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Predicting bioavailability and bioaccumulation of arsenic by freshwater clam *Corbicula fluminea* using valve daily activity

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Abstract There are many bioindicators. However, it remains largely unknown which metal–bioindicator systems will give the reasonable detection ranges of bioavailable metals in the aquatic ecosystem. Various experimental data make the demonstration of biomonitoring processes challenging. Ingested inorganic arsenic is strongly associated with a wide spectrum of adverse health outcomes. Freshwater clam *Corbicula fluminea*, one of the most commonly used freshwater biomomitoring organisms, presents daily activity in valve movement and demonstrates biotic uptake potential to accumulate arsenic. Here, a systematical way was provided to dynamically link valve daily activity in *C. fluminea* and arsenic bioavailability and toxicokinetics to predict affinity at arsenic-binding site in gills and arsenic body burden. Using computational ecotoxicology methods, a valve daily rhythm model can be tuned mathematically to the responsive ranges of valve daily activity system in response to varied bioavailable arsenic concentration. The patterned response then can be used to predict the site-specific bioavailable arsenic concentration at the specific measuring time window. This approach can yield predictive data of results from toxicity studies of specific bioindicators that can assist in prediction of risk for aquatic animals and humans.

Keywords Arsenic · Freshwater clam · *Corbicula fluminea* · Valve daily activity · Bioavailability · Bioaccumulation · Biomonitoring

Introduction

A key goal in biomonitoring is to offer a tool to assess metal pollution in the aquatic ecosystem. The use of the freshwater clam *Corbicula fluminea* as a species in metal toxicity testing has supported the hypothesis that *C. fluminea* is a bioindicator of impairment in aquatic ecosystems (Jou and Liao 2006; Liao et al. 2007a; Newton and Cope 2007; Tran et al. 2007). Valve daily activity in *C. fluminea* is able to detect specific waterborne metals at levels of ppb: 2.0–5.4 μg L^{-1} (95% confidence interval) for mercury (Tran et al. 2007), 2.3–8.8 μg L^{-1} for copper (Tran et al. 2004), 16 μg L^{-1} for cadmium (Tran et al. 2003), and

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300–400 μg L^{-1} for arsenic (As) (Liao et al. 2009) within 5 h.

One of the most popular commercialized devices of the early warning system based on valve daily movement of freshwater mussel is Mosselmonitor® (www.mosselmonitor.nl). Mosselmonitor® has been used intensively for many applications, mostly for water quality management (www.mosselmonitor.nl). Detection limits of the Mosselmonitor® by using zebra mussel (*Dreissena polymorpha*) for specific waterborne metals such as cadmium, copper, and zinc are 150, 10, and 500 μg L^{-1}, respectively. No information is available for arsenic.

World Health Organization (NRC 2001) considered arsenic is the top environmental chemical of concern. Arsenic also ranks first on the Agency for Toxic Substances and Disease Registry list of priority pollutants in the environment (http://www.atsdr.cdc.gov/cercla/05list.html). Many studies indicated that human-ingested inorganic arsenic caused significant potential risk for the occurrence of some complex diseases such as skin lesions, diabetes, cardiovascular disease, and cancers of several organs (lung, bladder, kidney) in blackfoot disease (BFD)-endemic areas (Chen et al. 2005).

Lin et al. (2001, 2005), Liao et al. (2003), Huang et al. (2003), and Liu et al. (2005, 2006, 2007) have conducted a long-term investigation during 1998–2007 in BFD-endemic areas of Taiwan. They indicated that arsenic was detected in many farm fish and shellfish ponds. Taken together, they reported that arsenic concentrations in aquaculture waters ranged from 40 to 900 μg L^{-1}, whereas arsenic levels in fish (tilapia *Oreochomis mossanbicus*, milkfish *Chanos chanos*, and large-scale mullet *Liza macrolepis*) and shellfish (hard clam *Meretrix lusoria* and oyster *Crassostrea gigas*) ranged from 1 to 350 and 4 to 23 μg g^{-1} dry wt, respectively.

Dell'Omo (2002) indicated that behavioral parameters reflect sublethal toxicity, suggesting that behavioral responses might be faster and more sensitive than mortality as the toxicity parameters. Gerhardt et al. (2005, 2006) indicated that behavioral parameters could add another dimension for providing more sensitivity and ecological relevance to standard toxicity testing, suggesting that behavioral parameters are particularly suitable to be used in online biomonitors. It is reported that *C. fluminea* present daily activity in valve movement (Ortmann and Grieshaber 2003; Liao et al. 2009). Gerhardt et al. (2005) suggested that it is urgently needed to define daily rhythm changes as a new behavioral test parameter to provide a candidate factor for biomonitoring the waterborne contaminants. Sebesvari et al. (2005) and Santos et al. (2007) suggested that *C. fluminea* is a suitable bioindicator for biomonitoring of arsenic in the aquatic ecosystem. Furthermore, Sebesvari et al. (2005) and Santos et al. (2007) both confirmed that *C. fluminea* might provide the capacity for arsenic regulation.

Generally, regulatory authorities rely on organism/water chemical concentration ratios measured in laboratory tests (bioconcentration factors (BCFs)) or in the field (bioaccumulation factors) to identify bioaccumulative substances (Kelly et al. 2007). The previous research (Liao et al. 2008) indicated that arsenic could bioaccumulate in *C. fluminea* with equilibrium BCF values of 17. Santos et al. (2007) also reported that *C. fluminea* exposed to 300 and 1,000 μg L^{-1} of arsenic concentrations resulted in the highest mean body burdens of 17 and 15 μg g^{-1} dry wt, respectively. This may have important implications for dietary exposure of arsenic to human-consumed contaminated clams because the soft tissue usually constitutes the majority of tissue consumed.

It is known that predicting the bioavailability of dissolved metals as a function of their speciation in the environment is an important goal in aquatic ecotoxicology. It is also recognized that the widely used biotic ligand model (BLM) that describes the bioavailability of metal causing toxicity in aquatic organisms was developed successfully to address this challenge (Paquin et al. 2002; Niyogi and Wood 2004).

