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A parsimonious AUC-based biokinetic method to estimate relative bioavailable zinc to abalone *Haliotis diversicolor supertexta*

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Abstract

We developed a mechanistic model based on the pharmacological area-under-curve (AUC) concept associated with a biokinetic rate model to predict relative bioavailable zinc (Zn) to abalone *Haliotis diversicolor supertexta*. We conducted a laboratory 14-day exposure experiment to obtain biokinetic parameters for soft tissue and shell of abalone and their food source, red alga *Gracilaria tenuistipitata* var. *liui*. The physiological parameters in terms of growth rate constants for abalone and algae were derived from literature. The present AUC-based model demonstrates that depuration rate constants and growth rate constants of abalone and algae are the critical parameters in predicting relative bioavailable Zn to abalone. During uptake phase of the exposure experiment, estimated relative bioavailable Zn to soft tissue and shell of *H. diversicolor supertexta* were 71.04 ± 9.71% (mean ± standard deviation) and 68.44 ± 8.29%, respectively. Sensitivity analysis indicates that relative bioavailable Zn to abalone is greatly affected by growth rate and depuration rate constants of abalone and is less dependent on algae growth rate. We also applied a Monte Carlo approach to estimate the impact of parameter uncertainty on relative bioavailable Zn predictions. Our results suggest that the probabilistic approach allows a range of possible outcomes and their likelihood; it better informs both aquacultural risk assessors and risk managers. Our study suggests that we have to take into account both biokinetic and physiological processes in predicting the relative bioavailable metals to aquatic animals.

Keywords: Abalone; Algae; Area-under-curve; Bioavailability; Zinc

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1. Introduction

The bioavailable and trophic transfer of metals in aquatic food chains has received considerable attention in recent years in an effort to better understand the geochemical cycling of metals and the nutritional and toxicological effects of metals in aquatic organisms (Wang et al., 1999; Lee et al., 2000; Reinfelder and Fisher, 2001). Assessment of bioavailable metals to aquacultural animals may help to determine if the metal levels are sufficiently high to be bioavailable and toxic, therefore requiring pond cleanup criteria. A mechanistic understanding of bioavailable metals to aquacultural species, however, is limited for most of these studies. The routes and rates of metal uptake are relatively unknown for most metals and most aquacultural species.

It is appropriate to develop a new approach to address bioavailable metals to aquacultural species by considering all processes responsible for metal bioaccumulation and the variations likely to be encountered in the field. We combine kinetic and pharmacological modeling approaches to develop a mechanistic model to predict bioavailable metals to aquacultural species. With this model, those physiological processes controlling bioavailable metals to aquacultural species can be identified and estimated under varying environmental conditions that are likely encountered by the species in the field.

The proposed mechanistic model, which incorporates all potential sources of metal uptake, appears to be the appropriate approach for understanding bioavailable metals to species and metal bioaccumulation in species, but its application has only recently been exploited. Using laboratory measurements of several key parameters, this proposed model can be readily used to quantify the relative importance of different pathways in bioavailable metals to aquacultural species.

In ecotoxicology, bioavailable fraction of a substance can be broadly defined as the portion of a chemical in the environment that is available for biological action such as uptake by an organism or that can cause a biological response (van Straalen, 1996). Thus, the bioavailable fraction of a substance is that part of the total concentration which can actually be taken by aquatic animals. The bioavailable metals to aquatic animals are determined by a complex interaction of biological and ecological factors such as habitat, feeding behavior, and digestion mechanisms. It is closely linked to the pharmacological definition of bioavailable fraction of a substance, which is the portion of a chemical in food that is available for uptake by an organism, e.g., reaches the systemic circulation of the organism (O'connell et al., 2000; Pelkonen et al., 2001).

In pharmacology, bioavailable fraction of a substance is most often calculated from the ratio of the absorbed dose representing by the areas under concentration–time curves (AUCs) of a toxicant to the ingested dose (Horsberg et al., 1996; McCloskey et al., 1998; Roberts et al., 2002). Martinsen et al. (1993a,b), and McCloskey et al. (1998) have used the pharmacological method to estimate the bioavailability of florfenicol and CH_3Hg from food in Atlantic salmon (*Salmo salar*) and channel catfish (*Ictalurus punctatus*), respectively.

In the biokinetic scheme, the conventional approach to estimate bioavailable metals to organisms is by quantifying a mathematical equation that relates metal content in the organisms with ambient dissolved metal concentration to produce the metal bioaccumulation factor (BAF) (Wang and Fisher, 1997; Reinfelder et al., 1998; Yan and Wang, 2002). Differences in BAF reflect differential relative bioavailable metals to organisms. Using BAF approach, bioavailable zinc (Zn) has been reported to between 70% and 80% to marine copepods, between 55% and 65% to bivalves (*Crassostrea virginica, Mercenaria mercenaria, Marcoma balthica*), and between 25% and 35% to marine mussel (*Mytilus edulis*) in which the elimination rate constants ranged from 0.01 to 0.1 day^{-1} (Reinfelder et al., 1998; Lee et al., 2000). McCloskey et al. (1998) pointed out that bioavailability estimates using the pharmacological methods were much lower than estimates using BAF approaches, suggesting that BAF approaches may overestimate the true bioavailability of toxicants in fish.

