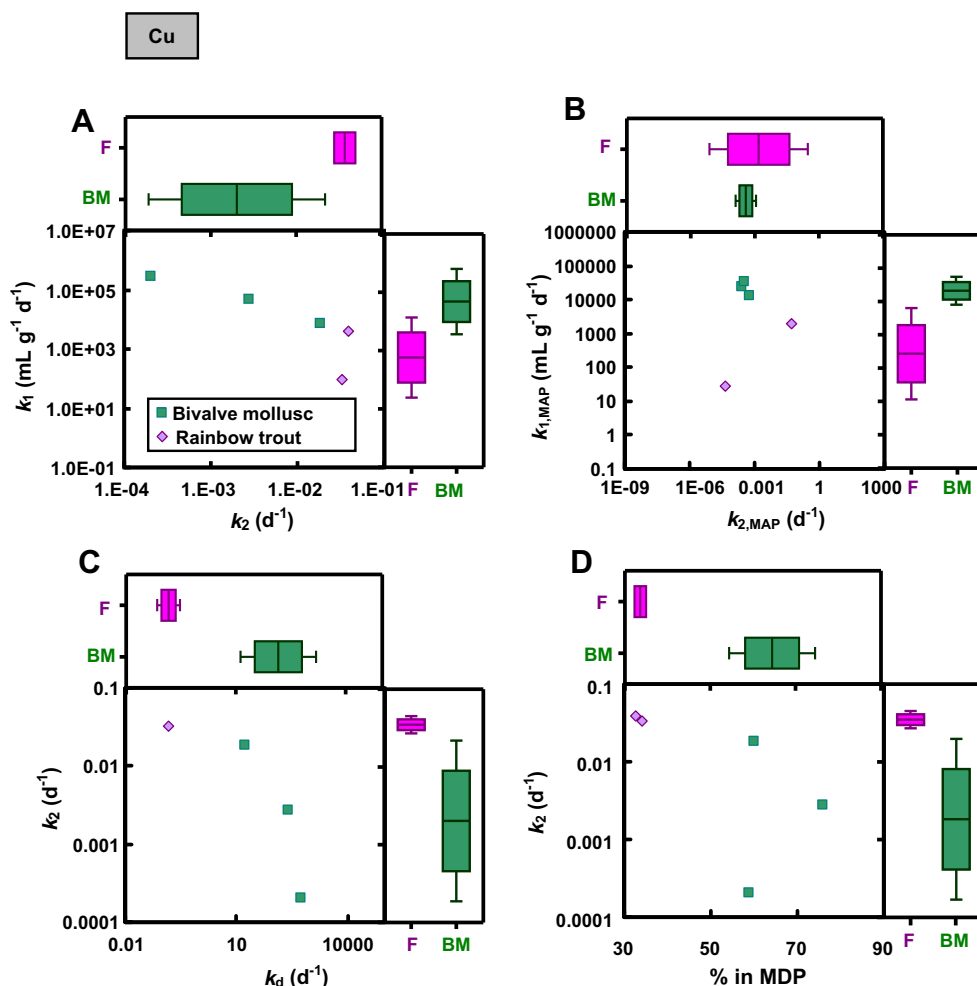

Fig. 4. Relationships between parameters with box-whisker plots of each parameter for bivalve mollusc (■) and rainbow trout (♦) exposure to cadmium. (A) k_1–k_2, (B) $k_{1,MAP}$–$k_{2,MAP}$, (C) k_2–k_d, and (D) k_2–% in MAP. Box and whisker represent the ranges of 25th–75th and 2.5th–97.5th percentiles, respectively. F = rainbow trout and BM = bivalve mollusc.

Fig. 5. Relationships between parameters with box-whisker plots of each parameter for bivalve mollusc (■) and rainbow trout (♦) exposure to copper. (A) k_1–k_2, (B) $k_{1,MAP}$–$k_{2,MAP}$, (C) k_2–k_d, and (D) k_2–% in MAP. Box and whisker represent the ranges of 25th–75th and 2.5th–97.5th percentiles, respectively. F = rainbow trout and BM = bivalve mollusc.

rate in a given environment. A theoretical understanding will improve our ability to interpret these parameters and design adequate experiments. Third, it provides an important direction for future study toward a quantitative estimation based on the metal- and species-specific toxicity.