The objective of this paper was to explore the potential of clam valve movement behavior, especially daily opening/closing rhythm, to predict bioavailability and bioaccumulation of arsenic in freshwater clam in site-specific settings. A computational toxicology method was developed to tune a valve daily rhythm model to the responsive ranges of valve daily activity system in response to varied bioavailable arsenic. It then used the patterned response to predict the site-specific bioavailable arsenic concentration at the specific measuring time window. This proposed method could be used to identify the responsive ranges of valve daily activity in response to waterborne metals. This result can provide a new method for probing the clam valve daily rhythm subsystem, and also suggests a novel method for identifying potential biomonitoring tools for assessment of metal pollution in the aquatic ecosystem.

Materials and methods

Mechanistic model

Theoretically, 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. At equilibrium thermodynamics, arsenate (As(V)) dominants in oxidative environments of the most surface water. As(V) thus is the most prevalent in most surface waters (Fergusion and Gavis 1972). The gill arsenic burden [AsBL] in the BLM scheme (De Schamphelaere et al. 2002) was linked with a one-compartment uptake-depuration model at a steady-state condition (Liao et al. 2007a, b) to estimate the concentration of unoccupied clam gill-BL sites,

$$[\mathrm{AsBL}]_{\mathrm{T}} = [\mathrm{BL}^-][\mathrm{a}]\{\mathrm{As(V)}\} \approx \mathrm{BCF}\{\mathrm{As(V)}\} = \frac{k_1}{k_2}\{\mathrm{As(V)}\}, \quad (1)$$

where $[\mathrm{AsBL}]_{\mathrm{T}}$ is the steady-state gill arsenic-BL burden (μg g^{-1}), $[\mathrm{BL}^-]$ is the concentration of unoccupied gill BL sites (μg g^{-1}), $\{\mathrm{As(V)}\}$ is the activity of the free arsenic ion (μg L^{-1}), k_1 (L g^{-1} day^{-1}) and k_2 (day^{-1}) are the uptake and depuration rate constants of *C. fluminea* to arsenic, respectively, and $[a]$ (L μg^{-1}) is an affinity (stability) constant-dependent parameter in the BLM scheme-based EC50 equation.

In the BLM scheme, EC50 can be mathematically expressed (De Schamphelaere et al. 2002),

$$\mathrm{EC50}_{\mathrm{AsBL}}(t_{\mathrm{R}}) = \frac{f^{50\%}_{\mathrm{AsBL}}(t_{\mathrm{R}})}{(1 - f^{50\%}_{\mathrm{AsBL}}(t_{\mathrm{R}}))}\left(\frac{[b]}{[a]}\right), \quad (2)$$

where $\mathrm{EC50}_{\mathrm{AsBL}}(t_{\mathrm{R}})$ is the fitted response time (t_{R})-varying function, which can represent effect concentration of [AsBL] causing 50% of total valve closure response in a group of clam, $[b] = 1 + K_{\mathrm{CaBL}}\{\mathrm{Ca}^{2+}\} + K_{\mathrm{MgBL}}\{\mathrm{Mg}^{2+}\} + K_{\mathrm{NaBL}}\{\mathrm{Na}^{+}\} + K_{\mathrm{HBL}}\{\mathrm{H}^{+}\}$, in that K_{CaBL}, K_{MgBL}, K_{NaBL}, and K_{HBL} are the stability constants for the binding of these cations to the BL (L μg^{-1}), and {ion} denotes the activity of each ion of water chemistry characteristics (μg L^{-1}), and $f^{50\%}_{\mathrm{AsBL}}(t_{\mathrm{R}})$ is the response time (t_{R})-dependent fraction of the total number of arsenic binding sites occupied by arsenic at 50% effect.

In Eq. 2, $f^{50\%}_{\mathrm{AsBL}}(t_{\mathrm{R}})$ can be well described by an exponential expression as: $f^{50\%}_{\mathrm{AsBL}}(t_{\mathrm{R}}) \approx c + d\exp(-t_{\mathrm{R}}/e)$ (Liao et al. 2007a). By incorporating $[a] = \mathrm{BCF} \times [\mathrm{BL}^-]^{-1}$ obtained from Eq. 1 into Eq. 2, it obtains

$$\mathrm{EC50}_{\mathrm{AsBL}}(t_{\mathrm{R}}) = \frac{c + d\exp(t_{\mathrm{R}}/e)}{(1 - (c + d\exp(-t_{\mathrm{R}}/e)))} \times A_0, \quad (3)$$

where $A_0 = [b][\mathrm{BL}^-]\mathrm{BCF}^{-1}$. The coefficients c, d, e, and A_0 can be estimated by fitting Eq. 3 to $\mathrm{EC50}(t_R)$ data obtained from the behavior assays. Subsequently, the concentration of unoccupied gill BL sites ($[\mathrm{BL}^-]$) and BLM parameter $[a]$ can also be calculated by known values of $[b]$ and BCF obtained from the laboratory uptake-depuration experiment.

A BLM-based Hill dose–response curve can thus be reconstructed without any prior knowledge of stability constant-dependent $[a]$ value describing the binding behavior of free arsenic ion,

$$R(t_{\mathrm{R}}, \{\mathrm{As(V)}\}) = \frac{R_{\max}(t_{\mathrm{R}})}{1 + \left(\frac{\mathrm{EC50}_{\mathrm{AsBL}}(t_{\mathrm{R}})}{\{\mathrm{As(V)}\}}\right)^{n(t_{\mathrm{R}})}}, \quad (4)$$

where $R(t_R, \{\mathrm{As(V)}\})$ is the time-dependent valve response (% response) based on As(V)-activity $\{\mathrm{As(V)}\}$ (mg L^{-1}) at any given clam response time t_{R}, $R_{\max}$ is the response time-specific maximum response (%), and $n(t_R)$ is a response time-dependent Hill coefficient, which is a measure of cooperativity. A value of $n > 1$ indicates positive cooperativity.

