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Linking valve closure behavior and sodium transport mechanism in freshwater clam *Corbicula fluminea* in response to copper

Chung-Min Liao [a,*], Chieh-Ming Lin [a], Li-John Jou [a,b], Kuo-Chin Chiang [a]

[a] *Department of Bioenvironmental Systems Engineering, National Taiwan University, 1, Sec. 4, Roosevelt Road, Taipei, Taiwan 10617, ROC*
[b] *Department of Biomechatronic Engineering, National Ilan University, Ilan, Taiwan 260, ROC*

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A new flux–biological response model can link valve closure and sodium transport mechanisms in freshwater clam in response to copper.

Abstract

The purpose of this study is to develop a mechanistic model to describe a conceptually new ''flux–biological response'' approach based on biotic ligand model (BLM) and Michaelis–Menten (M–M) kinetics to allow the linkage between valve closure behavior and sodium (Na) transport mechanism in freshwater clam *Corbicula fluminea* in response to waterborne copper (Cu). We test the proposed model against published data regarding Na uptake kinetics in rainbow trout and Na uptake profile in *C. fluminea*, confirming that the predictive model is robust. Here, we show that the predicted M–M maximum Cu internalization flux in *C. fluminea* is 0.369 μmol g^{-1} h^{-1} with a half-saturation affinity constant of 7.87×10^{-3} μM. Dynamics of Na uptake and valve closure daily rhythm driven by external Cu can also be predicted simultaneously. We suggest that this ''Na transport–valve closure behavior'' approach might provide the basis of a future design of biomonitoring tool.
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Keywords: Clam; *Corbicula fluminea*; Biotic ligand model; Valve closure behavior; Sodium transport; Copper

1. Introduction

Freshwater *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 potential risks on the health of clam, resulting in severe economic losses nation-wide due to bans on harvesting of contaminated clam and the need for costly monitoring programmes.

The development and implementation of effective remedial measures depend on our ability to predict the fate and effects of metals in these systems. The free ion activity model (FIAM) based on sound physicochemical and biological principles has shown great potential as predictive tools (Campbell, 1995; Morel and Hering, 1993; Hare and Tessier, 1996). FIAM suggests that the free ion concentration (or activity) plays a central role as a regulator of interactions (uptake, toxicity) between metals and aquatic organisms, postulating that only a small proportion of trace metals are found as free hydrated ions with the majority of the metal being complexed by ligands. Brown and Markich (2000) and Markich et al. (2003) have employed the extended FIAM to develop a conceptual model to quantify the effect of toxicity of Cd and Cu on valve movement behavior of freshwater bivalve *Hyridella depressa*. Markich et al. (2003) indicated that the valve movement behavior of *H. depressa* exposed to total Cd was directly proportional to

* Corresponding author. Tel.: +886 2 2363 4512; fax: +886 2 2362 6433.
E-mail address: cmliao@ntu.edu.tw (C.-M. Liao).

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the activity of the free metal ion (Cd^{2+}) in the linear region of the concentration–response profiles, indicating that the predictive acute toxicity to *H. depressa* supports the extended FIAM.

The biotic ligand model (BLM) is one of the new generation of models that describe the bioavailable fraction of metal causing toxicity in aquatic organisms (Paquin et al., 2002a,b; Niyogi and Wood, 2004). BLM quantifies the affinity and capacity of the gills (biotic ligand, BL) of aquatic organisms to bind metals and relates this binding to acute toxicity. BLM evolves from the gill surface interaction model (GSIM) (Pagenkopf, 1983) and the free ion activity model (FIAM) (Campbell, 1995). BLM linking with equilibrium geochemical modeling framework (i.e., Windermere humic aqueous mode (WHAM) (Tipping, 1994)) can describe the competition of the free metal ion with other naturally occurring cations (e.g., Ca^{2+}, Na^{+}, Mg^{2+}, H^{+}), together with complexation by abiotic ligands (e.g., DOM, chloride, carbonates, sulfide) for binding with the BL. The concentration of this metal–BL complex determines the magnitude of the toxic effect, independent of the physiochemical characteristics of the medium. 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., 2002a,b). This indicates that naturally occurring cations (e.g., Na^{+}) can offer protection by competing with Cu^{2+} for binding sites on the gill, whereas naturally occurring anions can bind Cu^{2+}, rendering it poorly available to gill sites.

One of the key problems of environmental toxicology remains how to best relate metal exposures to their biological effects. Most research has been based upon the assumption that equilibrium is attained between the metal in the bulk solution and that adsorbed to sensitive sites on the cell surface, i.e., metal internalization flux is rate-limiting. In such case, the metal-induced biological response can be related either to the free metal ion concentration in solution (basis of the FIAM), or to the metal bound to sensitive sites at the surface of the organism (basis of the BLM). Under such steady-state conditions, the metal internalization flux should follow a Michaelis–Menten (M–M) kinetic model (Paquin et al., 2002a,b; Chen and Liao, 2004; van Leeuwen et al., 2005). Our preliminary studies (Liao et al., submitted for publication) indicated that free ionic form of waterborne Cu bind specifically to a biotic ligand (i.e., clam gills) and impair normal valve closure behavior, indicating that a fixed-level of metal accumulation at a biotic ligand is required to elicit specific biological effects. Liao et al. (submitted for publication) also demonstrated that the time-dependent EC50(t) and valve closure behavior in response to Cu at any response time could be well predicted (Fig. 1A). Our preliminary results confirmed that BLM could be improved to analytically and rigorously describe the bioavailable fraction of metal causing toxicity to valve closure behavior in freshwater *C. fluminea.*

Bianchini et al. (2002) and Morgan and Wood (2004) indicated that acute metal toxicity is a function of Na^{+} uptake rate, implying that Na^{+} uptake data can be utilized to identify species sensitive to metal exposure and predict response time of biological behavior. Grosell et al. (2002) suggested that Na^{+} transport mechanism could be incorporated into BLM for risk management decisions. McCorkle and Dietz (1980) have examined the Na^{+} transport mechanism in pondwater-acclimated *C. fluminea*, indicating that Na influx in 0.5 mM Na_2SO_4 was estimated to be 7.90 ± 0.79 μmol Na g^{-1} dry tissue h^{-1}. Saturation of the transport system followed the M–M kinetics, demonstrating that maximum influx (J_{max}) was 12.90 ± 3.01 μmol Na g^{-1} dry tissue h^{-1} with a M–M affinity constant K_m of 0.05 mM Na representing the Na concentration at which the influx equals $J_{max}/2$ (Fig. 1B). They also indicated that Na movement in *C. fluminea* may be classified into passive diffusion, excretion, exchange diffusion, and active transport in which exchange diffusion comprises a substantial portion (67%) of Na movement of 5.91 ± 0.80 μmol Na g^{-1} dry tissue h^{-1}.

However, knowing that the Na^{+} turnover is a physiological process associated with the gill membranes and the key mechanism of acute metal toxicity consists of reduction in Na^{+} uptake by blockade of gill Na^{+}/K^{+}-ATPase in the gill of freshwater mussels (Saintsing and Towle, 1978; Dietz and Findley, 1980) and fish (Bianchini et al., 2002; Grosell et al., 2002; Paquin et al., 2002a,b; Morgan and Wood, 2004; Zhou et al., 2005), and recognizing that many of the factors that influence the valve closure behavior and Na^{+} transport mechanism would normally operate on endogenous and dietary substances, we decided to propose a hypothesis (Fig. 1C).

The aim of this paper is to test the hypotheses that FIAM and BLM associated with M–M model contain sufficient information to allow the linkage between valve closure behavior and Na^{+} transport mechanism and to allow the predictions of (1) the Cu internalization flux as a function of bioavailable Cu^{2+}-activity, (2) time-profile of internal sodium concentration, and (3) the relationships between sodium loss from clam and gill copper burden without any prior knowledge of the clam's physiological profile (Fig. 1C).

