Ecotoxicology
DOI 10.1007/s10646-010-0489-9

Dynamic features of ecophysiological response of freshwater clam to arsenic revealed by BLM-based toxicological model

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Accepted: 19 March 2010

Abstract The objective of this study was to use a quantitative process-based damage assessment model (DAM) associated with biotic ligand model (BLM) to examine the ecophysiological responses of freshwater clam *Corbicula fluminea* to waterborne arsenic. We carried out a 14-day exposure experiment to obtain bioaccumulation parameters and a 7-day acute toxicity bioassay to obtain survival data. To investigate the survival dynamics, we examined 2 key parameters characterizing bioaccumulation and damage regulation: capacity to eliminate body arsenic burden and reversible ability to recover the damage. Results show that uptake rate constant of 2.075 ± 0.442 (mean $\pm$ SE) ml g^{-1} d^{-1} during uptake phase and elimination rate constant was estimated to be 0.1995 ± 0.022 d^{-1}. The derived bioconcentration factor of 10.401 ml g^{-1} suggests that arsenic has a high potential for bioaccumulation in *C. fluminea*. Our results show that a negative association between bioaccumulation and the fraction of arsenic binding in biotic ligand at 50% mortality, indicating that *C. fluminea* with higher arsenic binding in gill biotic ligand at 50% mortality level gives a lower capacity to accumulate bioavailable arsenic. We found a linearly positive correlation between elimination rate and recovery rate constants. Yet, a potential tradeoff between ability to eliminate arsenic and ability to recover the damage is not found. We showed that an ecophysiological significance of *C. fluminea* exposed to arsenic can be revealed by the elimination–recovery regime. This research may also provide mechanistic insights into the development of biomonitoring organism such as *C. fluminea* mimicking metal bioaccumulation in a real situation.

Keywords Arsenic · Freshwater clam · *Corbicula fluminea* · Bioaccumulation · Bioavailability · Ecophysiology

List of symbols

As	Arsenic
$[a]$	Formation of the As complex with inorganic matter and binding to biotic ligand (M^{-1})
{ions}	Ions activity concentration (mg l^{-1})
BCF	Bioconcentration factor (ml g^{-1})
BCF{ions}	BCF of As to organism considering the competition of ions (ml g^{-1})
$[BL^-]$	Biotic ligand (μg g^{-1})
BLM	Biotic ligand model
C_b	As concentration in freshwater clam (mg g^{-1})
C_w	Waterborne As concentration (mg l^{-1})
DAM	Damage assessment model
DOC	Dissolved organic carbon
D	Cumulative damage level (–)
D_0	Threshold of cumulative damage level (–)
$D_{L,\ 50}$	Cumulative damage level for median lethal (–)
$D_{L,\ 50}/k_a$	Compound equivalent toxic damage level for median lethal
$f_{AsBL}^{50\%}$	Fraction of the total number of As binding sites occupied by As at median effect (–)
H	Cumulative hazard (–)
K_{ionsBL}	Stability constants for the binding of ions to the biotic ligand (M^{-1})

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Published online: 06 April 2010

k_a	Damage accumulation rate constant (g μg^{-1} d^{-1})
k_k	Killing rate constant (g μg^{-1} d^{-1})
k_r	Recovery rate constant (d^{-1})
k_1	Uptake rate constant (ml g^{-1} d^{-1})
k_2	Depuration rate constant (d^{-1})
POC	Particular organic carbon
LC50	External median lethal As concentration (mg l^{-1})
LT50	Median lethal time (d)
S	Survival probability (–)
ST50	Median survival time (d)
WHAM	Windermere humic aqueous model

Introduction

A fundamental property of organisms is their ability to respond to environmental stressors. Responses can be reversible and irreversible, depending on how organism reacts to a stressor that is first applied then withdrawn. If the mode of action in target organ of organism returns to its original state after the stressor, the response is reversible. If mode of action persists in a changed state after the stressor, the transition is irreversible. One of the important issues in ecotoxicological modeling is to model appropriately the mechanisms are responsible for reversible-irreversible responses while organisms exposed to contaminants.