Valve daily rhythm prediction

The 14-day continuous observation of individual valve opening under the status of free-range burrowing behavior was analyzed to obtain the variation of daily valve rhythm of the 16 *C. fluminea* (Liao et al. 2009). The processed valve daily rhythm data are based on the mean proportion of valve opening of 16 clams at hourly intervals during a 14-day observed duration (Liao et al. 2009). This study employed the BLM-based and Hill model-based time- and activity-specific valve closing response (Eq. 4) to predict the valve daily activity in *C. fluminea* in response to arsenic,

$$\begin{aligned}\psi(t,\{\mathrm{As(V)}\}) &= \psi(t,0)\\ &\quad - \psi(t,0) \times R(t_{\mathrm{R}},\{\mathrm{As(V)}\})\\ &= \psi(t,0)\times\left[1-R(t_{\mathrm{R}},\{\mathrm{As(V)}\})\right], \quad (5)\end{aligned}$$

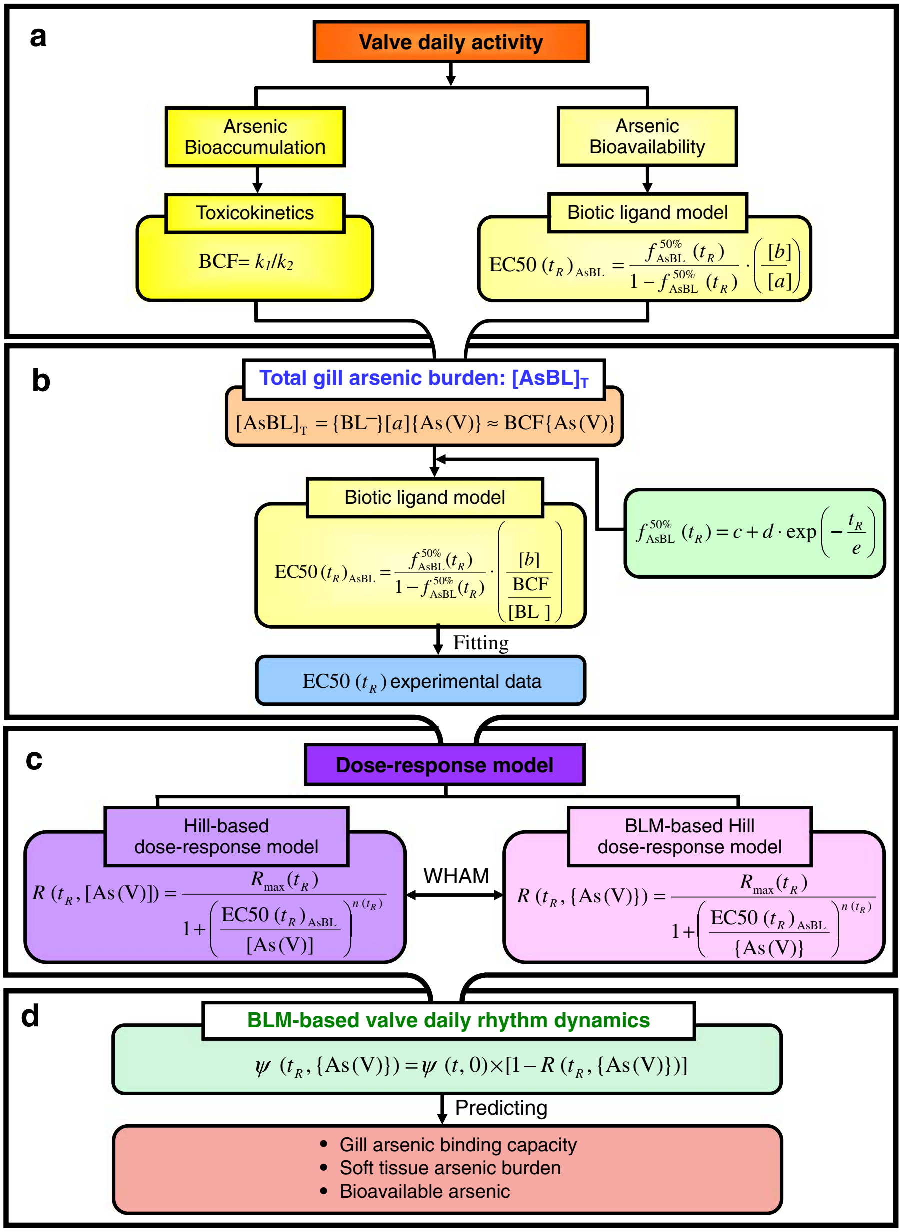

Fig. 1 Schematic showing a mechanistic modeling algorithm describing the linkage of valve daily subsystem in *C. fluminea*, BLM-based arsenic bioavailability, and bioaccumulation model to predict gill arsenic binding capacity and soft tissue arsenic burden (**a**–**d**) (see text for detail description of symbols)

where $\psi(t, \{As(V)\})$ is the daily rhythm function of valve opening at time t in response to arsenic activity and $\psi(t, 0)$ is the daily rhythm of valve opening at time t in the absence of arsenic and can be expressed as a sine function (Liao et al. 2009),

$$\psi(t, 0) = B + A \times \sin \frac{2\pi(t + \varphi)}{\tau} \tag{6}$$

with estimates of a baseline $B = 0.59$ as an average proportion of valve opening, an amplitude $A = 0.13$, a phase $\varphi = 0.034$ h, and a daily period $\tau = 21.32$ h (95% confidence interval (CI): 20.58–22.05 h) (Liao et al. 2009). Figure 1 illustrates the computational algorithm.

Furthermore, with the knowledge of site-specific key water chemistry characteristics, e.g., $\{Na^+\}$, $\{Mg^{2+}\}$, $\{Ca^{2+}\}$, and $\{H^+\}$, the free ion arsenic activity can be predicted followed by the inverse function of $R(t_R, \{As(V)\})$ in Eq. 4 as

$$\{As(V)\}(t_R) = \frac{\dfrac{f^{50\%}_{AsBL}(t_R)}{1 - f^{50\%}_{AsBL}(t_R)} \times \left(\dfrac{[b]}{[a]}\right)}{\sqrt[n(t_R)]{\dfrac{R_{max}(t_R) - R(t_R, \{As(V)\})}{R\left(t_R, \left\{As\left(V\right)\right\}\right)}}}, \tag{7}$$

where

$$R\left(t_R, \{As\left(V\right)\}\right) = \frac{\psi\left(t_R, 0\right) - \psi\left(t_R, \{As\left(V\right)\}\right)}{\psi\left(t_R, 0\right)}, \tag{8}$$

which is derived from Eq. 5.