2. Materials and methods

The Cu-BLM framework applied to *C. fluminea* associated with the overall rates of uptake and loss of Na from the clam is diagrammed in Fig. 2. Our approach for developing a mechanistic model to predict the linkage between valve closure behavior and Na^{+} transport mechanism in *C. fluminea* based on the concepts obtained from FIAM, BLM, and M–M kinetics is illustrated in Fig. 3 and is described in the subsequent sections.

2.1. Cu-BLM-clam model

In light of the concept of Cu-BLM framework (Fig. 2), a modified version of the basic Hill model equation (Liao et al., 2005) can be developed based on free Cu^{2+}-activity and BLM-based EC50 model and is referred to as the Cu-BLM-clam model (Fig. 3A),

$$R(\Delta t, Cu^{2+}) = \frac{R_{max} \times \{Cu^{2+}\}^{n(\Delta t)}}{[EC50(\Delta t)_{CuBL}]^{n(\Delta t)} + \{Cu^{2+}\}^{n(\Delta t)}}, \quad (1)$$

where $R(\Delta t, Cu^{2+})$ is the time-dependent valve response (% response) based on Cu^{2+}-activity $\{Cu^{2+}\}$ (M) at any given clam response time Δt, $EC50(\Delta t)_{CuBL}$ is the time-dependent BLM-predicted acute Cu EC50 value (M), R_{max} is

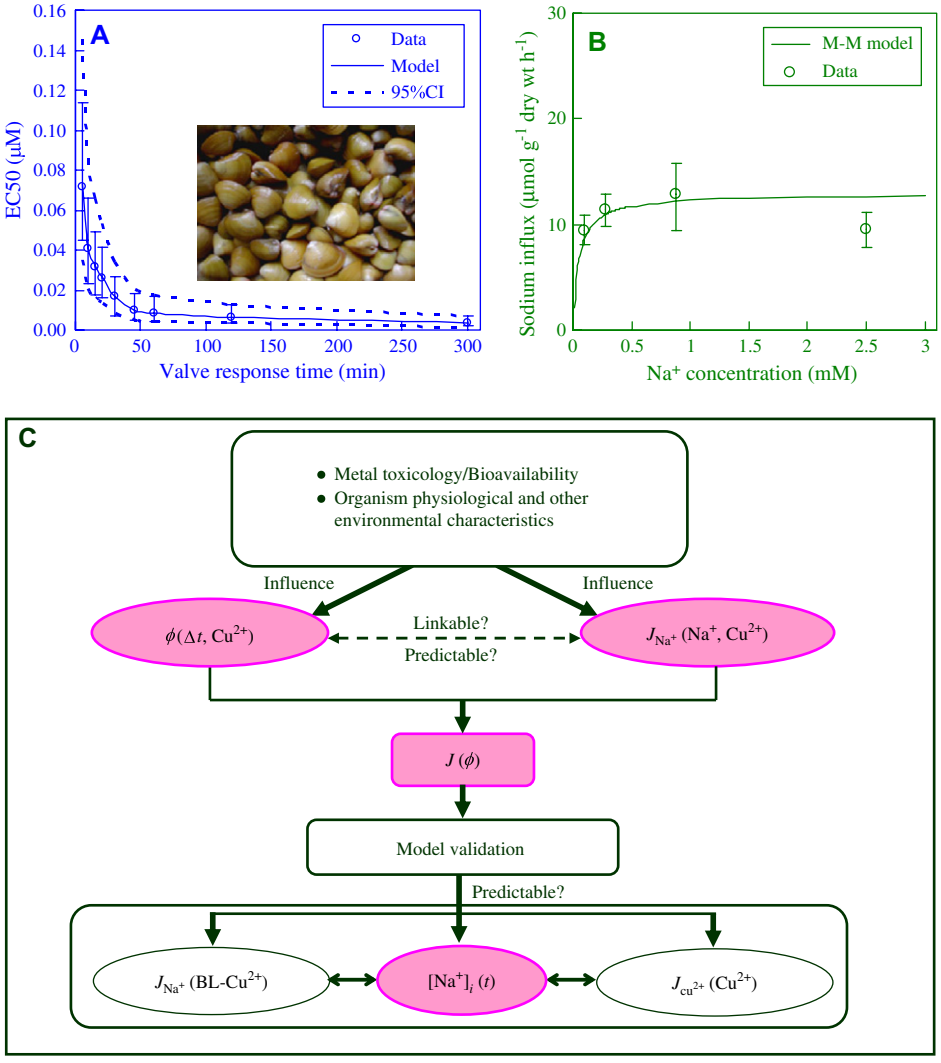

Fig. 1. Preliminary studies and the proposed "flux–biological response" hypothesis. (A) BLM-based response time-dependent EC50 values of valve closure behavior of *C. fluminea* in response to Cu. (B) Na transport mechanism in *C. fluminea* follows the Michaelis–Menten kinetics, showing that maximum influx J_{max} is 12.90 ± 3.01 μmol Na g^{-1} dry tissue h^{-1} with a half-saturation affinity constant K_m of 0.05 mM based on McCorkle and Dietz (1980). (C) Schematic of our "flux–biological response" hypothesis in that $\phi(\Delta t, Cu^{2+})$ is the response time (Δt)- and Cu^{2+}-dependent valve closure response, $J_{Na^+}(\Delta t, Cu^{2+}, Na^+)$ is the Na^+ uptake rate, $J(\phi)$ is our proposed integrated model, $J_{Na^+}(BL - Cu^{2+})$ is gill Cu burden-based Na loss rate, $[Na^+]_i$ is the internal Na concentration, and $J_{Cu^{2+}}(Cu^{2+})$ is the Cu internalization flux.

the maximum response (%), and $n(\Delta t)$ is a time-dependent Hill coefficient, which is a measure of cooperativity. A value of $n > 1$ indicates positive cooperativity.

Based on the refined Cu-BLM scheme (De Schamphelaere et al., 2002), $EC50(\Delta t)_{CuBL}$ in Eq. (1), taking into account the bioavailability and toxicities of $CuOH^+$ and $CuCO_3$, has the form as,

$$EC50(\Delta t)_{CuBL} = \frac{f^{50\%}_{CuBL}(\Delta t)}{\left(1 - f^{50\%}_{CuBL}(\Delta t)\right)}\left(\frac{[b]}{[a]}\right), \quad (2)$$

where $[a] = K_{CuBL} + K_{CuOHBL}K_{CuOH}\{OH^-\} + K_{CuCO_3BL}K_{CuCO_3}\{CO_3^{2-}\}$, $[b] = 1 + K_{CaBL}\{Ca^{2+}\} + K_{MgBL}\{Mg^{2+}\} + K_{NaBL}\{Na^+\} + K_{HBL}\{H^+\}$ in that K_{CuBL}, K_{CaBL}, K_{MgBL}, K_{NaBL}, K_{HBL}, K_{CuOHBL}, K_{CuCO_3BL} are the stability constants for the binding of these cations to the BL (M^{-1}); K_{CuOH} and K_{CuCO_3} are the stability constants for the formations of the $CuOH^+$ and $CuCO_3$, respectively (M^{-1}); and {ion} denotes the activity of each ion of water chemistry characteristics (M), and $f^{50\%}_{CuBL}(\Delta t)$ is the time-dependent fraction of the total number of Cu binding sites occupied by Cu at 50% effect. The evaluation of Eq. (2) for predicting time-dependent EC50 values expressed as $\{Cu^{2+}\}$ requires values of cation activities and known stability constants associated with the calculated fraction of the BL sites occupied by Cu. Practically, Eq. (2) is integrated into WHAM scheme by adding stability constants for the binding of metal species (i.e., Cu^{2+}, $CuOH^+$, and $CuCO_3$) and competing cations (Ca^{2+}, Mg^{2+}, Na^+, and H^+) onto the BL (Fig. 2). Through the linkage of Eqs. (1) and (2) and WHAM (WHAM VI, Centre for Ecology and Hydrology, Lancaster, UK) associated with calculated $f^{50\%}_{CuBL}(\Delta t)$ value that can be estimated by fitting Eq. (2) to the published $EC50(\Delta t)$ data, we could predict a site-specific concentration–time-response profile of valve closure behavior of *C. fluminea* in response to waterborne Cu.