In this study, we estimated bioavailable dietborne Zn to abalone *Haliotis diversicolor supertexta* by quantifying the trophic transfer of Zn in abalone from red alga *Gracilaria tenuistipitata* var. *liui. H. diversicolor supertexta* is the most abundant abalone species in Taiwan. The red alga *G. tenuistipitata* var. *liui* is the major forage for culturing the abalone. These two species are commercially important for fisheries and aquaculture in Taiwan. *H. diversicolor supertexta* is also appreciated for its delicacy and high market value; the aquaculture of *H. diversicolor supertexta* thus is a promising business. The coastal regions of Taiwan where abalone and algae farms are located, however, are subjected to polluted discharges from rivers. Zinc is available to abalone from both the dissolved phase and the diet. If waterborne Zn levels are elevated, however, toxicity can occur and have severe effects on the health of abalone, which will reduce the market price and cause closure of abalone farms. Previous investigations indicated that maximum Zn concentrations in contaminated aquacultural water are reported to be ranged from 60 to 300 μg l^{-1} in different areas of Taiwan (Lin and Liao, 1999).

The main purpose of the present study was to combine both first-order biokinetic and AUC-based models to estimate bioavailable Zn to *H. diversicolor supertexta*. The methods used in the present study will be distinct as the BAF approach. We estimated the abalone assimilation efficiency of Zn from ingested algae. We determined bioavailable Zn to soft tissue and shell of *H. diversicolor supertexta* and examined the controlling mechanisms. A kinetic rate equation was employed to determine assimilation efficiency and depuration rate constants of abalone and algae from an exposure experiment. This AUC-based biokinetic approach has been used rarely in ecotoxicology and has never been used for bioavailable Zn to *H. diversicolor supertexta.* There are uncertainties in the measurement of physiological and biokinetic parameters that are used to predict bioavailable Zn as conducted in this study. Thus, we performed the sensitivity and uncertainty analyses for the proposed bioavailable Zn prediction model.

2. Materials and methods

2.1. Experimental section

Live abalone *H. diversicolor supertexta*, and the alga *G. tenuistipitata* var. *liui* were collected from Toucheng located in northern Taiwan for the laboratory exposure experiments because this place was the most Zn-contaminated area. Abalone with a shell length of 3.4 ± 0.8 cm were selected for the experiments. The algal samples selected were mature, whole and healthy. A total of 180 abalone was transferred into four aquatic tanks of approximately 54-l volume, containing 50 l of artificial seawater. In order to imitate the environment of the abalone farms, the abalone were held in baskets. Each tank contained 10 baskets. Four abalone per basket were used for analysis.

To assure that at least four abalone would be alive at the end of the experiment, we put one extra abalone in each basket. Dissolved oxygen was maintained at close to saturation by aeration throughout the experiment. The temperature was maintained at 24 ± 2.3 °C under constant illumination (Yang and Ting, 1986, 1994). The salinity was maintained at 35 ± 1.5. The pH remained fairly constant during the assays (7.54 ± 1.07). Abalone were fed daily with *G. tenuistipitata* var. *liui*. The abalone and algae were acclimatized for 2 weeks before they were exposed to Zn.

In two tanks, Zn ($ZnCl_2$) was added to the seawater; in one tank, the abalone were fed with algae (water/food-exposed), and in the other tank, the abalone were kept without food (water-exposed). The Zn contamination level was determined by a preliminary test exposing abalone to different Zn concentrations of 0.25, 0.5, 1, 2, 4, and 6 mg l^{-1}. The median lethal tolerance (LT_{50}) of abalone at ≤ 1 mg l^{-1} Zn was longer than 3 weeks. Thus, the organisms were exposed to 1 mg l^{-1} Zn for 7 days. The algae and the abalone were reared in the contaminated environment for 7-day uptake, then transferred to clean seawater and reared for 7 days of depuration. To examine if starvation affects Zn depuration in abalone, the same procedure with abalone and algae was followed over 14 days using the other two tanks, but without Zn in the sea water.

Abalone, algae and water samples were collected at days 0, 1, 2, 4, and 7, starting from the day that those organisms were exposed to the contaminated seawater and from the day the organisms were transferred to clean seawater. Every time we took one basket along with 500 ml water out of each tank. From this basket, four pieces of algae and four abalone were collected. Because preliminary observation showed that *H. diversicolor supertexta* only feeds at night and has an empty gut in the evening, we collected the abalone at night to make sure the contents of gut would not influence the results. The experiments in the four tanks, described above, were repeated again. The water samples were fixed with 5 ml 1 N HNO_3, and the samples of abalone were stored in the dark at −20 °C until they were analyzed.

2.2. Metal analysis

The algae and dissected abalone including soft tissue and shell were freeze-dried overnight, and then grounded into a fine powder in a grinder (Tai-Hsiang S36-89, Taiwan). The 500-mg portions of the ground samples were digested in 10 ml of 65% concentrated HNO_3 (v/v) overnight at room temperature. The resulting solution was evaporated and redissolved in 0.1 N HCl. Zinc analysis was carried out by atomic absorption spectrophotometry using a Perkin-Elmer model 5000 atomic absorption flame spectrophotometer (Perkins-Elmer, Shelton, CT, USA) equipped with a graphite furnace. The detection limit was 5 μg Zn/l water and 0.5 μg Zn/g tissue. External quality control was achieved by digesting and analyzing identical amounts of rehydrated (90% water) standard reference materials (DORM-2, Dogfish Liver-2-organic matrix, provided by the NRC-CNRC, National Research Council, Canada). Recovery rate was 97 ± 1.7%.

2.3. Data analysis

Growth rates were calculated by fitting abalone shell length data obtained from Yang and Ting (1986, 1994) to an exponential model (ln shell length $(L) = a + gt$, where a is a constant, g is the growth rate (day^{-1}), and t is the time in days). Depuration rate constants (k_d) were determined by fitting concentration C to a first-order decay curve ($\ln C = c + (k_d + g)t$ where c is a constant and t is the time in days). Depuration half-lives ($t_{1/2}$) were calculated as $\ln 2/(k_d + g)$.