Table 1 Distributions and point values of affinity constants of BL-cation and bioaccumulation parameters used in the proposed model

Affinity constants (L g^{-1})	
log K^a_{MgBL}	1.93
log K^a_{HBL}	2.19
Log K^b_{NaBL}	LN(1.73, -0.32)[c]
log K^b_{CaBL}	LN(3.53, 1.03)[c]
Bioaccumulation parameter[d]	
k_1 (mL g^{-1} day^{-1})	1.718 ± 6.70[e]
k_2 (day^{-1})	0.392 ± 1.76[e]
BCF (mL g^{-1})	4.38

[a] Adopted from Niyogi and Wood (2004)
[b] Adopted from Liao et al. (2007a)
[c] Lognormal distribution with a geometric mean and a geometric standard deviation
[d] Adopted from Liao et al. (2008)
[e] Mean ± SE

Table 2 Measured pH and temperature values with the ion activities of key water chemistry characteristics calculated by WHAM from published data for two selected clam farms of Changhua and Hualien and laboratory conditions

	pH	Temp. (°C)	DO (mg l^{-1})	Ion activities (mg L^{-1})						
				Ca^{2+}	Mg^{2+}	Na^+	K^+	Cl^-	SO_4^{2-}	NO_3^-
Changhua[a]	8.01 ± 0.19	29.3 ± 0.9	–	16.4 ± 5.6	8.4 ± 5.7	9.9 ± 5.3	–	14.2 ± 8.9	9.4 ± 0.15	–
Hualien[a]	7.80	30.5	–	14.4	28.8	282.3	–	1,970.1	136.4 ± 14.4	–
Laboratory[b]	8.12 ± 0.06	22.14 ± 1.11	8.3 ± 0.28	24.8	0.98	4.8	2.7	7.44	–	0.31

[a] Adopted from Liao et al. (2007a) in data represented as mean ± SD ($n = 3$)
[b] Adopted from Liao et al. (2008)

Model parameterization

Niyogi and Wood (2003) summarized the estimated stability (or affinity) constants (log K) of BL-cation and inorganic complexes used in BLM developed for fathead minnow *Pimephales promelas* and *Daphnia magna*, respectively. To account for the uncertainty/variability of log K in different versions, we optimal fitted the lognormal distributions for log K_s (Table 1). The bioaccumulation parameters of k_1, k_2, and BCF for *C. fluminea* exposed to arsenic can be obtained from Liao et al. (2008) (Table 1). Behavioral assays have been conducted and fitted with the Hill equation to obtain the dose–response curves describing arsenic concentration-valve closing response relationships varied with different response times of 10, 15, 30, 60, 120, and 300 min (Liao et al. 2009). The experiments were carried out with 140 adult *C. fluminea* of a specific size class (mean shell length of 27.6 ± 2.4 mm (mean ± SD) and body weight of 6.19 ± 0.86 g wet wt). Based on the fitted does–response curves, the response time-specific EC50 values could be estimated to be 4.65, 3.48, 1.38, 0.60, 0.38, and 0.35 mg L^{-1} for valve response times of 10, 15, 30, 60, 120, and 300 min, respectively.

This study selected two clam farms located at Changhua and Hualien of southwestern and northeastern Taiwan regions, respectively, associated with laboratory experimental data of water chemistry characteristics to implement the proposed model. Distributions of measured metal ion concentrations were fit to the polled field observations obtained from clam farms and in the laboratory conditions (Table 2). It determined that the lognormal distribution model optimal fitted the observed data of ion activity concentrations in three selected clam farms favorably. All variables modeled as the lognormal distributions from which geometric mean and geometric standard deviation for each variable was calculated.

Monte Carlo simulation was used to obtain 2.5th- and 97.5th-percentiles as the 95% CI. TableCurve 2D (version 5, AISN Software, Mapleton, OR, USA) was used to optimal fit the published data to obtain the optimal statistical models. WHAM (Windermere humic aqueous model) version 6 (WHAM VI, Centre for Ecology & Hydrology, Lancaster, UK) was performed to calculate the activities of the competing cations considered in this study. The default inorganic arsenic form in WHAM is arsenate (AsO_4). Crystal Ball® software (version 2000.2, Decisioneering,

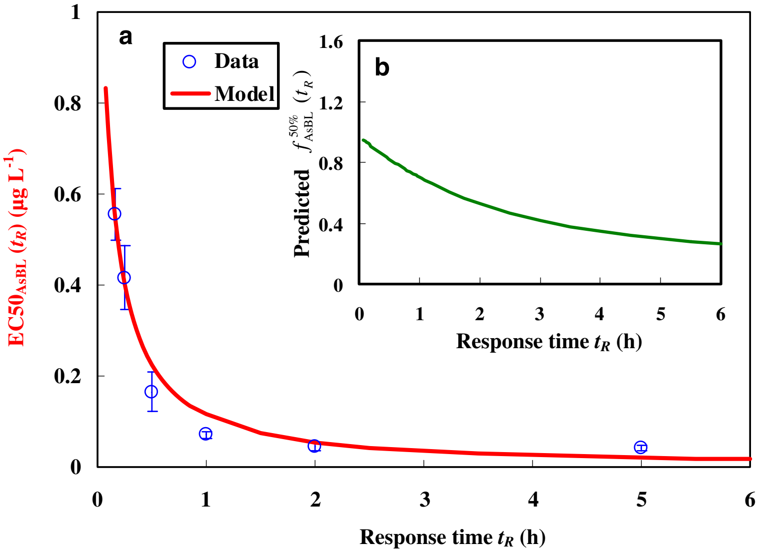

Fig. 2 **a** Fitting the proposed $EC50_{AsBL}(t_R)$ model (Eq. 3) to published experimental $EC50(t_R)$ data. **b** A relationship between the predicted $f^{50\%}_{AsBL}(t_R)$ and response time (t_R). *Error bars* denote standard deviation from mean

Denver, CO, USA) was employed to implement the Monte Carlo simulation.

