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A Simple Allometric Diffusion-Based Biokinetic Model to Predict Cu(II) Uptake Across Gills of Freshwater Clam *Corbicula fluminea*

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Abstract The purpose of this study was to link Fick's type mass transfer and biokinetics together with Michaelis-Menten kinetics to arrive at a simple predictive framework for quantifying biouptake mechanisms in gills of freshwater clam *Corbicula fluminea* exposed to Cu(II). A diffusion-based Cu(II) influx and permeability can be calculated using physiological and allometric-related parameters. Simulations indicate that Cu(II) bioconcentration factor of gills was 42. Estimated steady-state Cu(II) gill uptake influx and permeability were 0.097 nmol cm^{-2} s^{-1} and 0.48 cm s^{-1}, respectively. The proposed simple allometric diffusion-based biokinetic model meets the need for describing non-equilibrium aspects of biouptake mechanisms in bivalve gills.

Keywords Freshwater clam · Gills · Biouptake · Cu(II)

Freshwater clam *Corbicula fluminea* is a commercially important native species and has a high market value to Taiwan's aquaculture (http://www.fa.gov.tw) with wide farming distribution in the western and eastern coastal areas of Taiwan. Yet, human activities have greatly increased the flux of many potential toxic metals to aquatic ecosystems. Therefore, if waterborne metals are elevated, pollutant-induced changes in the mobility can occur, which has potentially risks on the health of clam, resulting in severe economic losses nation-widely due to bans on harvesting of contaminated clam and the need for costly monitoring programmes. Owing to direct contact with ambient water, gills are proposed to be the first and most important targets of fish/shellfish exposed to waterborne contaminants (Jorgensen 1996; Tao et al. 2000) and which is the most important mediation between whole body and contamination of water. Several studies also indicated that the major route of uptake for waterborne metals that concentrate in fish/shellfish is across the gill epithelium (Pelgrom et al. 1997; Bury et al. 1999). Moreover, the bioconcentration plays the important role in aquatic organism health, since the metal will accumulated in internal and induce adverse effect. Yet the accumulation capacity will dependent on the size and age of aquatic organism. Hence, in order to clarify the movement of a metal into an organism, it is fundamental to develop a clear understanding of the mechanism of metal uptake through gills of organisms with allometric relationships in the aquatic ecosystems.

Many studies have been reported that ion transport processes in freshwater bivalves exhibit saturation kinetics (McCorkle and Dietz 1980; Dietz and Byrne 1990; Zheng and Dietz 1998). Many researchers have suggested thereafter that waterborne metals are normally taken up via gills of bivalves by passive diffusion and can be described by Fick's first law of diffusion (McCorkle and Dietz 1980; Potts and Hedges 1991). McCorkle and Dietz (1980) indicated that solute transport mechanisms in *C. fluminea* dominated by passive and exchange diffusion. *C. fluminea* is a filter feeder; the gills serve dual functions of feeding and respiration, and are thus very complex organs. Many research efforts in biouptake flux have been exercised on a combination of both the diffusion mass transfer flux and actual biological uptake flux (Sijm and van der Linde 1995; Guyon et al. 1999; Kwon et al. 2006).

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The purpose of this paper was to develop a parsimonious mathematical model to predict Cu(II) uptake across gills of *C. fluminea* by linking biokinetic and diffusion mass transfer based on allometric relationships.

Materials and Methods

A linkage of a diffusion mass transfer model and a kinetic model can be used to estimate uptake and elimination of chemicals across the gills (Sijm and van der Linde 1995; Guyon et al. 1999). Generally, the uptake rate constant k_1 (mL g^{-1} day^{-1}) and elimination rate constant k_2 (day^{-1}) can be determined from the increase and decrease of the concentration in organism during the exposure time window, based on a bioaccumulation model that is assumed to follow a first-order biokinetic model as,

$$\frac{dC_m}{dt} = k_1 C_w - (k_2 + k_g) C_m, \quad (1)$$

where C_m is the Cu(II) concentration in *C. fluminea* (μg g^{-1}), C_w is the bulk concentration of Cu(II) in the water (M), and k_g is the growth rate of *C. fluminea* (day^{-1}).