The method to determine assimilation efficiency was by fitting concentration data to the integrated form of the kinetic equation for constant water and time-dependent dietary exposure, using iterative nonlinear regression (Reinfelder et al., 1998),

$$C_m(t) = C_m(0)e^{-(k_d+g)t} + \frac{(k_u C_w) + (\alpha f C_a(t))}{k_d + g}(1 - e^{-(k_d+g)t}), \tag{1}$$

where α is the assimilation efficiency of abalone, f is the abalone grazing rate (0.25 ± 0.05 g g^{-1} day^{-1} Chen and Lee, 1999), $C_m(t)$ is the time-dependent Zn concentration in abalone (μg g^{-1}), C_w is the dissolved Zn concentration in water (μg ml^{-1}), $C_a(t)$ is the time-dependent Zn concentration in algae (μg g^{-1}), t is the time (day), and k_u is the abalone uptake rate of Zn (ml g^{-1} day^{-1}) and can be obtained by fitting concentration data to the kinetic equation (i.e., the first-order one-compartment uptake-depuration model) for constant water exposure, using nonlinear iterative regression,

$$C_m(t) = C_m(0)e^{-(k_d+g)t} + \mathrm{BCF}C_w(1 - e^{-(k_d+g)t}), \tag{2}$$

where BCF is the bioconcentration factor of abalone for Zn and can also be expressed as $\mathrm{BCF} = k_u/(k_d + g)$. Biomagnification factor (BMF) was calculated from the equation $\mathrm{BMF} = \alpha f/(k_d + g)$. For food source of abalone, the red algae *G. tenuistipitata* var. *liui*, the uptake rate constant (k_{1a}) and depuration rate constant (k_{2a}) can also be calculated by fitting the concentration data to the kinetic equation for constant water exposure, using nonlinear iterative regression,

$$C_a(t) = C_a(0)e^{-(k_{2a}+g_a)t} + \frac{k_{1a}}{(k_{2a} + g_a)} C_w(1 - e^{-(k_{2a}+g_a)t}), \tag{3}$$

where g_a is the *G. tenuistipitata* var. *liui* growth rate (0.038 ± 0.013 day^{-1} Lee et al., 1999). Bioconcentration factor of algae (BCF_a) can be calculated by the equation $\mathrm{BCF} = k_{1a}/(k_{2a} + g_a)$.

We employed the nonlinear option of the Statistica® software (StatSoft, Tulsa, OK, USA) to perform all curve fittings. The Statistica® was also used to calculate the coefficient of determination (r^2) and to perform other statistical analyses (analysis of variance and Student's t-test). Statistical significance was determined if p values were less than 0.05.

2.4. Modeling bioavailable Zn to abalone

The amount of Zn absorbed through abalone (bioabailable Zn) can be described in absolute and relative terms. Absolute bioavailability (F_a), also referred to as the absorption fraction, is given by: F_a = absorbed dose/ingested dose (Schroder et al., 2003). For a consumer–resource system in the trophic transfer processes between abalone and algae, the relative bioavailability, F, is used and can be expressed as the ratio of the F_a of Zn present in consumer (abalone) to the F_a of Zn in resource (algae), that is (Schroder et al., 2003),

$$F = F_a\ (\text{abalone})/F_a\ (\text{algae}). \tag{4}$$

Bourne (1995) and de Vries (1996) suggested that in pharmacokinetic modeling, the AUC is commonly applied to estimate the total amount of substance eliminated from the whole body of organisms over a certain time period. Analogously, over a certain time period, the total amount of absorbed dose in the target tissue is equal to the amount of toxic compound that has been removed from the target tissue, i.e., AUC can be a surrogate measurement for the absorbed dose.

The concept of the relative bioavailability in this present research could be restated as follows: Relative bioavailable Zn to abalone is determined by assessing the proportion of Zn that can be taken up by abalone from their food source, the red algae *G. tenuistipitata* var. *liui*. Therefore, the time-dependent relative bioavailable Zn to abalone ($F(t)$) is calculated according to Eq. (4) as,

$$F \equiv \frac{[\text{AUC}/D_n]_{\text{abalone}}}{[\text{AUC}/D_n]_{\text{algae}}} \Rightarrow F(t) = \frac{\text{AUC}_m}{\text{AUC}_a} \times \frac{D_{n,a}(t)}{D_{n,m}(t)} = \frac{\int_0^t C_m(t)\mathrm{d}t}{\int_0^t C_a(t)\mathrm{d}t} \times \frac{D_{n,a}(t)}{D_{n,m}(t)}, \tag{5}$$

where AUC_m and AUC_a are the areas under the whole-body burden of Zn concentrations in abalone and in algae versus time curves, respectively (μg g^{-1} day), and $D_{n,m}$ and $D_{n,a}$ represent the doses normalized to the body mass of abalone and algae, respectively (μg g^{-1}) and can be seen as the whole-body burdens of Zn in abalone (i.e., $C_m(t)$) and in algae (i.e., $C_a(t)$), respectively.