2.2. Sodium transport–valve closure model

To understand how the "flux analysis–biological response" structure determines the dynamics of biouptake and bioavailability, we built a mathematical model constrained by experimental observations. This model can be deliberately incorporated in both the M–M kinetic model and the Cu-BLM-clam model in Eq. (1) to obtain a sodium transport–valve closure response model denoting as $J(\phi)$ (Fig. 3B),

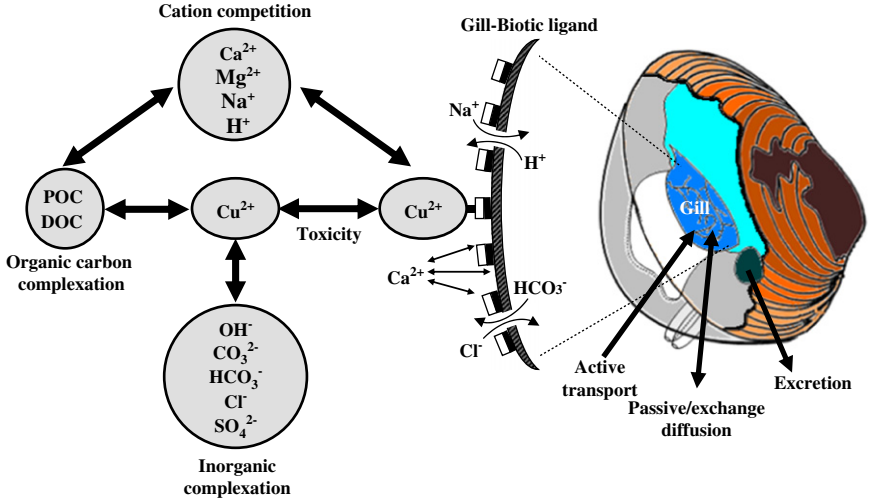

Fig. 2. Schematic diagram of the Cu-BLM framework applied to the freshwater clam *C. fluminea* associated with the overall rates of uptake and loss of Na from the clam. POC = particular organic carbon and DOC = dissolved organic carbon. (Modified from Paquin et al. (2002a,b) and Liao et al. (submitted for publication)).

$$J(\phi)\equiv J_{Na^+}\left(\Delta t,\phi\left(\Delta t,Cu^{2+},Na^+\right)\right)=J_{max}\times\left(1-I_{J_{Na^+}}\left(\Delta t,\phi\left(\Delta t,Cu^{2+},Na^+\right)\right)\right)$$
$$=J_{max}\times\left[1-\frac{1\times\phi^{m(\Delta t)}}{\left[ER50_\phi(\Delta t)\right]^{m(\Delta t)}+\phi^{m(\Delta t)}}\right], \quad (3)$$

where $\phi(\Delta t,Cu^{2+},Na^+)$ is a $\{Cu^{2+}\}$-dependent clam valve closure response function taking into account external Na^+ activity and can be rewritten based on Eq. (1) as,

$$\phi\left(\Delta t,Cu^{2+},Na^+\right)=\frac{\phi_{max}\times\left\{Cu^{2+}\right\}^{n(\Delta t)}}{\left[c(\Delta t)+d(\Delta t)\{Na^+\}\right]^{n(\Delta t)}+\left\{Cu^{2+}\right\}^{n(\Delta t)}}, \quad (4)$$

where $c(\Delta t)$ and $d(\Delta t)$ are the fitted parameters corresponding to $EC50(\Delta t)_{CuBL}$, J_{max} is the M–M maximum Na^+ uptake rate in the absence of $\{Cu^{2+}\}$ ($\mu mol\,g^{-1}\,h^{-1}$), $m(\Delta t)$ is the response time-dependent Hill coefficient, $ER50_\phi(\Delta t)$ is the 50% effective response due to the % inhibition of Na^+ uptake rate $I_{J_{Na^+}}$ that can be expressed by linking the M–M kinetic model as,

$$I_{J_{Na^+}}=1-\frac{J_{Na^+}\left(\Delta t,Cu^{2+},Na^+\right)}{J_{max}}, \quad (5)$$

where the Na^+ uptake rate $J_{Na^+}(\Delta t,Cu^{2+},Na^+)$ follows the M–M kinetics,

$$J_{Na^+}\left(\Delta t,Cu^{2+},Na^+\right)=\frac{J_{max}\left(\left\{Cu^{2+}\right\}\right)\times\left\{Na^+\right\}}{K_m\left(\Delta t,\left\{Cu^{2+}\right\}\right)+\left\{Na^+\right\}}, \quad (6)$$

where $\{Cu^{2+}\}$-dependent $J_{max}(\{Cu^{2+}\})$ and response time- and $\{Cu^{2+}\}$-dependent half-saturation affinity constant $K_m(\Delta t,\{Cu^{2+}\})$ can be obtained by fitting Eq. (6) to the data predicted from our proposed model in Eq. (3) subjected to various external $\{Cu^{2+}\}$.

2.3. Physiological processes predictions

Here, we link Cu biodynamics with transport physiology in the *C. fluminea* (Fig. 3C). Grosell et al. (2002) suggested that the internal sodium concentration decays exponentially if sodium uptake is inhibited,

$$[Na]_i(t)=[Na]_i(0)\exp(-kt), \quad (7)$$

where $[Na]_i$ is the internal Na concentration ($\mu mol\,g^{-1}$) and the decay rate constant k (h^{-1}) is defined as,

$$k=\frac{I_{J_{Na^+}}J_{Na^+}\left(\Delta t,Cu^{2+},Na^+\right)}{[Na]_i(0)}. \quad (8)$$

To obtain the relationships between Na uptake and gill Cu burden, we use fraction of the total number of Cu binding sites occupied by $Cu(f_{CuBL})$ to represent the BL–Cu burden in that f_{CuBL} is given by (De Schamphelaere et al., 2002),

$$f_{CuBL}=\frac{\left([a]\left\{Cu^{2+}\right\}\right)\left[BL^-\right]}{\left([a]\left\{Cu^{2+}\right\}+[b]\right)\left[BL^-\right]}. \quad (9)$$

We incorporate Eq. (9) into Eq. (4) to obtain the relationships between f_{CuBL} and $\phi(\Delta t,Cu^{2+})$ as $f_{CuBL}(\Delta t,\phi)$. Finally, the relationships between the inhibition of Na uptake and BL–Cu expressed by f_{CuBL} can be derived by incorporating $f_{CuBL}(\Delta t,\phi)$ into $J(\phi)$ model in Eq. (3) and expressed as a Hill model,

$$I_{J_{Na^+}}(\Delta t,f_{CuBL})=\frac{f_{CuBL}^{v(\Delta t)}}{\left[ER50_f(\Delta t)\right]^{v(\Delta t)}+f_{CuBL}^{v(\Delta t)}}, \quad (10)$$

where the Hill coefficient $v(\Delta t)$ and $ER50_f(\Delta t)$ can be obtained by fitting the model to the data.