The diffusion-based uptake rate constant k_u (mL g^{-1} s^{-1}) can be described as (Sijm and van der Linde 1995),

$$k_u = \left[\frac{\delta_w}{D_{aq}} + \frac{\delta_m}{D_m K_D}\right]^{-1} \frac{SA}{W}, \quad (2)$$

where δ_w is aqueous diffusion layer thickness (μm), D_{aq} is the diffusion coefficient of Cu(II) in aqueous solution (cm^2 s^{-1}), δ_m is the membrane diffusion layer thickness (μm), D_m is the diffusion coefficient in gill membrane (cm^2 s^{-1}), K_D is the distribution coefficient (dimensionless), SA is the gill surface area (cm^2), and W is the body weight (g wet wt).

The diffusion-based elimination rate constant k_e (s^{-1}) can be described as (Sijm and van der Linde 1995),

$$k_e = \left[\frac{\delta_w}{D_{aq}} + \frac{\delta_m}{D_m K_D}\right]^{-1} [(1-\alpha) + \alpha K_D]^{-1} \frac{SA}{W}, \quad (3)$$

where α is the lipid content that is an important parameter for the elimination rate constant.

Generally, the fish physiological data are most available for use in estimating model parameters. Since no general equations are available for bivalve could to found, it is assumed that equations used to estimate the physiological conformation for fish were also suitable for that of bivalve. We recognized that it is not possible to estimate all the physiological parameters based on the existed experimental data since individual experiments for *C. fluminea* were not available, such as thickness of gills lamellae, aqueous diffusion layer thickness, and membrane diffusion layer thickness. Therefore, the preliminary database adopted form Liao et al. (2008) regarding the shell length and body weight in *C. fluminea* is used to estimate a range of physiological model parameters.

The diffusion coefficient in gill membrane D_m can be estimated as 0.3 times the diffusion coefficient in the aqueous diffusion layer (Lien and McKim 1993) as: $D_m = 0.3 D_{aq}$. The aqueous diffusion layer thickness depends on the distance between two gills lamellae. The thickness of gills lamellae of *C. fluminea* (d, μm) can be estimated as the function of body weight as (Sijm and van der Linde 1995),

$$d = 20.5 W^{0.114}. \quad (4)$$

Thus, the aqueous diffusion layer thickness δ_w can be estimated as (Sijm and van der Linde 1995),

$$\delta_w = 0.1 d = 2.05 W^{0.114}. \quad (5)$$

On the other hand, the membrane diffusion layer thickness (δ_m) can be approximately estimated based on δ_w and bioconcentration factor (BCF) as (Sijm and van der Linde 1995) as

$$\delta_m = 0.3 \delta_w \mathrm{BCF}. \quad (6)$$

Parsimoniously, the distribution coefficient K_D can also be related to bioconcentration factor (BCF) as: $K_D = \mathrm{BCF} = k_1/(k_2 + k_g)$ (Sijm and van der Linde 1995; Del Vento and Dachs 2002; Bayen et al. 2006; Kwon et al. 2006). The allometic relationship between gill surface area (SA, mm^2) and shell length (SL, mm) for *C. fluminea* can be approximately described by a linear relationship based on a regression model (Payne and Miller 1995),

$$SA = a + b SL^2 \ (n = 34), \quad (7)$$

with $a = 63.2 \pm 9$ and $b = 0.78 \pm 0.16$.

Here we used the Fick's law to describe Cu(II) transport across gills of *C. fluminea* (Guyon et al. 1999),

$$J = -\frac{V}{SA}\left(\frac{dC_w}{dt}\right) = \left[\frac{\delta_w}{D_{aq}} + \frac{\delta_m}{D_m K_D} + \frac{1}{k_t}\right]^{-1} C_w, \quad (8)$$

where J is the internalization flux of Cu(II) (mol g^{-1} s^{-1}) V is the gill volume (cm^3), and k_t is the pseudo-first-order rate constant for the transfer of Cu(II). The Cu(II) internalization flux can also be expressed by a Michaelis-Menten (M-M) kinetics as (Liao et al. 2007),

$$J(C_w) = \frac{J_{\max} C_w}{K_m + C_w}, \quad (9)$$

where $J_{\max}$ is the M-M maximum Cu(II) uptake rate (μmol g^{-1} h^{-1}) and K_m is the half-saturation affinity constant (μM).