The mean residence time (MRT) of Zn in abalone is calculated by dividing the first moment of the whole-body Zn burden versus time profile by the AUC (Bourne, 1995),

$$\text{MRT} = \frac{\int_0^t tC_m(t)\mathrm{d}t}{\int_0^t C_m(t)\mathrm{d}t}. \tag{6}$$

We consider constant dietary exposure and $C_m(0) = 0$ in Eq. (1), AUC_m in Eq. (5) has the form as,

$$\text{AUC}_m = \int_0^t C_m(t)\mathrm{d}t = \left[\frac{\text{BCFC}_w + \text{BMFC}_a}{(k_d + g)}\right]((k_d + g)t + e^{-(k_d+g)t} - 1). \tag{7}$$

Similarly, $\mathrm{AUC_a}$ has the form as,

$$\mathrm{AUC_a} = \int_0^t C_\mathrm{a}(t)\mathrm{d}t = \frac{\mathrm{BCF_a}}{(k_\mathrm{2a}+g_\mathrm{a})} C_\mathrm{w}((k_\mathrm{2a}+g_\mathrm{a})t + \mathrm{e}^{-(k_\mathrm{2a}+g_\mathrm{a})t} - 1). \tag{8}$$

By substituting Eqs. (2), (3), (7) and (8) into Eq. (5), the mathematical expression of relative bioavailable Zn to abalone subject to constant water and dietary exposure has a following explicit form in terms of biokinetic and physiological parameters as,

$$F(t) = \left[\frac{(k_\mathrm{d}+g)t + \mathrm{e}^{-(k_\mathrm{d}+g)t} - 1}{(k_\mathrm{2a}+g_\mathrm{a})t + \mathrm{e}^{-(k_\mathrm{2a}+g_\mathrm{a})t} - 1}\right]\left[\frac{(k_\mathrm{2a}+g_\mathrm{a})(1-\mathrm{e}^{-(k_\mathrm{2a}+g_\mathrm{a})t})}{(k_\mathrm{d}+g)(1-\mathrm{e}^{-(k_\mathrm{d}+g)t})}\right], \tag{9}$$

$$= \frac{\dfrac{t}{(1-\mathrm{e}^{-(k_\mathrm{d}+g)t})} - \dfrac{1}{(k_\mathrm{d}+g)}}{\dfrac{t}{(1-\mathrm{e}^{-(k_\mathrm{2a}+g_\mathrm{a})t})} - \dfrac{1}{(k_\mathrm{2a}+g_\mathrm{a})}}.$$

Eq. (9) reveals that four parameters, abalone depuration rate constant for Zn (k_d), algae depuration rate constant for Zn (k_2a), algae growth rate (g_a), and abalone growth rate (g), are needed to predict the relative bioavailable Zn to abalone due to trophic transfer and water exposure.

3. Results and discussion

3.1. Physiological and biokinetic parameters

The best fit of growth rate model for shell length of 2–3.5 and 4–5 cm of abalone *H. diversicolor supertexta* shows relatively high r^2 values (Fig. 1 and Table 1). The 14-day water exposure experiment of Zn in soft tissue and shell of *H. diversicolor supertexta* and algae *G. tenuistipitata* var. *liui* had significant correlated nonlinear regression profiles ($p<0.05$, $r^2=0.68$–0.99) resulting from the best fit of the first-order one-compartment uptake-depuration model (Fig. 2). Table 2 summarizes the experimentally determined bioaccumulation parameters for Zn calculated from *H. diversicolor supertexta* and *G. tenuistipitata* var. *liui* exposure data.

The assimilation efficiency for Zn in *H. diversicolor supertexta* was in the range of 20–49% (Table 2). Wang and Fisher (1997), Reinfelder et al. (1998) and Lee et al. (2000) reported that the average assimilation efficiencies for Zn in marine copepods and mussels ranged from 32% to 57%. The calculated biological retention half-lives for soft tissue and shell of abalone were about 53 and 16 days, respectively (Table 2), indicating that Zn can be very efficiently retained in soft tissue than that in shell.

3.2. Relative bioavailable Zn to abalone

With known growth rate constants and depuration rate constants for abalone and algae, Eq. (9) was then used to calculate the relative bioavailable Zn to *H. diversicolor supertexta*, in that trophic transfer and water exposure account for the apparent accumu-

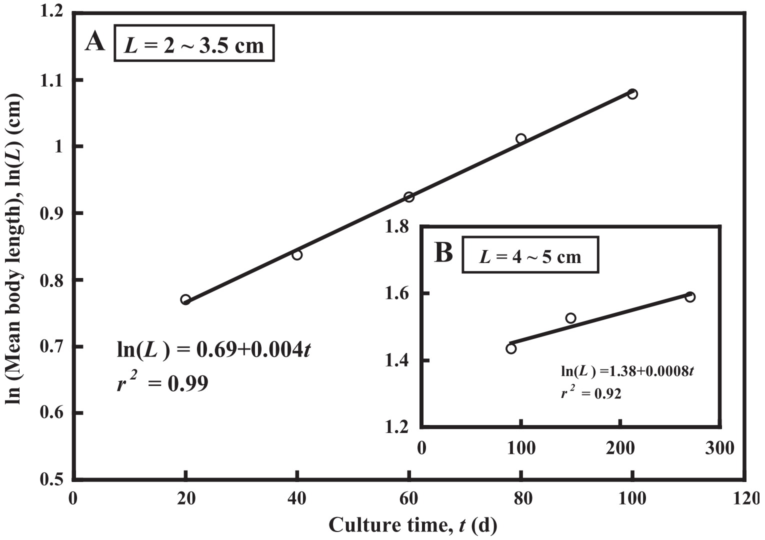

Fig. 1. Optimal fits of growth rate model for shell length (*L*) of abalone *H. diversicolor supertexta* at (A) $L=2-3.5$ cm and (B) $L=4-5$ cm in which open circles denote data points obtained from Yang and Ting (1986, 1994) and solid lines represent growth model fitting.

lation of Zn. The calculated relative bioavailable Zn to abalone during uptake phase in the exposure experiment is illustrated in Fig. 3. The relative bioavailable Zn to abalone decreased rapidly in the first 2 days then attained a steady-state by the end of the uptake phase. In the 7-day uptake experiment, the time-dependent relative bioavailable Zn to soft tissue and shell of *H. diversicolor supertexta* decreased from around 89% in the first day to around 60% in day 7 (Fig. 3A). Estimated average relative bioavailable Zn to soft tissue and shell of *H. diversicolor supertexta* were 71.04 ± 9.71% and 68.44 ± 8.29%, respectively, during uptake phase (Fig. 3B).