The concentration of unoccupied gill-BL sites can be estimated by linking the gill Cu burden [CuBL] in the BLM scheme (De Schamphelaere et al., 2002) and a one-compartment uptake-elimination model at a steady-state condition (Croteau et al., 2004),

$$[CuBL]_T=\left[CuBL^+\right]+\left[CuOHBL^0\right]+\left[CuCO_3BL^-\right]$$
$$=\left[BL^-\right][a]\left\{Cu^{2+}\right\}\approx\frac{k_1}{k_2}\left\{Cu^{2+}\right\}, \quad (11)$$

where $[CuBL]_T$ is the steady-state gill Cu–BL burden ($\mu mol\,g^{-1}$), $[BL^-]$ is the concentration of unoccupied gill BL sites ($\mu mol\,g^{-1}$), and k_1 ($L\,g^{-1}\,d^{-1}$) and k_2 (d^{-1}) are the uptake and depuration rate constants, respectively. Based on Eq. (11), we can directly estimate $[BL^-]$ from the knowledge of k_1/k_2 and $[a]$ provided by published data as $[BL^-]=(k_1/k_2)/[a]$ (Croteau and Luoma, 2005). By the definition of Cu internalization flux as $J_{Cu^{2+}}\equiv[CuBL]_T/\Delta t$ and under a steady-state condition, the Cu internalization flux as a function of external bioavailable $\{Cu^{2+}\}$ can be given by a M–M kinetics as,

$$J_{Cu^{2+}}\equiv\frac{[CuBL]_T}{\Delta t}=\frac{J_{Cu^{2+},max}\left\{Cu^{2+}\right\}}{K_{m,Cu^{2+}}+\left\{Cu^{2+}\right\}}, \quad (12)$$

where $J_{Cu^{2+},max}=[BL^-]/\Delta t=(k_1/k_2)[a]^{-1}/\Delta t$ is the M–M maximum Cu^{2+} uptake rate ($\mu mol\,g^{-1}\,h^{-1}$) and $K_{m,Cu^{2+}}$ is a half-saturation affinity constant

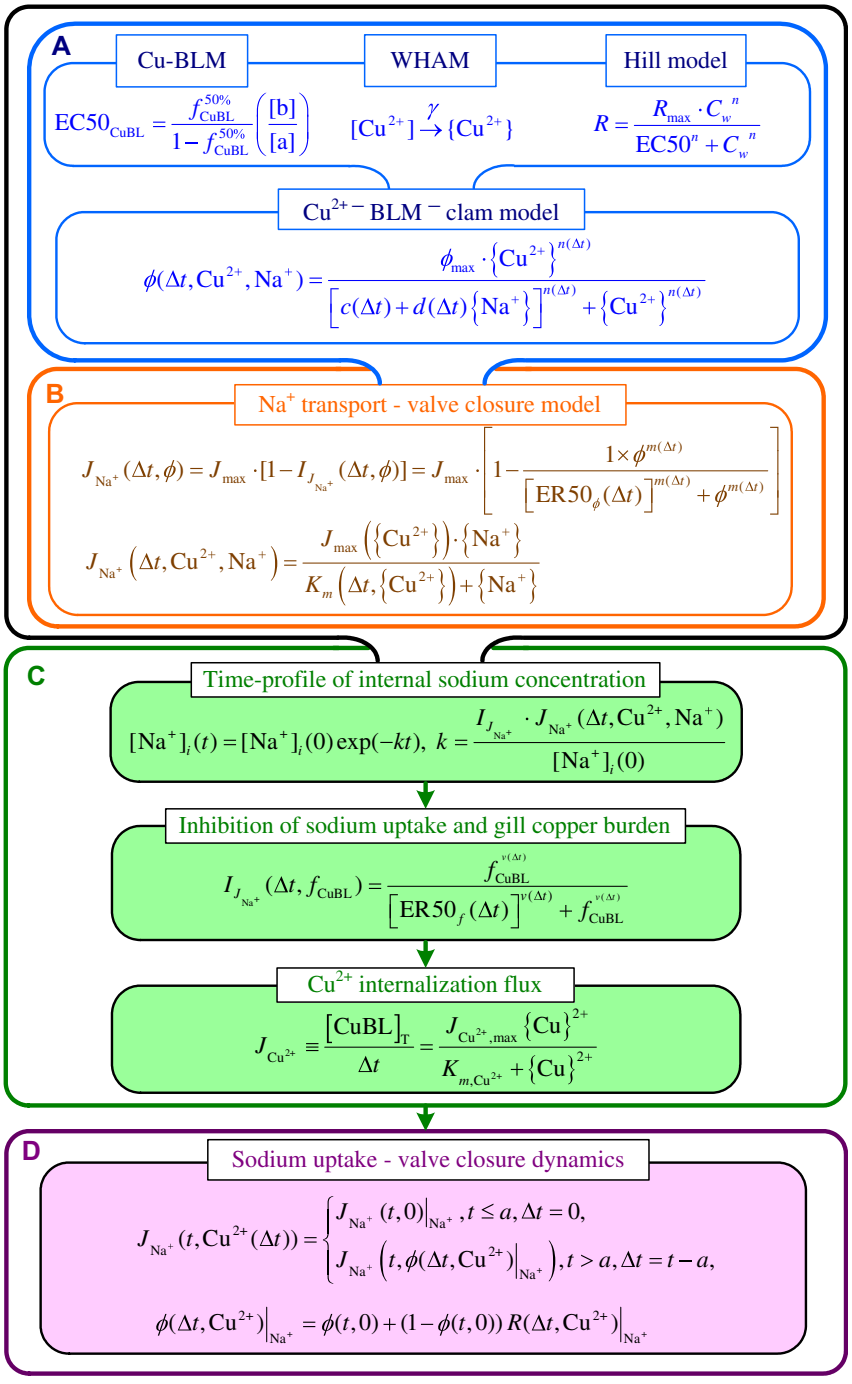

Fig. 3. A conceptual algorithm showing a mechanistic model to predict the linkage between valve closure behavior and Na transport mechanism in *C. fluminea*. (See text for detail descriptions of symbol).

(μM) that can be estimated from a limiting diffusion flux of free metal expressing as a linear function (Jansen et al., 2002),

$$J_{Cu^{2+},diff} = \frac{[BL^-][a']\{Cu^{2+}\}}{\Delta t} = \frac{(k_1/k_2)[a]^{-1}[a']\{Cu^{2+}\}}{\Delta t}, \quad (13)$$

where $J_{Cu^{2+},diff}$ is a diffusion-limited Cu-uptake rate and $[a']$ can be obtained from the published water chemistry characteristics by McCorkle and Dietz (1980). Finally, by linking Na^+ uptake rate $J_{Na^+}(\Delta t, Cu^{2+}, Na^+)$ in Eq. (6) with Eq. (12), we can capture the relationships between Na uptake and Cu internalization flux expressed as $J_{Na^+}(\Delta t) - J_{Cu^{2+}}$ profile varied by different external $\{Na^+\}$.

2.4. Sodium uptake–valve closure dynamics

We link the $J(\phi)$ model shown in Eq. (3) and the fitted model of clam valve opening/closing daily rhythm to predict the Na uptake and bivalve closure behavior dynamics in response to waterborne Cu (Fig. 3D),

$$J_{Na^+}(t, Cu^{2+}(\Delta t)) = \begin{cases} J_{Na^+}(t,0)|_{Na^+} = J_{max}, & t\le a,\ \Delta t = 0, \\ J_{Na^+}(t,\phi(\Delta t, Cu^{2+})|_{Na^+}), & t > a,\ \Delta t = t-a, \end{cases} \quad (14)$$

and the valve closure behavior dynamics can be predicted as,

$$\phi(t, Cu^{2+})|_{Na^+} = \phi(t,0)+(1-\phi(t,0))R(\Delta t, Cu^{2+})|_{Na^+}, \quad (15)$$

where $\phi(\Delta t, Cu^{2+})|_{Na^+}$ is the valve closure daily rhythm at time t in response to $\{Cu^{2+}\}$ subjected to a specific external $\{Na^+\}$, a is an initiation time of clam in response to waterborne Cu, and $\phi(t, 0)$ is the daily rhythm function of valve closure exposed to unpolluted water and has the form modeled as a three-parameter lognormal model (Liao et al., 2005),

$$\phi(t,0) = \begin{cases} \phi_1(t,0) = 12.3\exp\left[-0.5(\ln(t/4)/0.20)^2\right] + 3.8, & 0 \le t \le 7,\ r^2 = 0.84, \\ \phi_2(t,0) = 14.8\exp\left[-0.5(\ln(t/18.2)/0.083)^2\right] + 3.6, & 7 \le t \le 24,\ r^2 = 0.92. \end{cases} \quad (16)$$

Eq. (16) has a bimodal distribution that is separated at 07:00 a.m. based on the suggestion by Tran et al. (2003).