Table 1 Input published data used in the proposed model

	Range value	Reference
Shell length (SL, mm)	27.6 ± 2.4[a]	Liao et al. (2008)
Body weight (W, g wet w)	6.19 ± 0.86[a]	Liao et al. (2008)
Diffusion coefficient of Cu(II) (D_{aq}, $cm^2\ s^{-1}$)	7.8×10^{-6}	Guyon et al. (1999)
Uptake rate constant (k_1, $mL\ g^{-1}\ day^{-1}$)	224	Croteau and Luoma (2005)
Elimination rate constant (k_2, day^{-1})	0.058	Croteau and Luoma (2005)
Lipid content (α, $kg_{fat}\ kg^{-1}$)	0.0189 (0.0158–0.0225)[b]	Yang et al. (2006)
M-M maximum Cu(II) influx (J_{max}, $\mu mol\ g^{-1}\ h^{-1}$)	0.369 (0.26–0.51)[b]	Liao et al. (2007)
M-M affinity constant (K_m, μM)	7.87×10^{-3} (5.72×10^{-3}–11.2×10^{-3})[b]	Liao et al. (2007)

[a] Mean ± SD (number of clams = 140)

[b] 95% CI

In light of Eq. (9), the permeability of Cu(II) in clam gill membrane can be describe approximately (i.e., k_t approaches infinity) as (Guyon et al. 1999; Kwon et al. 2006),

$$P = \frac{J}{C_w} = \left[\frac{\delta_w}{D_{aq}} + \frac{\delta_m}{D_m K_D}\right]^{-1}, \tag{10}$$

where P is the permeability of Cu(II) across gills membrane of *C. fluminea* ($\mu m\ s^{-1}$).

Table 1 summarizes the published data used in the simulation study.

Results and Discussion

Table 2 lists the estimated parameter values used in the present paper. The predicted diffusion-based biokinetics in gill membrane of *C. fluminea* varied with waterborne Cu(II) (0–400 nM) is illustrated in Fig. 1a. The diffusion-based biokinetic parameters of the Cu(II) biouptake and elimination rate constants were calculated to be $0.026\ mL\ g^{-1}\ s^{-1}$ and $6.19 \times 10^{-4}\ s^{-1}$, respectively, based on body weight of 6.19 g wet wt and shell length of 27.6 mm. The BCF of gills was calculated to be $41.69\ mL\ g^{-1}$, indicating that gills pose the strong potential to accumulate Cu while *C. fluminea* exposed to a threshold waterborne Cu concentration. Elimination half-life was calculated to be nearly 50 min, indicating that it will take a relative long time to exchange Cu(II) across the gills. Figure 1b shows Cu(II) levels in gills membrane as a function of membrane lamellae thickness varied with response times (0–20 s) at the constant waterborne Cu(II) of 200 nM. Our estimated gills lamellae thickness of 25.24 μm is closed to measured value of 23.3 ± 0.7 μm of relaxed gills of *C. fluminea* (Medler and Silverman 2001). The Cu(II) concentration gradient increases with response time 0–20 s. The result shows that membrane lamellae Cu(II) levels are nearly constant with a nonlinear fashion, suggesting that Cu(II) transfer across the gills lamellae is fast. Therefore, diffusion in the gill membrane is the limiting step. This finding was consistent with McCorkle and Dietz (1980) for Na transport in *C. fluminea*.