A negative correlation was observed between k_2 and k_d for rainbow trout, whereas a positive correlation was found for bivalve mollusc in this study. The different relations for different aquatic organisms may due to the different subcellular compartments of metal for detoxification and storage (Vijver et al., 2004; Rainbow, 2007). When ambient metal accumulated in organisms, it may be removed by excreting from organism or be sequestrated by binding metallothionein-like proteins and storing in granules as the non-toxic form to store in body (Klaassen and Liu, 1998; Wallace et al., 2003; Li et al., 2008). Our study indicated that trade-offs between elimination and detoxification could only be found in rainbow trout (Fig. 1C and G), implying that the ability of detoxifying metal may complement the insufficient ability of eliminating metal.

Moreover, we found that the relationship between k_d and % metal in MDP was positive for rainbow trout and negative for bivalve molluscs, implying different detoxified strategies. Rainbow trout seems to prefer to use metallothionein-like proteins as the binding ligand for detoxifying metal (Kraemer et al., 2005; Kamunde and MacPhail, 2008; Kamunde, 2009; Ng et al., 2009). However, metal-lothionein-like proteins can be immediately induced by ambient

metals, such as Cd, Cu, Zn, and Hg, and then bind specifically the incoming metals (George and Olsson, 1994; Isani et al., 2000). Thus, as long as rainbow trout produces metallothionein, the mechanism of detoxification is active (Vijver et al., 2004).

On the contrary, metals majorly bind to metal-rich granules in bivalve molluscs (Cheung and Wang, 2005; Blanchard et al., 2009; Rainbow and Smith, 2010). Metals bind to metal-rich granules in the beginning, and subsequently metal-bound granules are eliminated from cell (Gibbs et al., 1998), decreasing the fractions of metal in granules. Therefore, we found an opposite relationship between k_d and % metal in MDP for bivalve molluscs.

4.2. Regulation under essential/non-essential metals exposures

Recent researches indicated that rainbow trout had a higher accumulation in MAP exposed to essential metals of Cu and Zn (∼60–90%); whereas nearly 10–50% accumulation of non-essential metals of Cd and Tl were observed (Kamunde and MacPhail, 2008; Ng and Wood, 2008; Lapointe and Couture, 2009; Kamunde, 2009; Sappal et al., 2009). On the contrary, however, bivalve molluscs had nearly 40–70% metal accumulated in MDP for essential and non-essential metals (Serafim and Bebianno, 2007; Pan and Wang, 2008; Blanchard et al., 2009; Lapointe and Couture, 2009). Such high bioaccumulation in MAP for rainbow trout may be caused by Cu and Zn being the physiologically essential metals. Rainbow trout may need relative higher essential metals to maintain basic
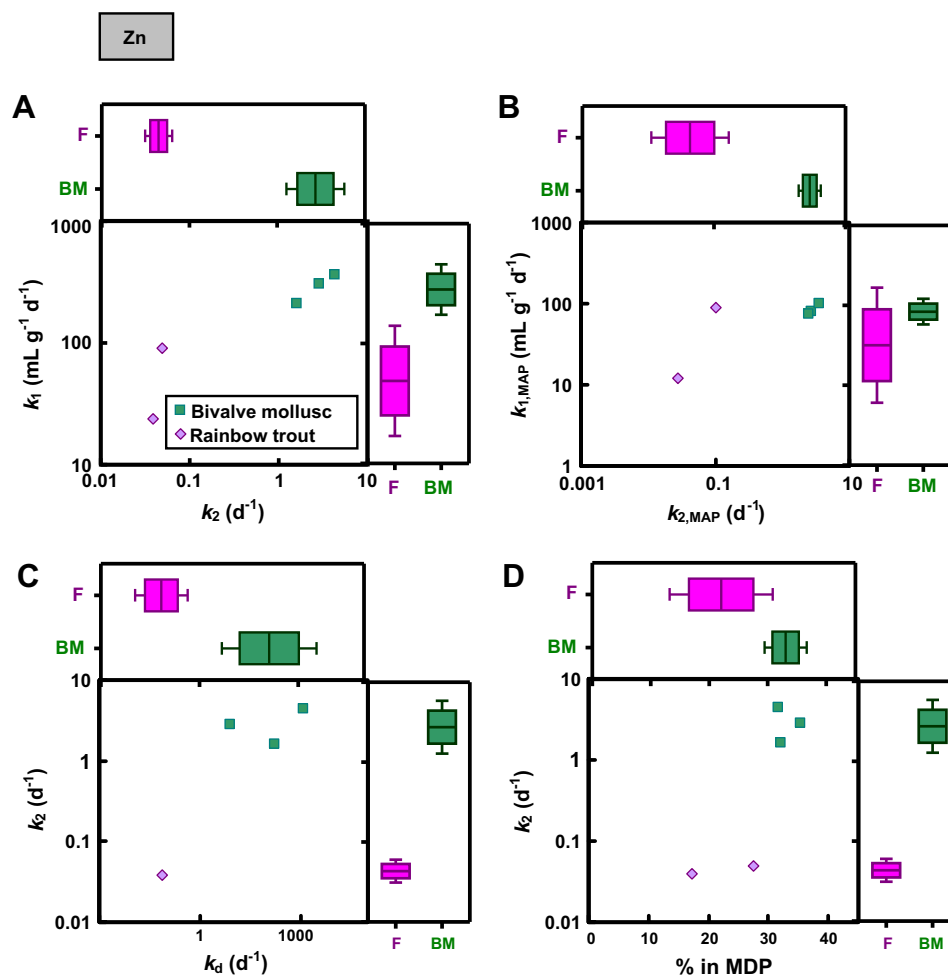
Fig. 6. Relationships between parameters with box-whisker plots of each parameter for bivalve mollusc (■) and rainbow trout (♦) exposure to zinc. (A) k_1–k_2, (B) $k_{1,MAP}$–$k_{2,MAP}$, (C) k_2–k_d, and (D) k_2–% in MAP. Box and whisker represent the ranges of 25th–75th and 2.5th–97.5th percentiles, respectively. F = rainbow trout and BM = bivalve mollusc.