Reinfelder et al. (1998) reported that relative bioavailable Zn to marine copepods and to bivalves (*C. virginica, M. mercenaria, M. balthica*) ranged between 70–80% and 55–65%, respectively, when depuration rate constants varied between 0.01 and 0.1 day^{-1}. Therefore, using *H. diversicolor supertexta* collected from coastal water-cultured abalone farms in Taiwan as an example, these estimated relative bioavailable Zn are remarkably comparable to the field-measured values (55–80%), suggesting that the parameters identified in the AUC-based model can account for relative bioavailable Zn to abalone.

Table 1
Growth rate constants (mean ± 1 S.E.) for *H. diversicolor supertexta*[a]

Shell length (cm)	Growth rate (10^{-3} day^{-1})[b]
2–3.5	4.0 ± 0.12 ($r^2=0.99$)
4–5	0.8 ± 0.23 ($r^2=0.92$)

[a] Observation data obtained fromYang and Ting (1986, 1994).

[b] Growth rates calculated using the equation ln lenght = $a+g$ (time) (day), where g is the growth rate and r^2 is the coefficient of determination.

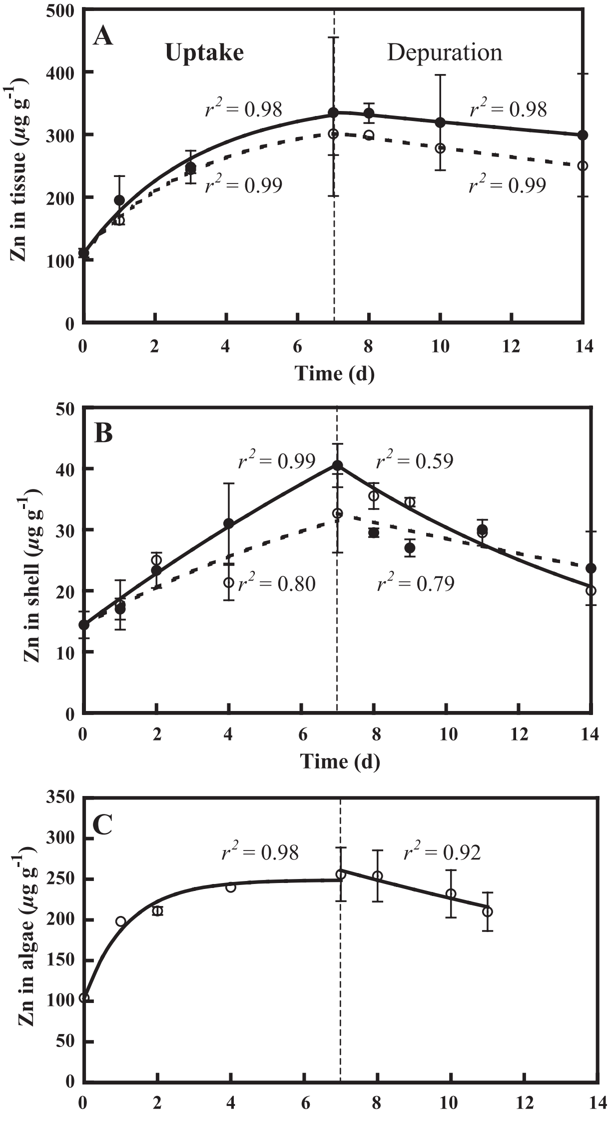

Fig. 2. Optimal fits of laboratory 14-day exposure experiment of uptake and depuration of Zn by (A) soft tissue and (B) shell of *H. diversicolor supertexta* during 7-day exposure and then 7-day depuration period. The measurements are shown with symbols (●: fed with algae; ○: kept without algae); and model fittings are shown in lines (—: fed with algae; · · ·: kept without algae) and (C) uptake/depuration of Zn by red alga *G. tenuistipitata* var. *liui* where measurements are shown with symbols and the model fitting is shown in solid line. Error bars show one standard deviation from the mean.

Table 2
Physiological and bioaccumulation parameters (mean ± 1 S.E.) for Zn calculated from abalone *H. diversicolor supertexta* and algae *G. tenuistipitata* var. *liui* exposure experiment

Parameters	Values
Soft tissue of H. diversicolor supertexta	
Assimilation efficiency, α (%)[a]	34.57 ± 14.60 (0.199–0.492)
Depuration rate constant, k_d (day^{-1})[b]	0.013 ± 0.0015 (with algae) 0.024 ± 0.002 (without algae)
Depuration half-life, $t_{1/2}$ (d)[c]	53.31
Uptake rate constant, k_u (ml g^{-1} day^{-1})[a]	99.13 ± 7.48
BMF[a]	4.95 ± 2.05
BCF (ml g^{-1})[d]	328.25
MRT (day)[e]	3.99
Shell of H. diversicolor supertexta	
Depuration rate constant, k_d (day^{-1})[b]	0.044 ± 0.044 (with algae) 0.063 ± 0.022 (without algae)
Depuration half-life, $t_{1/2}$ (day)[c]	15.75
Uptake rate constant, k_u (ml g^{-1} day^{-1})[a]	4.84 ± 1.51
BCF (ml g^{-1})[b]	48.36
G. tenuistipitata var. *liui*	
Depuration rate constant, k_{2a} (day^{-1})[f]	0.811 ± 0.21
Uptake rate constant, k_{1a} (ml g^{-1} day^{-1})[g]	211.62 ± 47.09
BCF_a (ml g^{-1})[g]	261
MRT (day)[e]	3.75

[a] Assimilation efficiency, uptake rate constant, and biomagnification factor (BMF) calculated from Eq. (1).