Table 2 Estimated parameter values used in the proposed model

	Range value	Equation
Growth rate of *C. fluminea* (k_g, day^{-1})	0.045[a]	
Membrane lamellae thickness (d, μm)	25.24	Eq. (4)
Aqueous diffusion thickness (δ_w, μm)	2.52	Eq. (5)
Membrane diffusion thickness (δ_m, μm)	445.64	Eq. (6)
Gill surface area (SA, cm^2)	6.57	Eq. (7)
Diffusion coefficient in gill membrane (D_m, $cm^2\ s^{-1}$)	2.34×10^{-6}[b]	
Distribution coefficient (K_D, $mL\ g^{-1}$)	2153.85[c]	
Diffusion uptake rate (k_u, $mL\ g^{-1}\ s^{-1}$)	0.026	Eq. (2)
Diffusion elimination rate (k_e, s^{-1})	6.19×10^{-4}	Eq. (3)

[a] Estimated based on data adopted from Chen (2007)

[b] $D_m = 0.3 D_{aq}$

[c] $K_D = BCF = k_1/(k_2 + k_g) = 224\ mL\ g^{-1}\ day^{-1}/(0.058\ day^{-1} + 0.045\ day^{-1}) = 2153.85\ mL\ g^{-1}$

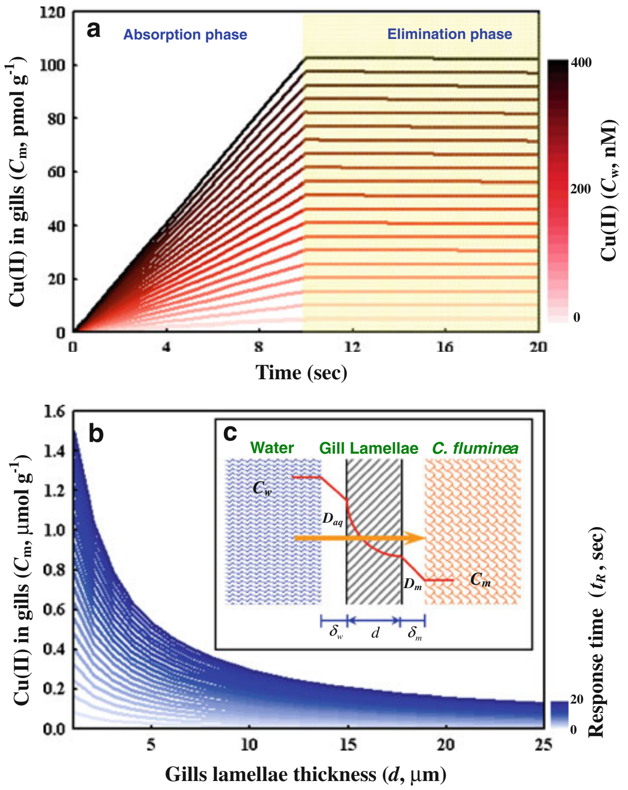

Fig. 1 Simulation of **a** absorption and elimination dynamics of Cu(II) in gills of *C. fluminea* varied with waterborne Cu(II) 0–400 nM. **b** Cu(II) in gills lamellae as the function of lamellae thickness varied with response times 0–20 s at waterborne Cu(II) of 200 nM based on transport pathway shown in (**c**)

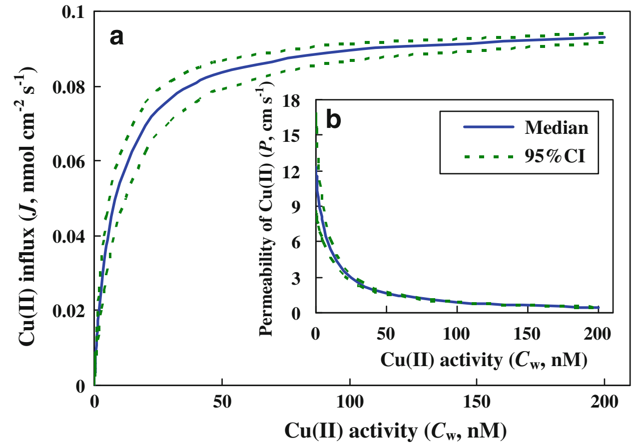

Fig. 2 Cu(II) influx and permeability across gill membrane of *C. fluminea* as the function of waterborne Cu(II) ranging from 0–200 nM. **a** Cu(II) influx based on M–M kinetics (Eq. (9)). **b** Cu(II) permeability across gill membrane