To model how the reversible-irreversible interactions in aquatic organisms exposed to metals, the recently developed biologically-based damage assessment model (DAM) is a suitable candidate (Lee et al. 2002). The DAM describes the mode of action of compounds with rapid reversible binding to the target site as well as to those that act with irreversible binding. DAM assumes that death occurs when the cumulative damage reaches a critical level. Damage is assumed to accumulate in proportion to the accumulated residue and damage recovery in proportion to the cumulative damage when damage is reversible. When initial damage overwhelms threshold damage, then the damage is irreversible. Therefore, the recovery rate in DAM is species and metal specific, ranging from 0 to infinity. In DAM, the recovery rate constant characterizes all processes leading to recovery such as repair mechanisms on a cellular scale or adaptation of the physiology and other compensating processes. The DAM model thus provides a more comprehensive framework to investigate the time-dependent toxicity of chemical incorporating the co-influence of chemical accumulation and damage accumulation. This is particular true for real field exposures.

Available experimental evidence (Lee et al. 2002; Ashauer et al. 2006, 2007a, b, c) indicates that a process-based DAM is capable of simulating the survival of aquatic organisms exposed to contaminants. It is not inconceivable that this conclusion might be better suited for understanding aquaculture species exposed to toxic metals. Understanding the existing published experimental evidence (Lee et al. 2002; Reinert et al. 2002; Diamond et al. 2006; Ashauer et al. 2006, 2007a, b, c) enhances our confidence in the estimates of the dynamic physiological response and recovery of aquaculture species to toxic metals. Tsai et al. (2009) have also developed a bioavailability- and mode of action-based bioenergetic growth model that link biotic ligand model (BLM) and DAM to assess how arsenic affects the tilapia growth in entire life span in site-specific field conditions. They concluded that BLM-based DAM well described the water chemistry-dependent toxicokinetic and toxicodynamic variations of arsenic to tilapia. Luoma and Rainbow (2005) and Buchwalter et al. (2008) further indicated that depuration rate constants are the most important determinant in bioaccumulation processes, yet bioaccumulation alone is not necessarily a good predictor in survival modeling of aquatic organisms. Therefore, by linking other biologically-based approach to bioaccumulation model is crucial for better understanding the inherent ecophysiological responses of accumulated metals in aquatic organisms.

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 *C. fluminea* and the need for costly monitoring programmes. 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).

The purpose of this paper was to use a quantitative process-based DAM associated with BLM to examine the ecophysiological responses of *C. fluminea* to waterborne arsenic. A mechanistic model based on cation-dependent toxicokinetics was formulated to serve as a bridge between the experimentally measured responses to standard constant concentrations and predictions of the survival of *C. fluminea*. To obtain the essential bioaccumulation and acute toxicity data, two experiments with *C. fluminea* were

carried out in this paper. One experiment involved conduction of a 14-day exposure experiment to obtain bioaccumulation parameters. The second experiment conducted a 7-day acute toxicity bioassay to obtain survival data. We aimed to motivate and develop effective process oriented BLM-based DAM that determines in a fully quantitative fashion to develop robust approximate relations that convey how environmental contaminants impacts on species ecophysiological responses. Further, an important of this study is that physiological and recovery rate estimates must, at least partly, rely on DAM and that pure estimates from experiments are currently not possible.

Materials and methods

Experiments

Juvenile *C. fluminea* ($\sim$26–30 mm shell length with $\sim$5–7 g wet wt), collected from freshwater clam farms situated at Hualien of eastern Taiwan, were acclimated (23–26°C, 10‰ salinity, fed with algae *Platymonas* sp.) for at least 2–3 weeks before experiments. Water ionic compositions were Ca^{2+} 24.8, Mg^{2+} 1.0, Na^{+} 4.9, K^{+} 2.7, H^{+} 7.21, NH_4^{+} 0.26, Cl^{-} 7.6, NO_2^{-} 0.047, and NO_3^{-} 0.318 mg l^{-1}.