[b] Depuration rate constant calculated using the equation $\ln C = c + (k_d + g)t$.

[c] Depuration half-life calculated from $t_{1/2} = \ln 2 / k_d$.

[d] Bioconcentration factor (BCF) calculated from Eq. (2).

[e] Mean residence time (MRT) calculated from Eq. (6).

[f] Depuration rate constant of algae calculated using the equation $\ln C = c + (k_{2a} + g_a)t$.

[g] Algae uptake rate of Zn and bioconcentration factor of algae (BCF_a) calculated from Eq. (3).

3.3. Effect of parameters

A typical example of the simulation carried out for the relative bioavailable Zn to *H. diversicolor supertexta* under various biokinetic and physiological conditions is shown in Fig. 4. In calculating the effect of each parameter for relative bioavailable Zn to abalone in Eq. (9), the mean numeric values of other parameters (Table 2) were used. Among the four parameters considered, relative bioavailable Zn to abalone is greatly affected by abalone depuration rate constant for Zn (k_d) and abalone growth rate (g) and is less dependent on algae growth rate (g_a) (Fig. 4).

Fig. 4 reveals that the predicted relative bioavailable Zn to abalone varies by a difference change of 26–36% when k_d and g increasing by one order of magnitude, as compared to a 0–7% difference change for g_a and k_{2a}. The result therefore suggests that k_d and g should not be ignored in the AUC-based modeling, whereas g also should not be ignored in the biokinetic modeling of Zn concentration in abalone. Our results thus further indicate that trophic transfer plays an important role in determining the relative bioavail-

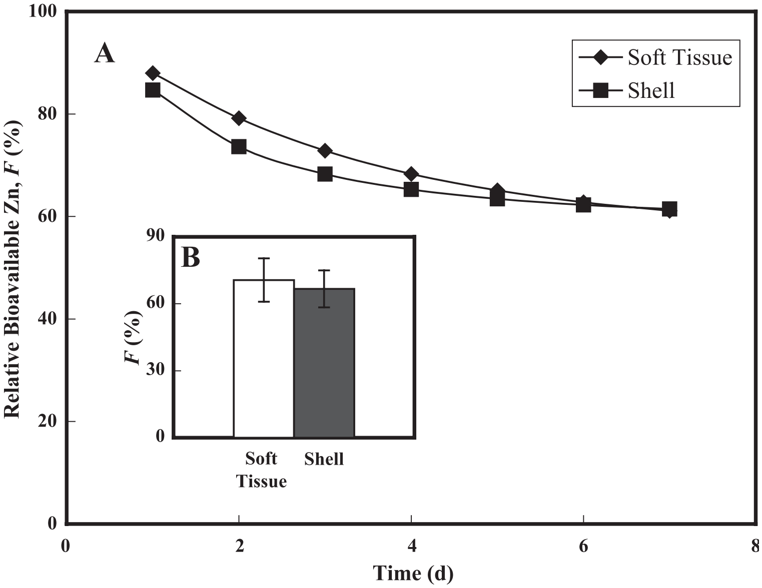

Fig. 3. Estimated relative bioavailable Zn to soft tissue and shell of *H. diversicolor supertexta* during uptake phase of exposure experiment: (A) Time profile of relative bioavailable Zn calculated by Eq. (9) and (B) average relative bioavailable Zn to soft tissue (71.04 ± 9.71% (mean ± S.D.)) and to shell (68.44 ± 8.29%).

able Zn to abalone. Thus, it is possible to interpret relative bioavailable Zn to abalone in a mechanistic way based on certain important physiological and biokinetic parameters that can be obtained from deliberately laboratory measurements.

3.4. Uncertainty analysis

Because of limitations in data and theories to support proposed modeling, there is a need to characterize uncertainty and variability in the model approach and input parameters. In the current study, we explicitly address parameter uncertainty.

We applied a Monte Carlo approach to estimate the impact of parameter uncertainty on predictions of relative bioavailable Zn to abalone in Eq. (9). Lognormal distributions are used to represent abalone depuration rate of Zn (k_d), algae depuration rate of Zn (k_{2a}), algae growth rate (g_a), and abalone growth rate (g). To test the convergence and the stability of the numerical output, we performed independent runs at 1, 4, 5, and 10 thousand iterations with each parameter sampled independently from the appropriate distribution at the start of each replicate. Largely because of limitations in the data used to derive model parameters, inputs were assumed to be independently. The result shows that 5000 iterations are sufficient to ensure the stability of results. In this case, the numerical error on the 95th percentile is equal to 2%. The simulation was implemented using @RISK (Version 4.5, Professional Edition, Palisade, USA).