Results

BLM-based dose–response profile

The time-dependent fraction of the total number of arsenic binding sites occupied by arsenic at 50% effect, $f^{50\%}_{\mathrm{AsBL}}(t_{\mathrm{R}})$, could be estimated by fitting Eq. 3 to published EC50(t_R) data (Liao et al. 2008) associated with known ionic compositions and $\log K$ values (Fig. 2a). Figure 2b demonstrates the relationship between estimated $f^{50\%}_{\mathrm{AsBL}}(t_{\mathrm{R}})$ and response time (t_{R}) in that predicted $f^{50\%}_{\mathrm{AsBL}}(t_{\mathrm{R}})$ has a form as $f^{50\%}_{\mathrm{AsBL}}(t_{\mathrm{R}}) = 0.21 + 0.76\exp(-t_{\mathrm{R}}/2.31)$ ($r^2 = 0.97$). The concentration of unoccupied clam gill BL sites ($[\mathrm{BL}^-]$) can also be estimated from the relationship of $A_0 = [b][\mathrm{BL}^-]\mathrm{BCF}^{-1}$ with fitted $A_0 = 48.62\ \mathrm{ng\ L^{-1}}$ (0.35 nM), $\mathrm{BCF} = 4.38\ \mathrm{mL\ g^{-1}}$, and $[b] = 1 + K_{\mathrm{CaBL}}\{\mathrm{Ca}^{2+}\} + K_{\mathrm{MgBL}}\{\mathrm{Mg}^{2+}\} + K_{\mathrm{NaBL}}\{\mathrm{Na}^{+}\} + K_{\mathrm{HBL}}\{\mathrm{H}^{+}\} = 1.16$, resulting in

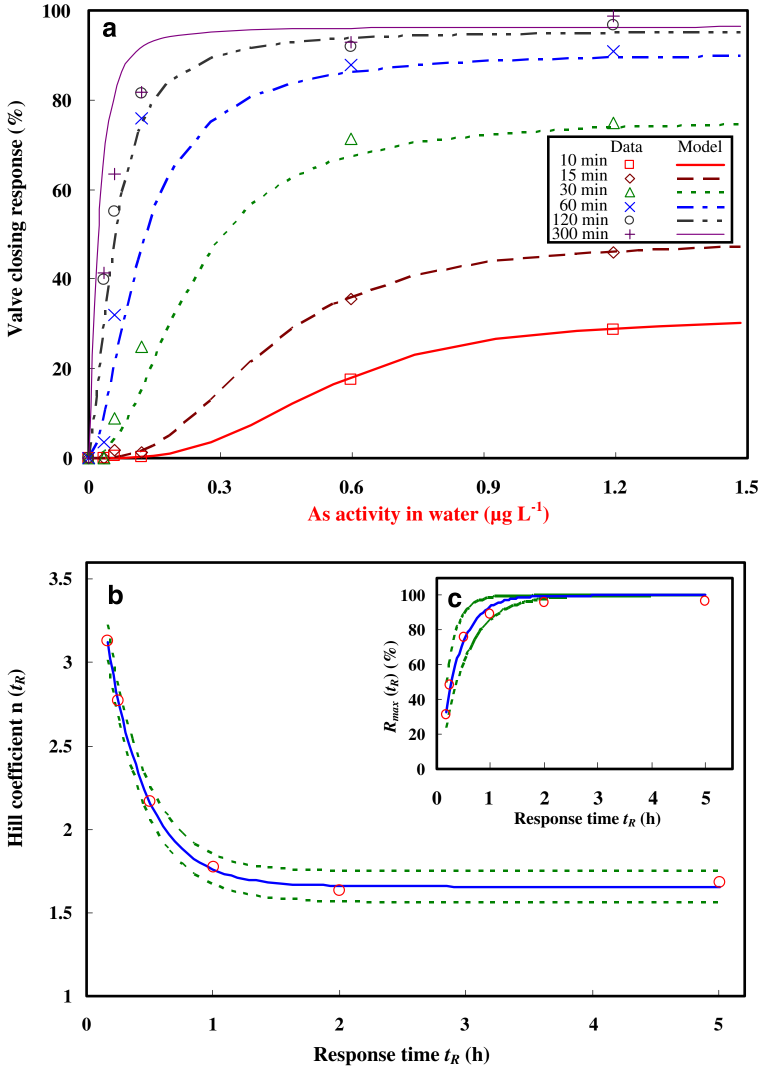

Fig. 3 **a** Fitting the proposed BLM-based Hill dose–response model (Eq. 4) to published experimental data describing valve closing response and waterborne arsenic. **b** Predicted relationship between Hill coefficient and response time (t_{R}). **c** Predicted relationship between maximum response ($R_{\max}$) and response time (t_{R})

$[\mathrm{BL}^-] = 0.18$ ng g^{-1} (1.32 pmol g^{-1}). Consequently, $[a]$ value in BLM scheme-based EC50 model (Eq. 2) can be estimated to be $[a] = 2.39 \times 10^7$ L g^{-1} (3.32×10^9 M^{-1}) followed the relationship of $[a] = \mathrm{BCF} \times [\mathrm{BL}^-]^{-1}$.

Equation 4 was fitted to published response time-specific dose–response curves with higher r^2 values (0.89–0.99), describing the relationship between valve closing response and arsenic activity in water (Fig. 3a), to obtain $n(t_\mathrm{R})$ and $R_{\max}(t_\mathrm{R})$ (Fig. 3b, c). The relationship between estimated $n(t_\mathrm{R})$ (Fig. 3b) and response time (t_R) has a form as $n(t_\mathrm{R}) = 1.65 + 2.5\exp(-t_\mathrm{R}/0.31)\ (r^2 = 0.98)$ with n ranging from 1.63 to 3.13. The relationship between estimated $R_{\max}(t_\mathrm{R})$ and response time (t_R) has a form as $R_{\max}(t_\mathrm{R}) = 1.00 - 1.06\exp(-t_\mathrm{R}/0.36)(r^2 = 0.98)$ (Fig. 3c). Based on the fitted arsenic-activity–time–response curves (Fig. 3a), the estimated EC50$_{\mathrm{AsBL}}(t_\mathrm{R})$ values were 0.22 (1.62 nM), 0.12 (0.83 nM), and 0.05 μg L^{-1} (0.39 nM) for valve response times of 30, 60, and 120 min, respectively. Therefore, low arsenic concentrations caused a significant change in the valve closing/opening activity, suggesting that valve movement is suitable for the biologically sensitive endpoint. The results also show that there were profound differences in sensitivity to arsenic in different response times for $n(t_\mathrm{R})$, $R_{\max}(t_\mathrm{R})$, $f^{50\%}_{\mathrm{AsBL}}(t_\mathrm{R})$, and EC50$_{\mathrm{AsBL}}(t_\mathrm{R})$ estimates.