For the measurement of survival, ten *C. fluminea* were transferred randomly into each experimental tank (5 l water) exposed to arsenic. The nominal arsenic concentrations were 0 (control), 2, 5, 10, 25, 50, 100, and 200 mg l^{-1}. Only *C. fluminea* exhibiting obvious signs of death (blackened and decomposing tissues, odor) were removed and included in mortality counts. *C. fluminea* exposed in parallel to control suffered no mortalities over the course of the experiment. Gross mortality of *C. fluminea* to each concentration were recorded every 30 min for the first 24 h and every 1 h thereafter to the end of the experiment, and the dead *C. fluminea* being removed every 0.5–1 h. Tanks were treated as replicates and mortalities were averaged.

To determine arsenic accumulation, a preliminary test was firstly conducted, revealing that 50% lethal tolerance arsenic concentration of *C. fluminea* was found at $\leq$5 mg l^{-1}. Thorsen et al. (2007) indicated that uptake in freshwater bivalves generally were very rapid when bivalves first exposed to environmental contaminants. Thus, an exposure experiment was conducted by using 5 mg l^{-1} of water arsenic concentration for 3 days uptake and then 11 days depuration in clean water. During the exposure experiment, experimental *C. fluminea* were not fed and mortality was less than 5%. Five *C. fluminea* were sequentially removed from each of two tanks at daily basis to determine arsenic uptake. The frozen soft tissue of *C. fluminea* were dehydrated in an oven (105°C) for 24 h and grounded into fine powder. Aliquots of dry soft tissue powder weighting 100 mg were placed into a 250 ml beaker. Nitric acid (SIGMA, Taiwan, purity = 65%, 10 ml) was added and then covered with a glass for an overnight digestion. After the initial digestion, the beaker was heated with a water bath at 95°C for 2–4 h to reduce the total volume to 3 ml. This volume of solution was transferred to a volumetric flask (50 ml). The rinsed solution (5 ml of 0.01 N of HNO_3) for the watch glass was also added to the flask. The flask was then filled with 0.01 N of HNO_3 to dilute to a 25 ml of final solution with double deionized water.

Individual arsenic was analyzed by a Perkin–Elmer Model 5100PC atomic absorption spectrometer (Perkins–Elmer, Shelton, Connecticut, USA) equipped with an HGA-300 graphite furnace atomizer. Analytical quality control was achieved by digesting and analyzing identical amounts of rehydrated (90% H_2O) standard reference materials (dogfish muscle, DORM-2; NRC-CNRC, Canada). Recovery rate was 95.3 $\pm$ 2.6%, and the levels of detection were 0.62 μg As l^{-1} for water samples and 0.05 μg As g^{-1} for soft tissue samples.

Mechanistic models

Here the BLM is used to predict the degree of arsenic binding at the site of action causing toxicity in gill of *C. fluminea* (Fig. 1a, b). In light of BLM scheme (Fig. 1a, b), the lethal concentration for 50% response over time (LC50(t)) can be expressed in terms of the time course fraction of the total number of arsenic binding sites occupied by arsenic at 50% effect (i.e., $f_{\mathrm{AsBL}}^{50\%}(t)$) (De Schamphelaere and Janssen 2002),

$$\mathrm{LC50}(t) = \frac{f_{\mathrm{AsBL}}^{50\%}(t)\left(1 + \sum K_{\mathrm{ionsBL}}\{ions\}\right)}{\left(1 - f_{\mathrm{AsBL}}^{50\%}(t)\right)[a]}, \quad (1)$$

where $\sum K_{\mathrm{ionsBL}}\{ions\} = K_{\mathrm{CaBL}}\{Ca^{2+}\} + K_{\mathrm{MgBL}}\{Mg^{2+}\} + K_{\mathrm{NaBL}}\{Na^{+}\} + K_{\mathrm{HBL}}\{H^{+}\}$in that K_{CaBL}, K_{MgBL}, K_{NaBL}, and K_{HBL} represent the affinity constants for the binding of these cations to the biotic ligand (M^{-1}), $\{ions\}$ denotes the activity of each ion of water chemistry characteristics (M), and $[a]$ represents the formation of the arsenic complex with inorganic matter and binding to biotic ligand (M^{-1}).