Fig. 5 illustrates the probability density functions (pdfs) and cumulative distribution functions (cdfs) of Zn bioavailavility in soft tissue and shell of abalone. Probabilistic

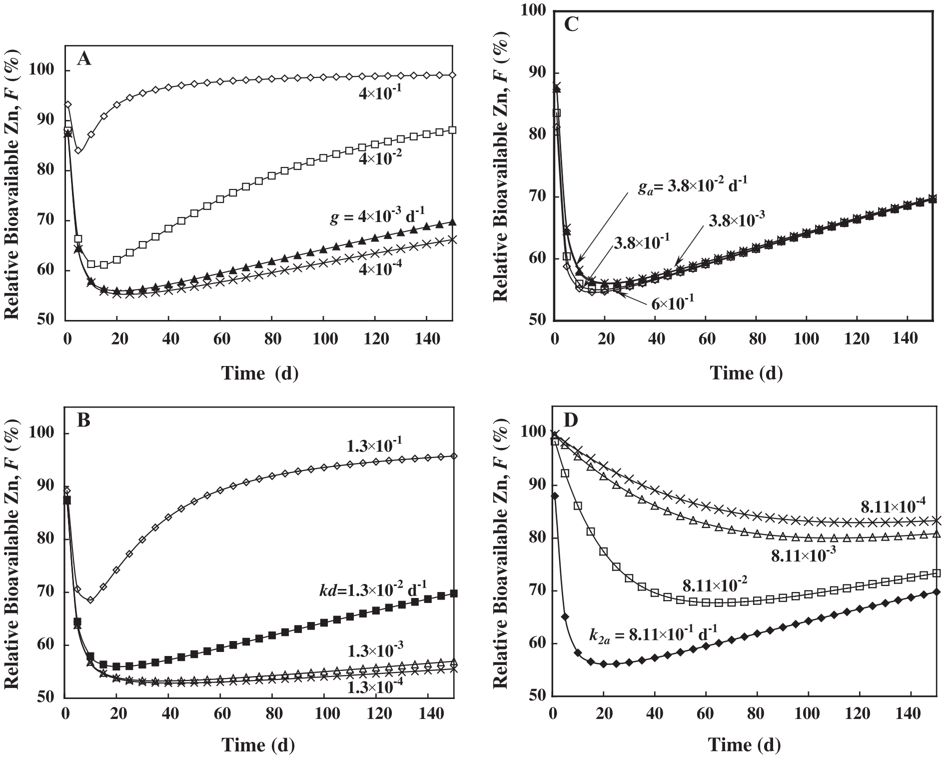

Fig. 4. Simulations carried out for relative bioavailable Zn to *H. diversicolor supertexta* under various biokinetic parameters (Zn depuration rate constants for abalone (k_d) and for algae (k_{2a})) and physiological parameters (growth rate constants for abalone (g) and for algae (g_a)). The solid symbols indicate mean numeric values shown in Table 2 and the open symbols represents increased and reduced order of magnitude of the parameter considered.

simulations of the AUC-based model produce skewed distributions of predicted relative bioavailable Zn to abalone. Percentile predictions of relative bioavailable Zn to abalone could be determined from cdfs illustrated in Fig. 5B and D. Fig. 6 shows box plots of interquartile- and 50th-percentile predictions associated with whisker plots indicating 10th- and 90th-percentile predictions of relative bioavailable Zn to soft tissue and shell of abalone.

Fig. 5A and C shows that the pdfs of relative bioavailable Zn to *H. diversicolor supertexta* have a lognormal distribution of a geometric mean (gm) of 0.55 and a geometric standard deviation (gsd) of 1.13 (LN(0.55, 1.13)) for soft tissue and a gm 0.63 with gsd 1.41 (LN(0.63, 1.41)) for shell, respectively. The relative bioavailable Zn to shell has a higher uncertainty as quantified by the variance, i.e., parameter variability of gsd. Fig. 5A and C also demonstrates that relative bioavailable Zn to soft tissue has a greater likelihood of occurrence than the same relative bioavailable Zn to shell in that the relative skewness and spread in modeled output associated with a long tail at higher bioavailable Zn.

Compared with the field observations (Reinfelder et al., 1998), measured mean relative bioavailable Zn to bivalves ($F = 55–65\%$) all fell within the 10th and 90th percentile