Model applications

Model application was performed (Eq. 3) with site-specific water chemistry characteristics (Table 2) to predict site-specific EC50(t_R) as water quality criteria for *C. fluminea* in response to waterborne arsenic in clam farms located at Changhua and Hualien with site-specific water chemistry characteristics (Fig. 4). Figure 4a, b indicates that predicted 30-min-EC50s are 0.26 (1.86 nM) (95% CI: 0.23–0.31 (1.62–2.23 nM)) and 1.91 (3.71 nM) μg L^{-1}, whereas 120-min-EC50s are 0.06 (0.45 nM) (95% CI: 0.05–0.07 (0.40–0.54 nM)) and 0.46 μg L^{-1} (3.34 nM) for Changhua and Hualien clam farms, respectively. The results indicate that clam valve opening/closing rhythm in Changhua clam farm is more sensitive than that in Hualien.

Model application was also performed to generate data sets of valve daily activity dynamics in response to waterborne arsenic activities ranging from 0 to 0.28 μg L^{-1} (0–2 nM) that occurred at specific times of 02:00, 09:00, and 16:00 hours,

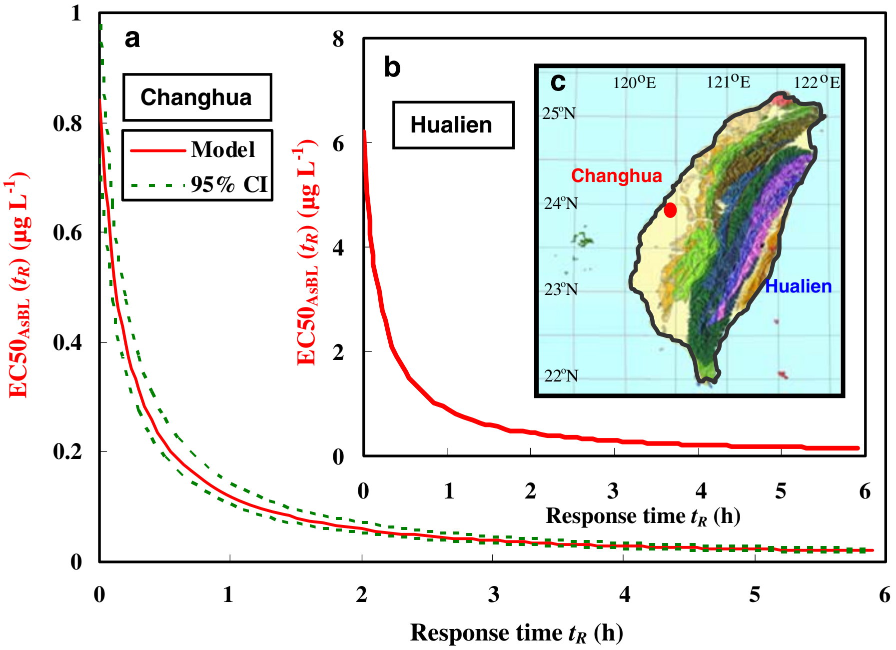

Fig. 4 Predicted site-specific EC50(t_R) of clam farms in **a** Changhua and **b** Hualien. **c** Map shows the locations of study clam farms

respectively, in association with a baseline valve daily rhythm in the absence of arsenic (Fig. 5a–d). Each value shown in Fig. 5a is the mean proportion of valve opening of 16 clams of a 14-day observed period at every 5 min (14 readings per 5 min) (Liao et al. 2009). It therefore would be possible to extrapolate from that rich data set typically presented in Fig. 5b–d to predict waterborne arsenic activities. Consequently, equilibrium arsenic body burden of *C. fluminea* could also be estimated with known BCF estimate as: $C_b(\infty) = \mathrm{BCF} \times C_w$.

Figure 5e, f gives an example demonstrating site-specific valve daily rhythm changes in response to an input total arsenic concentration of 2 mg L^{-1} that occurred at 18:00 hours at clam