3. Results

3.1. Clam Na uptake predictions in response to Cu

The reconstructed clam valve closure response function incorporating external Na^+ activity can be obtained by fitting $\phi(\Delta t, Cu^{2+}, Na^+)$ in Eq. (4) to the reported data of response time-specific concentration–% valve closure response profiles (Tran et al., 2004), resulting in the fitted parameters of $n(\Delta t) = 1.221 + 0.988\exp(-\Delta t/37.703)$ ($r^2 = 0.89$), $c(\Delta t) = 1.05 \times 10^{-8} + 6.44 \times 10^{-8}\exp(-\Delta t/31.743)$ ($r^2 = 0.99$), and $d(\Delta t) = 4.46 \times 10^{-6} + 2.76 \times 10^{-5}\exp(-\Delta t/31.75)$ ($r^2 = 0.99$). Linking estimated $\phi(\Delta t, Cu^{2+}, Na^+)$ with fitted M–M kinetic model from McCorkle and Dietz (1980) (Fig. 1B), the fitted parameters in Na transport–valve closure model in Eq. (3) are estimated to be: $m(\Delta t) = 24.33 - 778.43/\Delta t$ ($r^2 = 0.97$) and $ER50_\phi(\Delta t) = 84.15 - 1103.27/\Delta t$ ($r^2 = 0.95$). Table 1 gives the reported data of used water chemistry characteristics employed in our study.

The result shows that the proposed $J(\phi)$ model (Eq. (3)) agrees well with the Na uptake profile in the absence of Cu (Fig. 4A). Based on our $J(\phi)$ model, we can straightforwardly predict the Na uptake profiles as a function of external Na^+ activity varied with different waterborne Cu^{2+} concentrations ranging from 1–20 $\mu g\,L^{-1}$ (Fig. 4B) in that the Na^+ uptake rate $J_{Na^+}(\Delta t, Cu^{2+}, Na^+)$ has a M–M kinetics shown in Eq. (6) in that $\{Cu^{2+}\}$-dependent maximum uptake rates and half-saturation affinity constants can be, respectively, estimated to be,

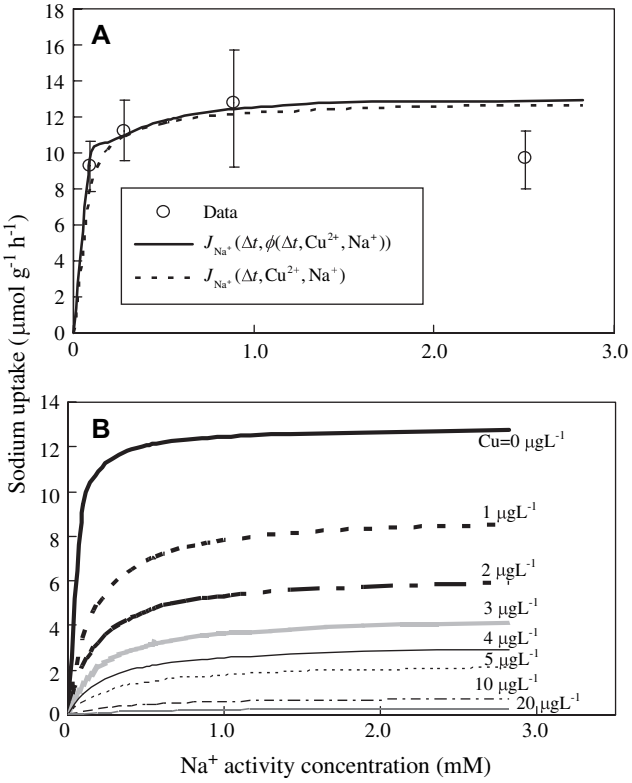

Fig. 4. Clam Na uptake prediction in response to Cu. (A) Validation of our proposed model of (i) response in valve closure behavior depends on external Cu and Na activity concentrations by Eq. (3) and (ii) a modeled M–M kinetics by Eq. (6) agrees well with the Na uptake profile in the absence of Cu estimated by McCorkle and Dietz (1980). (B) Predicted Na uptake rate in response to waterborne Cu of 0, 1, 2, 3, 4, 5, 10, and 20 $\mu g\,L^{-1}$, respectively.

$$J_{max}(\{Cu^{2+}\}) = 0.345 + 12.90 \times \exp(-\{Cu^{2+}\}/6.154 \times 10^{-8}), \quad r^2 = 0.85, \quad (17)$$

$$K_m(\Delta t, \{Cu^{2+}\}) = a_1(\Delta t)\{Cu^{2+}\}^{a_2(\Delta t)}, \quad (18)$$

where $a_1(\Delta t) = 3.84 + 193.66 \times \exp(-\Delta t/136.23)$ ($r^2 = 0.99$) and $a_2(\Delta t) = 0.862 \times \exp(-\Delta t/2875.88)$ ($r^2 = 0.68$). Fig. 4A also demonstrates that derived Na^+ uptake rate model of $J_{Na^+}(\Delta t, Cu^{2+}, Na^+)$ also has a good fit with the published data in the absence of Cu.

Table 1
Published data of water chemistry characteristics used in the study

Water chemistry characteristics	Tran et al. (2004)	McCorkle and Dietz (1980)	Marr et al. (1998)	Matsuo et al. (2004)	Croteau and Luoma (2005)
pH	8.2	7.2	7.5	7.8	6
Temperature (°C)	15	25	9.8	12	15
$[Ca^{2+}]$ (mM)	0.24	0.4	0.14	0.10	0.048
$[Mg^{2+}]$ (mM)	0.16	0.20	0.12	0.15	0.062
$[Na^+]$ (mM)	1.4	0.70	0.039	0.60	0.14
$[K^+]$ (mM)	0.092	0.05	0.10	0.05	0.0067
$[CO_3^{2-}]$ (mM)	0.35	0.00018	–	0.90	0.14
$[SO_4^{2-}]$ (mM)	0.037	–	0.037	–	0.11
$[Cl^-]$ (mM)	1.0	1.4	0.02	0.70	0.0067

3.2. Validation of $J(\phi)$ model

We further test the present $J(\phi)$ model to the reported experimental observations of Na uptake kinetics in rainbow trout to validate the model. We reconstruct the response time-specific mortality–Cu profiles adapted from Marr et al. (1998) (Fig. 5A) and Na uptake profiles varied with different Cu concentrations reanalyzed from Matsuo et al. (2004) (Fig. 5B) associated with the reported water chemistry characteristics (Table 1), we can obtain a relationship between mortality and inhibition of Na uptake $I_{J_{Na^+}}$ expressed as a Hill model (Fig. 5C). Based on the proposed $J(\phi)$ model incorporated with Fig. 5A–C, the model test results show that the predicted M–M type Na uptake profiles in response to waterborne Cu ranging from 0–300 $\mu g\,L^{-1}$ agree well with the published data with an average root mean square error of predictions 77 $nmol\,g^{-1}\,h^{-1}$ (Fig. 5D and Table 2). Thus, our validation (Fig. 5D) confirms that the predictive model for Na uptake kinetics in response to waterborne Cu is robust.

3.3. Model predictability of transport physiology

The predictability of our proposed model is reflected on predicting three transport physiological processes of *C. fluminea*: (1) time-profile of internal Na^+ concentration, (2) Na^+ uptake as a function of gill Cu burden, and (3) Cu^{2+} internalization flux.

The time-profile of internal Na concentration in *C. fluminea* in response to waterborne Cu can be well predicted based on our present $J(\phi)$ model (Fig. 6A), whereas the relationships between valve closure response and internal Na concentration can also be depicted (Fig. 6B). Our simulation assumes that Na^+ uptake equals the diffusive Na loss associated with a change in uptake by an inhibition parameter $I_{J_{Na^+}}$ (Eq. (8)). Fig. 6 indicates that % valve closure response will decrease for clam with higher internal sodium concentration. The inversion of decay rate constant k in Eq. (8) can be defined as the time constant τ, indicating that % valve closure response will occur earlier in clam with higher relative inhibition of sodium uptake.