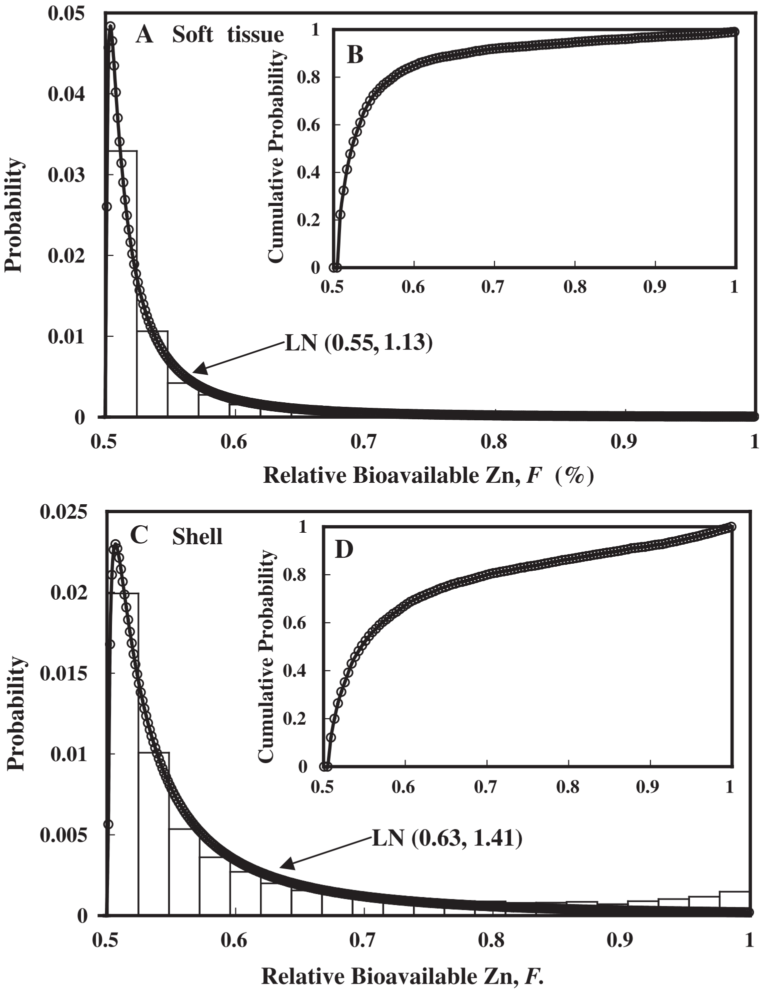

Fig. 5. Uncertainty simulations show probability density functions (pdfs) and cumulative distribution functions of relative bioavailable Zn to (A) soft tissue and (B) shell of *H. diversicolor supertexta*. Results indicate that pdfs of LN(0.55, 1.13) and LN(0.63, 1.41) for soft tissue and shell, respectively, in which LN(x,y) denotes lognormal distribution with geometric mean x and geometric standard deviation y.

prediction range (Fig. 6). Relative to minimum and maximum field data, however, lower and upper probabilistic predictions were more conservative. This is evident that the modeling framework and the distributional parameters and assumptions in the model are appropriate for estimating relative bioavailable Zn to *H. diversicolor supertexta*.

3.5. Implications

Our study suggests that relative bioavailable Zn to *H. diversicolor supertexta* can be predicted with reasonable accepted ranges by a parsimonious AUC-based biokinetic

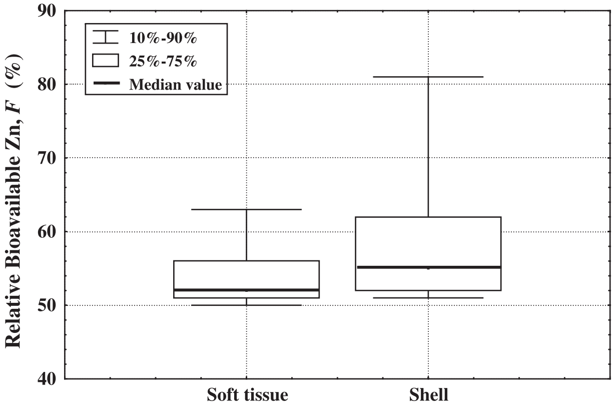

Fig. 6. Box and whisker plot representations of percentile predictions of relative bioavailable Zn to soft tissue and shell of *H. diversicolor supertexta*.

model if important metal-related biokinetic parameters (assimilation efficiency and depuration rate constant) and animal's physiological parameters (grazing rate and growth rate) are properly identified and determined. The AUC-based biokinetic model can also be used to assess the significance of trophic transfer and to understand the important biological processes governing relative bioavailable metals to aquatic animals.

Our results show that the depuration rate constant and growth rate constant of abalone and algae are critical in affecting relative bioavailable Zn to abalone. Consequently, variation of relative bioavailable Zn to abalone may reflect the influence of abalone growth rate, grazing rate, depuration rate, and assimilation efficiency, which are in turn dependent on food availability, season, and other environmental conditions. Wang et al. (1999) also pointed out that metal uptake may be more dependent on the physiological conditions of the aquatic animals than on the metal concentration in ingested food source, particularly for metals such as Zn, which may be regulated in prey organisms.

The AUC-based biokinetic model may also be useful in ecological and human assessment when predicting the bioaccumulation of toxicants in aquacultural species from food, as it relates to consumption by humans or other species. As ecological risk assessment requirements demand more predictive power and accuracy, better methods are needed to accurately measure the concentration reaching internal target organs. The AUC concept derived from pharmacology in the proposed model in predicting relative bioavailable Zn to abalone meets this demand.

This present study revealed that metal-related biokinetics and physiological processes due to a stochastic environment are typically in rather complex ways within species and ecosystems. Such complex interactions present major challenges and require the proper experiments for exploring the underlying complex mechanisms. Thus, understanding the exposure pathways is critical for the setting of aquacultural water quality criteria. Our study highlights the significance of trophic transfer in the overall relative bioavailable metals to aquatic animals. Future work should focus on incorporating consumer–resource dynamics (Liao et al., 2002) and AUC-based biokinetics in a variety of circumstances to conduct more detailed mechanistic models in predicting more accurate metal bioavailability to aquacultural animals.

4. Conclusions

A mechanistic model based on an AUC-based biokinetic rate model can predict relative bioavailable Zn to abalone *H. diversicolor supertexta*. This present relative bioavailable metal prediction model indicates that growth rate constants and depuration rate constants of abalone and algae are the critical controlling parameters in predicting relative bioavailable Zn to abalone.

A sensitivity analysis of the model reveals that relative bioavailable Zn to abalone is greatly affected by growth rate and depuration rate constants of abalone and is less dependent on algae growth rate. An uncertainty analysis of the model shows that the probability density functions of relative bioavailable Zn to abalone followed a lognormal distribution of geometric means of 0.55 and 0.63 with geometric standard deviations of 1.13 and 1.41 for soft tissue and shell, respectively.

Before *H. diversicolor supertexta* can be designated as an appropriate biomonitor of Zn contamination in aquacultural and coastal waters, relative bioavailable metal information must be fully perceived. Our study suggests that relative bioavailable metals to aquatic animals can be predicted only when both physiological and biokinetic processes are considered.

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