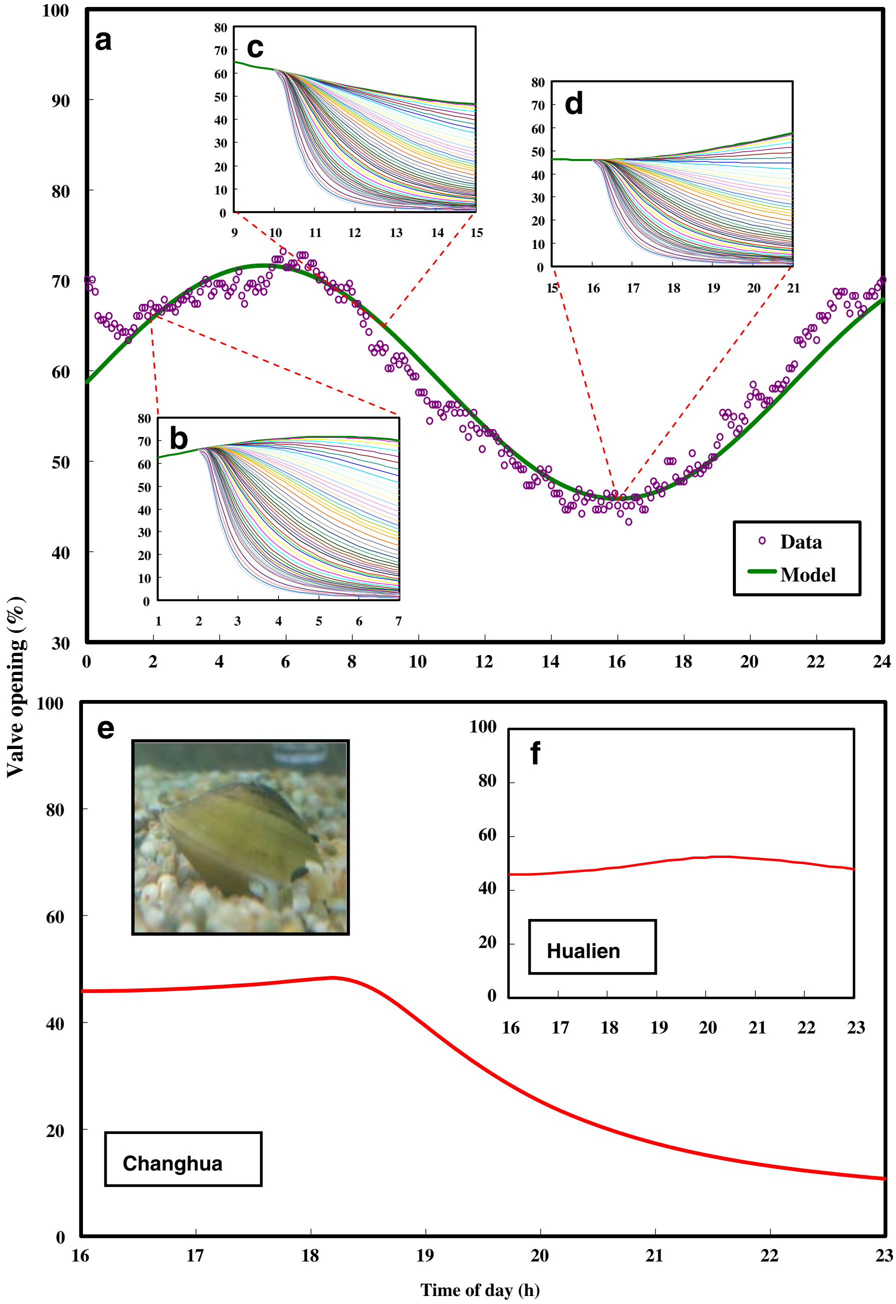

Fig. 5 **a** A baseline valve daily rhythm in *C. fluminea* in the absence of arsenic (Liao et al. 2009). **b–d** Data sets generated from the proposed model (Eq. 5) showing valve daily rhythm dynamics in response to arsenic activities ranging from 0–0.28 μg L^{-1} occurred at 02:00, 09:00, and 16:00 hours, respectively. **e**, **f** Site-specific valve daily rhythm changes to total arsenic concentration of 2 mg L^{-1} occurred at 18:00 hours at clam farms of Changhua and Hualien, respectively

farms of Changhua and Hualien, respectively. Simulation results show that free ion activities of arsenate ({As(V)}) are estimated to be 0.24 (1.73 nM) and 0.17 μg L^{-1} (1.25 nM) at Changhua and Hualien clam farms, respectively. The arsenic body burdens were calculated to be 1.05 and 0.76 ng g^{-1}, respectively. It thus suggested that the present algorithm can be used as a strategy for predicting waterborne bioavailable arsenic concentration and arsenic body burden of *C. fluminea*. The application can be extended to test the bivalve biological response ability to close its shell as an alarm signal to reflect health of clam when exposed to waterborne arsenic.

Discussion

Ecological risk assessment implications

The purpose of this study was to provide a mechanistic model that incorporates explicitly the factors controlling bioavailability and bioaccumulation to enhance predictive ability of arsenic toxicity to protect health of *C. fluminea*. The reconstructed dose–response curves shown in Fig. 3a, describing valve closing response and arsenic free ion activity, can be used to provide a regulatory policy in recommending the site-specific water quality criteria for clam farms. The US Environmental Protection Agency (2000) recommended that EC10 could be used as a surrogate threshold of regulatory endpoint in ecological risk assessment. It may employ Fig. 2a to derive $\mathrm{EC}X_{\mathrm{AsBL}}(t_{\mathrm{R}})$, the response time-dependent BLM-predicted arsenic ECX value. Consequently, the response time-dependent fraction of the total number of arsenic binding sites occupied by arsenic at X% effect, $f^{X\%}_{\mathrm{AsBL}}(t_{\mathrm{R}})$, can also be obtained by fitting Eq. 2 to ECX data.

Therefore, clam gill arsenic binding capacity can be represented explicitly as a response surface function of $f^{X\%}_{\mathrm{AsBL}}(t_{\mathrm{R}}, \phi)$, where ϕ denotes the valve closing response (Fig. 6) and has a general form

$$f^{50\%}_{\mathrm{AsBL}}(X, t_{\mathrm{R}}) = 0.105 + 0.003X + 0.733\exp\left(-\frac{t_{\mathrm{R}}}{d}\right), \tag{9}$$

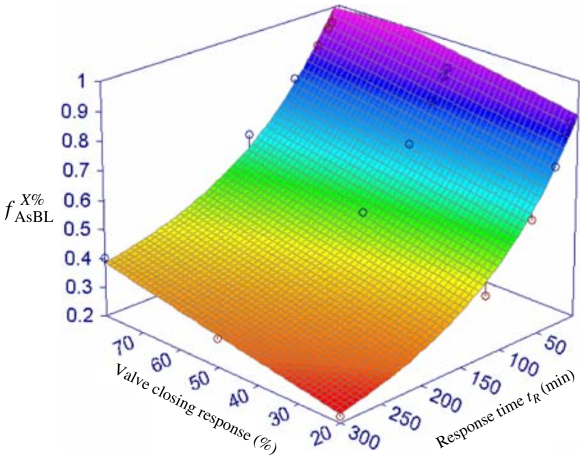

Fig. 6 A response surface showing the relationship between $f^{X\%}_{\mathrm{AsBL}}(X, t_{\mathrm{R}})$ and valve closing behavior

where $d = 131.72$ (95% CI: 97.56–165.87) ($r^2 = 0.99$). Thus, a site-specific water quality criterion was obtained for waterborne arsenic based on recommended EC10 guideline by incorporating Fig. 5 with $X = 10$ into Eq. 2 with $[a] = \mathrm{BCF} \times [\mathrm{BL}^-]^{-1}$,

$$\mathrm{EC10}_{\mathrm{AsBL}}(t_{\mathrm{R}}) = \frac{f^{10\%}_{\mathrm{AsBL}}(t_{\mathrm{R}})}{\left(1 - f^{10\%}_{\mathrm{AsBL}}(t_{\mathrm{R}})\right)} \times \left(\frac{[b]}{\mathrm{BCF} \times \left[\mathrm{BL}^-\right]^{-1}}\right). \tag{10}$$

In view of Eq. 10, the proposed valve daily rhythm-based EC10 guideline accounts for arsenic bioavailability and bioaccumulation parameter associated with site-specific water chemistry characteristics. Therefore, the predictive $\mathrm{EC10}_{\mathrm{AsBL}}(t_{\mathrm{R}})$ model (Eq. 10) may provide a good predictor of arsenic bioavailability and toxicity and is adequate to accurately assess the potential impact of arsenic on the ecological quality of aquatic ecosystems. The results have broad applicability to other metals to overcome frequently over-protective, and occasionally under-protective, on-site-specific water quality criteria. Thereby, it provided a means for estimating the effect of site-specific factors on metal toxicity. The predicted accumulation of arsenic in *C. fluminea* also points to bioavailability and BCF as important risk factors for human health resulting from the consumption of this species.