physiological metabolism, and result in the accumulation of more Cu and Zn in MAP. However, non-essential metal such as Cd mainly accumulating in detoxified fraction was a crucial strategy for decreasing toxicity for rainbow trout (Kraemer et al., 2005). Rainbow and Smith (2010) indicated that metal-rich granule binding was a major fraction of metal assimilation by bivalves collected in field.

We found that rainbow trout had different strategies for handling metal stresses. When rainbow trout exposed to waterborne Cd, the capacities of elimination and detoxification are switched on in gills to reduce the Cd toxicity, whereas for dietary exposure, the detoxification is the dominant strategy in gut. Due to Cu and Zn are the essential metals, how to maximize the uptake capacity is the leading physiological regulation for rainbow trout. For clam (*R. decussatus*) and scallop (*C. nobilis*) exposed to Cd, detoxification is more important than elimination to reduce the Cd toxicity. Not surprisingly, a higher ability of metal intake is found in oyster (*C. virginica*) exposed to Cu and in scallop (*C. nobilis*) exposed to Zn. Moreover, the ability of detoxification can also be activated.

For classifying selected study data based on the similarities within estimated parameters, we used a cluster analysis to designate a set of estimated parameter into subsets. The groups in the same subsets are similar in regulating metal stressor for organisms, which is an effective approach for finding an analogy between numerous experimental data. We found a distinct cluster in Cu exposure group from Cd and Zn. Voets et al. (2009) indicated that

zebra mussels stored a higher fraction of Cd and Zn in detoxified form. In contrast, more Cu was found in sensitive fractions. Furthermore, some studies have reported that Cu had low induction potential of MT synthesis in aquatic organisms (Brown et al., 2004; Lecoeur et al., 2004; Bouskill et al., 2006; Ivanković et al., 2010). Thus, the strategy of regulation for Cu is different from that of Cd and Zn.

4.3. Ecotoxicological implications

Previous studies have quantified the foodborne assimilation efficiencies of metals in predators fed on different preys (Cheung and Wang, 2005; Rainbow, 2007; Huang et al., 2008; Dubois and Hare, 2009; Lapointe and Couture, 2009). This factor may control metal trophic transfer level. The metal subcellular partitioning in organisms will trophically transfer available metal through food chains (Cheung and Wang, 2005). Subcellular partitioning fractions of the major trophically available metal include organelles, heat labile protein, and metallothionein-like protein; that could be used as the indicators to predict trophic transfer (Wallace and Luoma, 2003). If this critical information of metal subcellular partitioning can be further quantified, we can identify which subcellular partitioning fraction causes biomagnification in food chains to achieve the critical harmful metal concentrations in predator organisms.

In conclusion, quantification of trade-offs between subcellular partitioning and detoxification provides valuable insights into the

ecotoxicology of aquatic organisms and enhances our understanding of the subcellular biology of trace metals. Our framework could be used to assess the physiological traits related to Cd, Cu, and Zn bioaccumulation, compartmentalization, and susceptibility in rainbow trout and generic bivalve molluscs. Our study also provides a predictive model by which detoxification can be predicted by an elimination–detoxification scheme with known elimination rate constant. Our results underscore the importance of considering the subcellular partitioning-detoxification linkage as a determinant of susceptibility strength, and the dynamic interplay between bioaccumulation and coping mechanisms for the predictions of metal susceptibility among aquatic organisms.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2011.07.033.

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