The primary focus of the present study is on the process-based viewpoint of the organism damage response. Insights into the DAM associated with the process-based properties, damage response of organism and its environment can be described by three dynamic variables: the water arsenic concentration (the input), the internal damage (the hazard), and the survival (the output). Figure 1c illustrates the block diagram of continuous representation of process-based DAM.

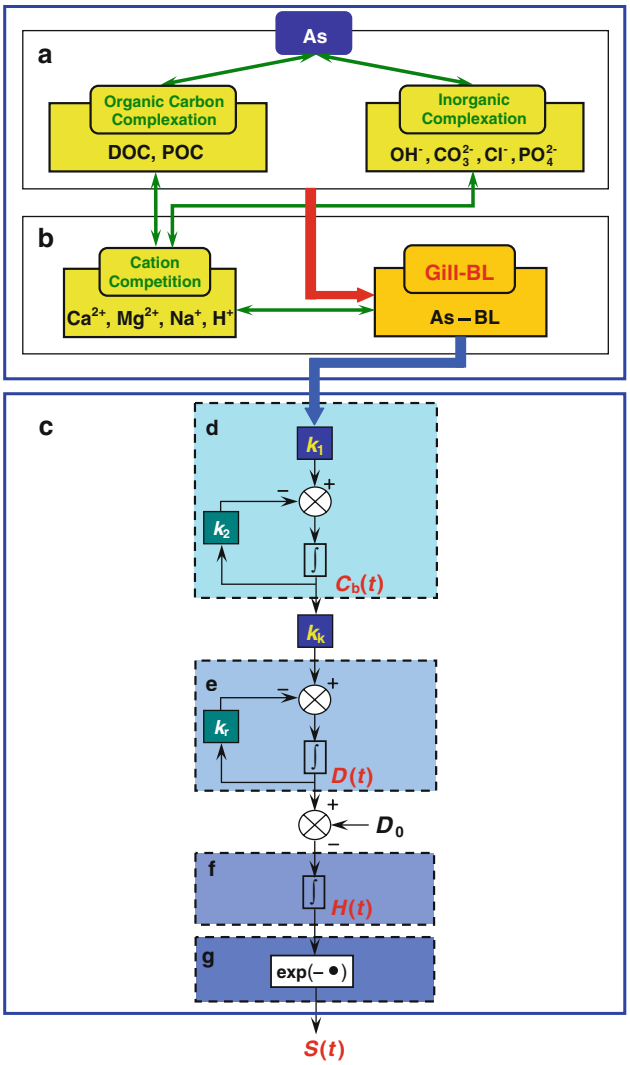

Fig. 1 **a** and **b** Schematic representation of the biotic ligand model for arsenic bioavailability where DOC: dissolved organic carbon and POC: particular organic carbon. **c–g** Block diagram of BLM-based damage assessment model. (See text for the symbol descriptions)

First, first-order bioaccumulation model can be used to predict the organism body burden followed the exposure to water arsenic concentrations (Fig. 1d),

$$\frac{dC_b(t)}{dt} = k_1 C_w - k_2 C_b(t), \tag{2}$$

where $C_b(t)$ is the time-dependent arsenic concentration in the soft tissue of *C. fluminea* (μg g^{-1} dry wt), k_1 is the *C. fluminea* uptake rate constant (ml g^{-1} d^{-1}), k_2 is the depuration rate of arsenic (d^{-1}), and C_w is the dissolved arsenic concentration in the water (mg l^{-1}), t is the time in day.

Second, the time-dependent cumulative damage can be derived from the first-order damage accumulation model as (Ashauer et al. 2007a, b, c) (Fig. 1e),

$$\frac{dD(t)}{dt} = k_k C_b(t) - k_r D(t), \tag{3}$$

where $D(t)$ is the damage at time t (dimensionless), k_k is the killing rate constant (g μg^{-1} d^{-1}), and k_r is the damage recovery or repair rate constant (d^{-1}). The killing rate constant in Eq. 5 is the proportionality factor describing the relation between the cumulative damage and hazard.