Biomonitoring implications

This study thus proposes an approach that accounts for the interaction among clam valve movement and arsenic bioavailability and arsenic toxicity. This idea is to seek an integrated approach describing the dynamic link between valve daily activity and bioavailability associated with bioaccumulation that predicts the clam gill arsenic binding capacity and soft tissue arsenic burden (Fig. 7). It thus would be useful to link valve daily activity in *C. fluminea* for arsenic bioavailability and bioaccumulation directly. This approach further confirmed that valve daily rhythm-based biomonitoring linking with toxicokinetics and BLM is a useful tool to describe arsenic–gill binding interactions and to predict arsenic burden of soft tissue in the field situations.

Cherry and Soucek (2007) have intensively reviewed the practical uses of *C. fluminea* as an *in situ* monitoring test organism, underscoring the increasing importance of integrating *in situ* bioassays using field-caged bivalves with traditional measures of ecological integrity. Newton and Cope (2007) further pointed out that valve activity in freshwater bivalves has promise as a biological response to contaminants because it is relatively easy and inexpensive to monitor, mirroring responses at ecologically relevant concentrations. Daily activities are vital for physiological and behavioral homeostasis in multi- and unicellular organisms (Rosato 2007). These endogenous daily activities allow organisms to coordinate a myriad of physiological and developmental processes such as growth with predictable daily environmental changes like day length, temperature, and food availability (Rosato 2007). Gerhardt et al. (2005) suggested that it is urgently needed to define daily rhythm changes as a new behavioral test parameter to provide a candidate factor for biomonitoring the aquatic contaminants.

For such a dynamic clam biomonitoring system performed in any field situation, a further limitation with respect to the uncertainty/variability problem provoked by various environmental conditions (e.g., photoperiod, light intensity, DO, and trophic additions) must be considered. Those factors affect the bivalve behavioral rhythm response and further disturb the sensitivity of the signal when false responses occur (Ortmann and Grieshaber 2003). The influences and complexities of the environment are a key problem and also have to be taken into consideration, especially, the influence of organic functional groups and oxidation state of arsenicals (Shaw et al.

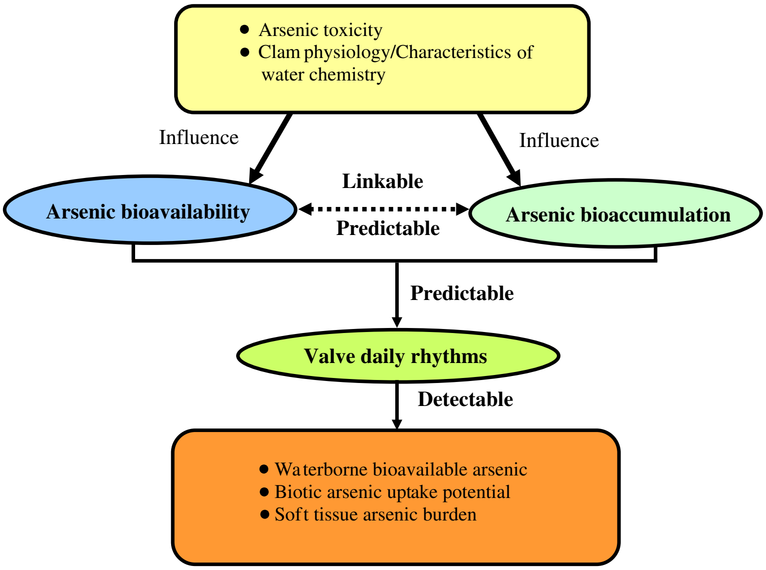

Fig. 7 Conceptual model of proposed "valve daily activity-bioavailability-bioaccumulation" approach describing environmental monitoring and assessment of freshwater clam *C. fluminea* exposed to arsenic

2007). The possibilities of additive, synergistic, potentiation, and antagonism interact between other contaminations and arsenic may act on the free sites of the BL. Recently, researches have been investigating the combined toxicity effects of metal mixtures on some aquatic organism used in BLM (Hatano and Shoji 2008; Kamo and Nagai 2008). Hence, an application of the integration among BLM, toxic unit, and toxicological models to predict multi-metal toxicities on freshwater clam under such conditions at environmentally relevant situations should be considered in future research. Furthermore, the site-specific bivalve daily rhythm exposed to uncontaminated aquatic environments should be built in the database of a valve daily subsystem associated with auxiliary water quality sensors such as temperature, pH, conductivity, and dissolved oxygen. Furthermore, we have to carefully process the observation of response-time-specific valve daily activity in the field to precisely evaluate the cause of provoking abnormal response of valve movement to reliably provide minimal false estimation of arsenic bioavailability.

This study demonstrated the first link between the function of a clam valve daily subsystem and arsenic bioavailability and bioaccumulation to predict affinity at the arsenic-binding site in gills and clam arsenic body burden. The study can assist the government to evaluate the biomagnification of arsenic in food chains and detect harmful concentration thresholds in high-trophic-level organisms, including humans. It is hoped that this proposed BLM-based valve daily subsystem can offer a convenient and useful tool to accurately detect free ion activity of arsenic to account for the bioavailability and the bioaccumulation potential of *C. fluminea* exposed to arsenic. This present method may also set a path toward more mechanistic understanding as well. Such understanding may be achieved by in-depth examination of the particular biosensors that dominate the response in one case or another, or by systematic examination of receptor–ligand interactions in behavioral ecotoxicology settings (Dell'Omo 2002). This research may provide insight into the development of biomonitoring organisms such as *C. fluminea*, mimicking metal bioaccumulation in a real situation. This study therefore suggested that freshwater clam valve activity could be used as an interface sensor between aquatic ecosystems and ecologically relevant arsenic exposure.

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