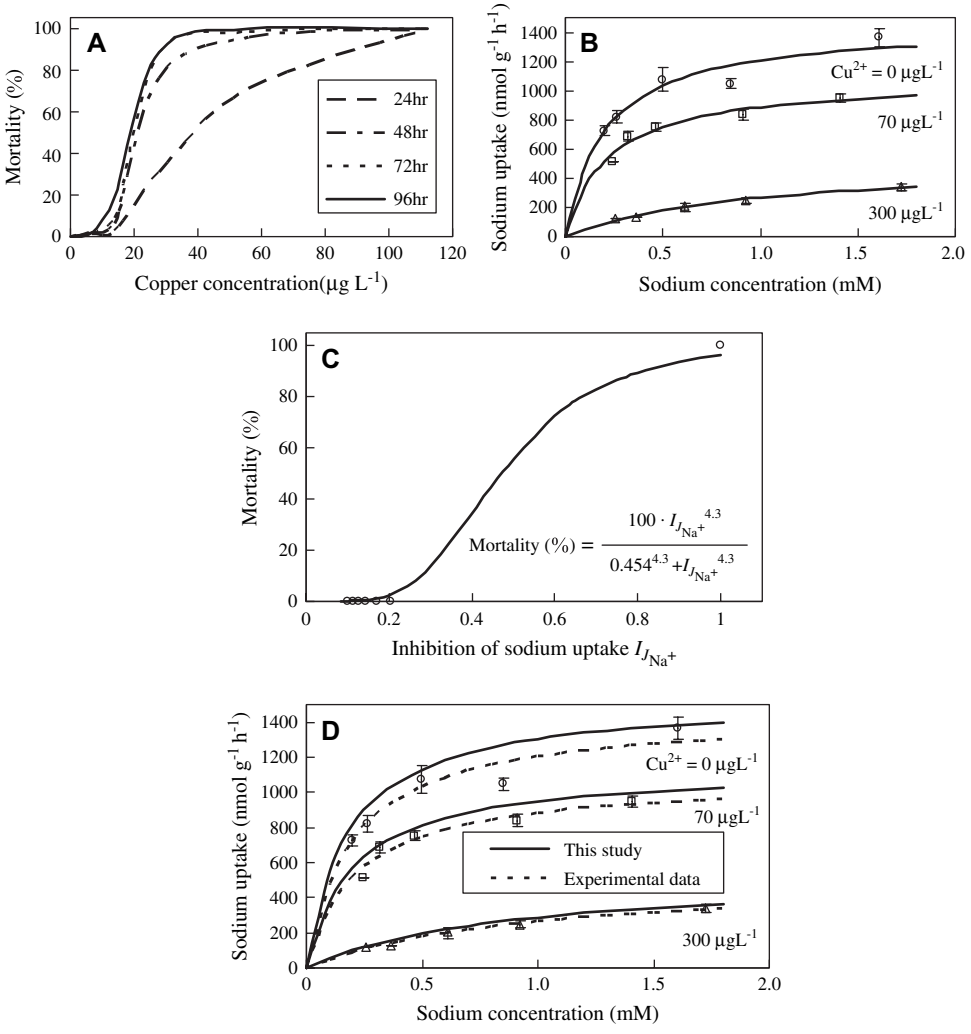

Fig. 5. Validation of $J(\phi)$ model to the reported data of Na uptake kinetics in rainbow trout. (A) Reconstructed Cu concentration–mortality profile at 24, 48, 72, and 96 h, respectively based on Marr et al. (1998). (B) Published M–M Na uptake profiles in response to Cu concentrations of 0, 70, and 300 $\mu g\,L^{-1}$ based on Matsuo et al. (2004). (C) By linking BLM-based dose–response and Na uptake mechanism into our proposed model to predict the relationship between inhibition of Na uptake and mortality as a Hill model. (D) Model validation results show that our predicted M–M kinetics agrees well with the published data.

Table 2
A comparison of M–M kinetics-based J_{max} and K_m value between experimental data (mean ± SD) and predictions by the present model applied to sodium uptake profile of rainbow trout varied with different Cu concentrations

Copper concentration ($\mu g\,L^{-1}$)	J_{max} (nmol $g^{-1}\,h^{-1}$)	K_m (mM Na^+)	r^2	RMSE (nmol $g^{-1}\,h^{-1}$)[b]
Experimental data[a]				
0	1453 ± 116	0.204 ± 0.058	0.91	
70	1082 ± 72	0.221 ± 0.051	0.93	
300	522 ± 30	0.967 ± 0.010	0.99	
Model prediction				
0	1540	0.180	0.99	117.3
70	1140	0.200	0.99	63.5
300	550	0.912	0.94	14.7

[a] Adapted from Matsuo et al. (2004).
[b] Root mean square error of predictions (RMSE) $= \sqrt{\sum_{n=1}^{N}(C_{m,n}-C_{s,n})^2/N}$ where N denotes the number of measurements, $C_{m,n}$ is the measurement data, and $C_{s,n}$ is the simulation result corresponding to data point n.

Followed by the BLM scheme in Eq. (9), we can obtain a relationship between the fraction of the total number of Cu binding sites occupied by Cu (f_{CuBL}) and external bioavailable Cu activity (Fig. 7A) based on the reported water chemistry characteristics from McCorkle and Dietz (1980) (Table 1). We incorporate Fig. 7A into published concentration–% valve closure response profiles expressed by Eq. (4) to estimate a response time-specific $f_{CuBL}-\phi(\Delta t, Cu^{2+})$ relationship as $f_{CuBL}(\Delta t,\phi) = -0.109 + (0.0068\phi) + 0.571\exp(-\Delta t/38.24)$ ($r^2 = 0.98$). We integrate estimated $f_{CuBL}(\Delta t,\phi)$ into the present $J(\phi)$ model in Eq. (3) to capture the relationships between the inhibition of Na uptake and BL–Cu expressed by f_{CuBL} as a Hill model (Eq. (10)) where fitted parameters of $v(\Delta t) = 18.457 - 411.744/\Delta t$ ($r^2 = 0.99$) and $ER50_f(\Delta t) = 0.421 + 1.853/\Delta t$ ($r^2 = 0.94$) (Fig. 7B). We validate the derived $J_{Na^+}(\Delta t, f_{CuBL}(\Delta t, \phi(\Delta t, Cu^{2+}, Na^+)))$ model to the published Na uptake profile in *C. fluminea*, showing a good agreement not only with the data but also with the other two models of $J_{Na^+}(\Delta t, Cu^{2+}, Na^+)$ in Eq. (6) and $J_{Na^+}(\Delta t, \phi(\Delta t, Cu^{2+}, Na^+))$(Fig. 7C).

We adapt published k_1 and k_2 values of Cu accumulation and elimination in *C. fluminea* ($k_1 = 0.224 \pm 0.038\ L\,g^{-1}\,d^{-1}$ and $k_2 = 0.004 \pm 0.054\ d^{-1}$) associated with the reported water chemistry data from Croteau and Luoma (2005) (Table 1) to estimate $[BL^-]$ based on Eq. (11), resulting in $[BL^-] = 1.107\ \mu mol\,g^{-1}$ with a 95% confidence interval (CI) of 0.780–1.524 performed by the Monte Carlo simulation technique. Thus, $J_{Cu^{2+},max} = [BL^-]/\Delta t = (k_1/k_2)[a]^{-1}/\Delta t = 0.369(95\%CI: 0.26–0.51)\ \mu mol\,g^{-1}\,h^{-1}$ where the response time $\Delta t = 3$ h. The half-saturation affinity constant $K_{m,Cu^{2+}}$ can then be estimated from $J_{Cu^{2+},diff}$ profile based on Eq. (13) (Fig. 8B) where $[a']$ is estimated to be $6.35 \times 10^7\ M^{-1}$, resulting in $K_{m,Cu^{2+}} = 7.87 \times 10^{-3}(95\%CI: 5.72 \times 10^{-3}–11.2 \times 10^{-3})\ \mu M$. The Cu internalization flux profile as a function of external $\{Cu^{2+}\}$, therefore, can be predicted by Eq. (12) based on the reported water chemistry data from McCorkle and Dietz (1980) (Fig. 8A). We finally predict the relationships between Na uptake and Cu internalization flux by linking Eq. (6) as illustrated in Fig. 4B and Eq. (13) to depict the $J_{Na^+}(\Delta t) - J_{Cu^{2+}}$ profile (Fig. 8C).