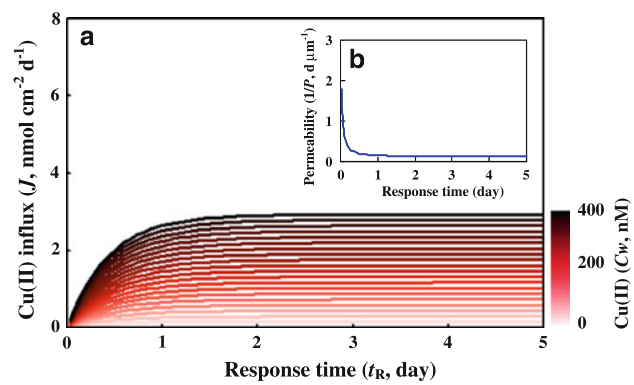

Fig. 3 Simulation of the dynamics response in gills of *C. fluminea* exposed to waterborne Cu(II). **a** Cu(II) internalization flux dynamics in gills as the function of waterborne Cu(II) 0–400 nM. **b** Time-varying Cu(II) permeability across gills membrane

M-M kinetics in Eq. (9) was used to determine the biouptake flux of Cu(II) by *C. fluminea* varied with waterborne Cu(II) concentration based on $J_{\max} = 0.348$ μmol cm^{-2} h^{-1} and $K_m = 7.87 \times 10^{-3}$ μM (e.g., 0.5 mg L^{-1}) based on $SA/W = 1.06$ cm^2 g^{-1} (Fig. 2a). Waterborne Cu(II) concentration dependent membrane permeability across gills of freshwater clam therefore could be derived (Fig. 2b). The steady-state Cu(II) biouptake flux and permeability were estimated to be 0.097 nmol cm^{-2} s^{-1} and 0.48 cm s^{-1}, respectively. To the best of our knowledge, information on biouptake parameters of Cu(II) in *C. fluminea* gills is limited (Liao et al. 2007). The K_m value represents inverse of binding affinity of transport metal. The binding affinity of a metal for biotic ligand is a function of ligand chemistry and type of bond formation, yet the characteristics of actual binding sites on gills of *C. fluminea* are not entirely known (Zheng and Dietz 1998).

To investigate the dynamic response of Cu(II) internalization flux across gills of *C. fluminea*, Eq. (8) can be used to simulate the response time-dependent $J(t_R)$ by incorporating non-steady state $K_D(t_R) = k_u/k_e(1 - \exp(-k_e t_R))$ into Eq. (8) (Fig. 3a). The time-varying membrane permeability therefore can be predicted based on Eq. (10) (Fig. 3b). Generally, a steady-state approached after 5 h for Cu(II) internalization flux in gills for waterborne Cu(II) ranging from 0–400 nM. Specifically, Cu(II) permeability across gills membrane of *C. fluminea* decreased from 1.78 to a steady-state value of 0.13 d μm^{-1} after 5 h, indicating the nonequilibrium aspects of the biouptake processes involved (Fig. 3b).

Looking forward, our analytical framework could quantify a dynamics of biouptake processes instead of the traditionally steady-state condition. Ideally, this could provide an assessment of the relative contributions of different mechanisms to Cu(II) bioavailability in bivalves. We showed that for a relative high bioaccumulated and bioavailable circumstance, such as Cu(II) biouptake by gills of *C. fluminea*, the equilibrium distributions are not generally achieved and the time-varying biouptake processes involved. The proposed simple allometric diffusion-based biokinetics may meet the need of clarifying the nonequilibrium aspects of biouptake mechanisms in bivalve gills. The dynamics of such system could be captured by appropriate future research focusing on absorption of Cu(II) to the body surface and uptake by the gut as well as on a more through evaluation of particulate or dissolved phases of Cu(II) in freshwater clam farms. Understanding the processes controlling metal biouptake in organisms is also a need for enhancing dynamic risk assessment in ecotoxicology. Further, our study may highlight the role of underlying physical limits on the biological design of artificial membrane systems inspired by gills of *C. fluminea*. We recommend that future research focus on a more thorough evaluation of the interactions of geoscience with biophysiology in *C. fluminea*.

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