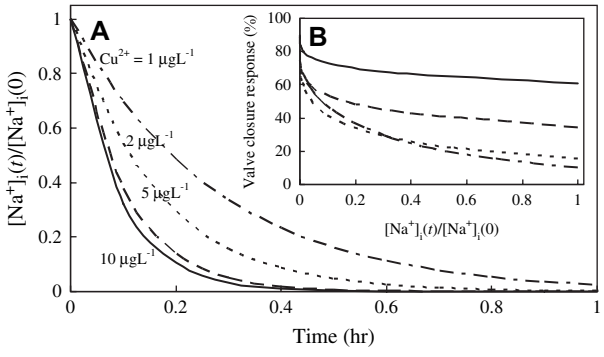

Fig. 6. (A) Time-dependent internal Na^+ concentration in *C. fluminea* in response to waterborne Cu of 1, 2, 5, and 10 $\mu g\,L^{-1}$ corresponding to decay rate constants k of 3.64, 6.14, 9.80, and 11.24 h^{-1}, respectively. (B) Relationship between valve closure responses and internal Na concentration.

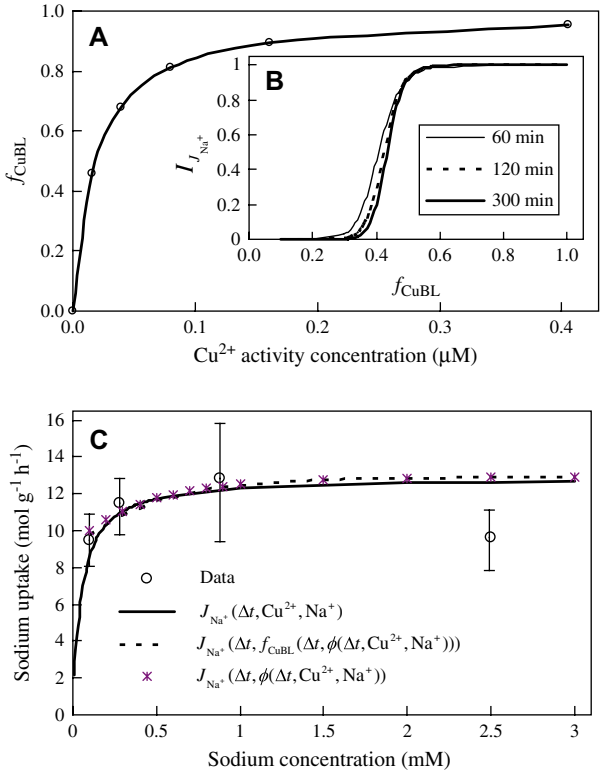

Fig. 7. (A) BLM-based predicted relationship between the fraction of the total number of Cu binding sites occupied (f_{CuBL}) and external Cu activity based on the reported water chemistry characteristics from McCorkle and Dietz (1980). (B) Response time–specific response profile of inhibition of Na uptake and f_{CuBL} expressed as a Hill model. (C) Validation of the derived $J_{Na^+}(\Delta t, f_{CuBL}(\Delta t, \phi(Cu^{2+}, Na^+)))$ model with published Na transport profile in *C. fluminea* in the absence of Cu ($r^2 = 0.91$).

3.4. Na uptake–valve closure dynamics

The dynamic behavior of clam valve closure behavior associated with Na uptake in response to waterborne Cu (0.03–5 $\mu g\,L^{-1}$) at a specific external Na concentration can be

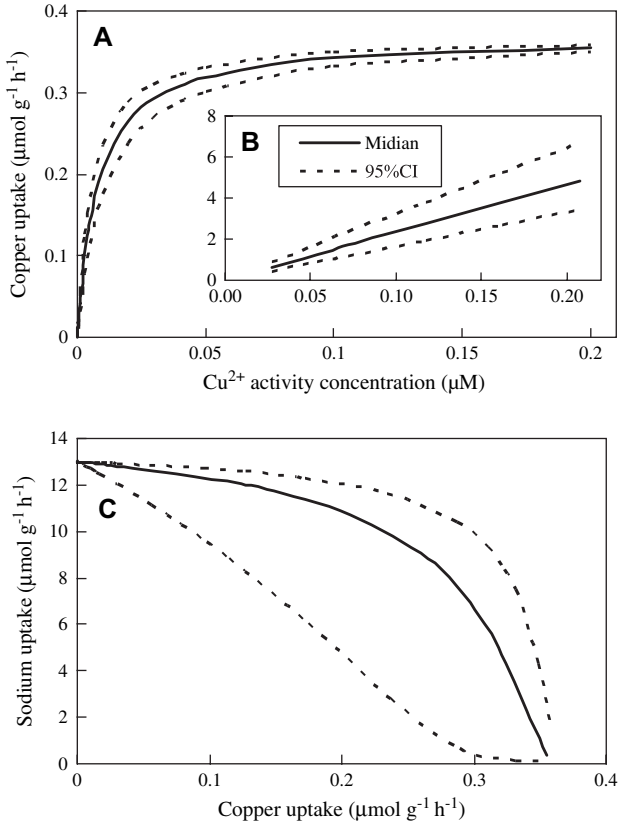

Fig. 8. Copper internalization flux in *C. fluminea*. (A) The Cu internalization flux profile as a M–M kinetic model. (B) Diffusion-limited Cu internalization flux. (C) Predicted profile of the relationship between Na uptake and Cu internalization flux varied with different external Cu concentration.

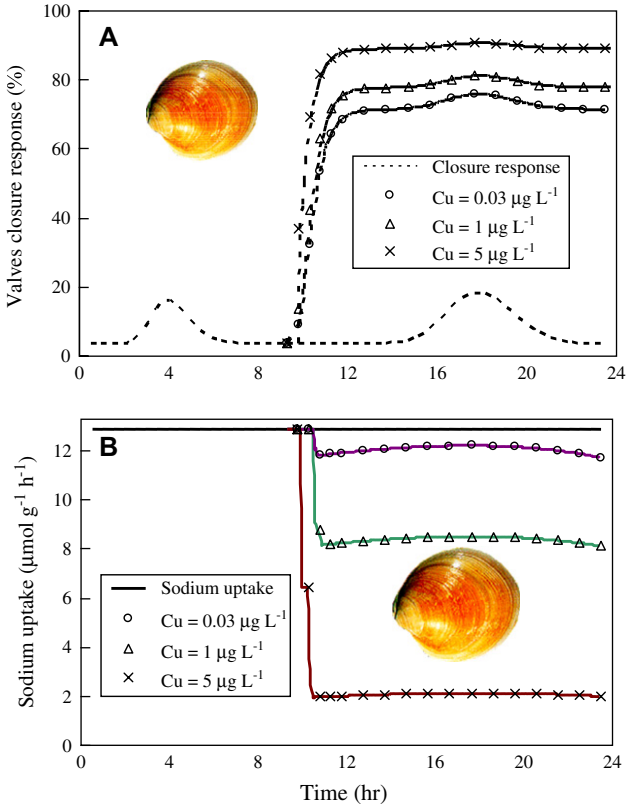

Fig. 9. Simulations of dynamic response of (A) clam valve closure behavior associated with (B) Na uptake rate in response to waterborne Cu of 0.03, 1, and 5 μg L^{-1} at 9:00 a.m. at a specific external Na concentration of 0.7 mM.

predicted from Eqs. (14) and (15) for appropriate parameters where $J_{Na^+}(t,0)|_{Na^+} = J_{max} = 12.90$ μmol g^{-1} dry wt h^{-1} (Fig. 9). Fig. 9 implicates that our proposed models can not only quantitatively describe the Na-specific Na uptake dynamics along with the valve closure behavior when clams are exposed to waterborne Cu but can also be used as a tool to test the bivalve biological and physiological response abilities to close its shell as an alarm signal to reflect clam's health when they are exposed to Cu.

4. Discussion

4.1. Applications to biomonitoring protocols

The use of novel biomonitoring strategy, including those exploiting simulation techniques, to better characterize aquatic ambient pollutant distributions and quantify source fluxes are required to understand and address the water quality problems they create. This is particularly true in the aquatic ecosystems, where the temporally and spatially undersampled data from conventional fixed water quality measurement sites is either limited or unavailable.

Continuous and rapid detection of environmental toxicity caused by waterborne metals is of great value for conserving aquatic ecosystems and protecting species health. Bivalves have been extensively used for monitoring environmental changes and the effect of these changes on the performance of aquatic organisms (Sloof et al., 1983; Doherty et al., 1987; Borcherding, 1994; Sluyts et al., 1996; Borcherding and Jantz, 1997; Curtis et al., 2000; Tran et al., 2003, 2004; El-Shenawy, 2004). Recent technological advances have allowed the development of robust, relatively compact, low cost, rapid response biomonitors with sufficient sensitivity and specificity to quantify many waterborne metals in the aquatic ecosystems.

Dynamic metal speciation analysis in aquatic ecosystems is emerging as a powerful basis for development of predictions of bioavailability and reliable risk assessment strategies (Kahru et al., 2005; van Leeuwen et al., 2005; Unsworth et al., 2006). Our Na uptake–valve closure dynamic analysis establishes the conditions under which complex chemical species will contribute to biouptake and furthermore to identify the domain of validity of BLM-based approach. The validity of our proposed concept has been established by application to published measurements of Cu biouptake fluxes in rainbow trout. Our proposed model therefore can be used to develop a dynamic biosensor to assess Cu-uptake process and to measure Cu bioavailability based on the Na uptake–valve closure structure.

Since natural waters may contain trace metals (e.g., Zn, Al, Fe) other than Cu at appreciable concentrations, it is possible that these metals act to increase Cu bioavailability by competitive interactions with dissolved humic substances.

Further investigation of these interactions is possible using the concept of our proposed model as the analytical tool to assess Cu bioavailability.

Therefore, our study, by integrating the M–M rigorous flux analysis and BLM-based biological response to environmental chemical stressor, provides insight into the contribution of ion transport kinetics to bioavailability as sensed by freshwater organisms. Although assessment of Cu bioavailability in aquatic ecosystems remains difficult, the use of this Cu-BLM-clam associated with Na uptake model is promising. Further development and simultaneous use of other dynamic sensors will further advance our understanding of Cu bioavailability to freshwater organisms.

4.2. Applications to water quality criteria

Ion transport associated with valve movement is a characteristic feature of *C. fluminea* and part of their natural behavior and it cannot be neglected as it is an important physiological factor for their survival (Zheng and Dietz, 1998; Tran et al., 2004). Transport of Na in *C. fluminea* is efficient and the affinity of the transport system for Na ions in these animals is greater than the affinity in the unionid mussels (McCorkle and Dietz, 1980). The K_m in *C. fluminea* is low relative to literature K_m values of 0.2–0.7 mM in other freshwater animals and the exchange diffusion is characteristic of Na transport (McCorkle and Dietz, 1980). Changes in the valve movement rhythm and Na transport of *C. fluminea* can therefore be used as suitable endpoints in ecotoxicological risk assessment.

The *C. flumenia* are filter-feeder animals. They extend siphon from their bivalve shells to filter waterborne plankton or organic matter for uptake. Siphon extension is related to the magnitude of shell gape (%) that was proportioned to the valve position as well as percentage of the shell span (Ortmann and Grieshaber, 2003). When valve closure behavior in response to waterborne contaminants reduces Na uptake activity by closing their shells to escape toxicant damage and exclude themselves from the outside contaminated environment for maintaining their biotic faculty and increasing their survivability (Wildridge et al., 1998; Kadar et al., 2001).

Based on the valve position, we may use two different physiological responses with respect to behavioral activities of the clam as biological endpoints, i.e., valve closure and Na influx decreasing. Using different biological endpoints to develop BLM would be ideal for formulating predictive models of chronic low-level metal exposures in aquatic ecosystems. The present Na–Cu-BLM-clam model offers a conceptual framework for integrating the influence of environmental factors such as water chemistry and bioavailability on biological response as monitored using valve movement behavior and Na uptake mechanism. We suggest that developing novel methods through BLM for interpreting biological response data will increase its utility in environmental risk assessment of toxicant exposure for aquatic species. Escher and Hermens (2004) pointed out that by linking bioavailability to effects could improve risk assessment and bridge the gap between human and environmental risk assessment.

Our $J(\phi)$ model has the ability to predict interplay relationships among valve closure response and internal Na level (Fig. 6B), inhibition of Na uptake and external Cu activity (Fig. 7B), and Cu internalization as a function of external Cu activity (Fig. 8A). By integrating Figs. 6B, 7B, and 8A, we can extend our model to a physiologically-based model of the survival time of *C. fluminea* exposed to Cu to provide a potential utility to longer-term ongoing efforts to develop and refine water quality criteria (WQC) (Paquin et al., 2002a,b; Grosell et al., 2002; Escher and Hermens, 2004). Meaningful WQC are needed to serve as a basis for development and implementation of a site-specific risk management strategy that will protect the aquatic environment, whereas at the same time result in the cost-effective implementation of control measures. From the perspective of the aquatic ecosystems, rather than developing a single-value waterborne metal concentration for establishing the WQC, it is better to derive a mechanistic model that explicitly incorporates the factors controlling bioavailability and bioaccumulation to enhance predictive ability to protect aquatic organisms.

Therefore, the present $J(\phi)$ model integrates knowledge of water chemistry with physiological transport mechanisms and biological response of organisms to generate a site-specific assessment of the toxicity of a given metal to the biota therein and provides a direct and quantitative method for evaluation of metal bioavailability in ecological risk assessment as a function of water chemistry and organism sensitivity to overcome frequently over-protective, and occasionally under-protective on site-specific WQC, thereby providing a means for estimating the effect of site-specific factors on metal toxicity.

4.3. Implications

Looking forward, we propose that this Na transport–valve behavior approach, which amounts to metal biodynamics–transport physiology–biological response of freshwater *C. fluminea* within the BLM framework, might provide the basis of a future design of biomonitoring tool for measuring metal bioavailability to important freshwater species. Furthermore, this approach should have certain potential to provide a means to predict toxicity by influx rates for dissolved metals. A further inherent benefit of the Na transport–valve behavior approach is the identification of new bioindicators of dietary toxicity as a function of metal influx rates in the digestive tract that are predictive of individual responses.

The main potential application we envisage for Na transport–valve behavior approach is with respect to the health of freshwater clam to promote economic benefits on the development of bivalve extract that are now available on the market with a widely varying ornithine (Uchisawa et al., 2004). Wu and Shiau (2002) indicated that a freshwater clam extract or referred to as clam essence contained more ornithine than that in a chicken or beef essence. Recently, it has been reported that ornithine promotes the secretion of the growth hormone and builds muscle (Davenport et al., 1990; Bucci et al., 1990). Ornithine is thus attractive as an ingredient of dietary supplements.

In principle, by using this methodology, metal-binding characteristics could be quantified upon exposures to waterborne Cu, allowing evaluation of the relative contribution of the physiological mechanisms to the influx of metals. We envisage that optimal prediction of metal toxicity may eventually involve a variety of response-prediction approaches. However, by linking ion transport mechanism and BLM-based metal biodynamics to investigate clam biological response has an important theoretical advantage over traditional toxicity models (Luoma and Rainbow, 2005; Tsai and Liao, 2006) in that it can potentially take into account of both clam physiological and environmental factors affecting metal-induced biological responses. Furthermore, although our proposed model would normally relate to predicting xenobiotic-induced biological responses of freshwater species from influx rates, we envisage that similar methodology could also be applied to broaden the knowledge of the molecular and cellular mechanisms involved in the main physiological processes of interest including growth, reproduction, and immunity (Neumann and Galvez, 2002; Bricelj et al., 2005).

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