Therefore, when a threshold for damage is exceeded, the time change of hazard (i.e., hazard rate) rises above zero, indicating the probability of the organisms suffering injure at a give time t (Fig. 1f),

$$\frac{dH(t)}{dt} = D(t) - D_0, \tag{4}$$

where $H(t)$ is the cumulative hazard (dimensionless) and D_0 presents the threshold of damage (dimensionless). Thus, the survival probability $S(t)$ can be derived directly from DAM and is given by the exponential of cumulative hazard (Lee et al. 2002; Ashauer et al. 2007a, b, c) (Fig. 1g),

$$S(t) = e^{-H(t)}. \tag{5}$$

Tsai et al. (2009) linked the BLM and DAM to construct a relation for estimating time-dependent $f^{50\%}_{\mathrm{AsBL}}(t)$and bioconcentration factor (BCF($\{ions\}$, t)) by assuming that the free ion activity concentration resulting in 50% effect calculated by DAM ($\mathrm{LC50}_{\mathrm{DAM}}$ (t)) equals to that predicted by the BLM ($\mathrm{LC50}_{\mathrm{BLM}}(t)$),

$$\frac{D_{L,50}/k_a}{\left(\frac{e^{-k_r t}-e^{-k_2 t}}{k_r-k_2}+\frac{1-e^{-k_r t}}{k_r}\right)}\mathrm{BCF}^{-1}(\{ions\},t) = \frac{f^{50\%}_{\mathrm{AsBL}}(t)(1+\sum K_{\mathrm{ionsBL}}\{ions\})}{(1-f^{50\%}_{\mathrm{AsBL}}(t))[a]}, \tag{6}$$

where $D_{\mathrm{L},50}$ is the cumulative damage for 50% effect, k_a is the damage accumulation rate ((μg g^{-1})$^{-1}$ d^{-1}), $D_{\mathrm{L},50}/k_a$ is a coefficient reflecting the compound equivalent toxic damage level required for 50% effect (μg d g^{-1}), BCF($\{ions\}$, t) is the BCF of arsenic to organism considering the competition of cations $\{ions\}$ at time t. In view of Eq. 6, BCF($\{ions\}$, t)has the form as (Tsai et al. 2009),

$$\mathrm{BCF}(\{ions\},t) = \frac{(1-f^{50\%}_{\mathrm{AsBL}}(t))[a](D_{L,50}/k_a)}{f^{50\%}_{\mathrm{AsBL}}(t)(1+\sum K_{\mathrm{ionsBL}}\{ions\})\left(\frac{e^{-k_r t}-e^{-k_2 t}}{k_r-k_2}+\frac{1-e^{-k_r t}}{k_r}\right)}. \tag{7}$$

Thus, the commonly employed one-compartmental toxicokinetic model can be modified by the BLM concept for linking the relationship between BCF($\{ions\}$, t) and $f^{50\%}_{\mathrm{AsBL}}(t)$.

Data analysis

A Hill equation was used to best fit the mortality data over time to determine 50% lethal time (LT50). The relationships between LT50 and exposure arsenic concentration can then be established. Uptake and depuration rate constants were determined by fitting the integrated form of the kinetic rate equation to concentration data for constant arsenic exposure, using nonlinear regression technique,

$$C_b(t) = C_b(t=0)e^{-k_2 t} + \frac{k_1}{k_2}C_w(1 - e^{-k_2 t}), \quad (8)$$

where $C_b(t=0)$ is initial concentration of arsenic in *C. fluminea* soft tissue (μg g^{-1} dry wt). The depuration rate constant (k_2) could be estimated by the linear regression of log-transformed soft tissue arsenic concentrations on depuration time (days) as $\ln C_b(t) = \ln C_b(t=T) - k_2 t$ where T is the time when depuration begins.

TableCurve 2D (Version 5.0) and 3D (Version 4.0) (AISN Software Inc., Mapleton, OR, USA) packages were used to perform all curve fittings. WHAM (Windermere humic aqueous model) Version 6 (WHAM VI, Center for Ecology and Hydrology, Lancaster, UK) was performed to calculate the activities of competing and complex ions considered in BLM scheme. The default inorganic arsenic form in WHAM is arsenate (As(V)). Fergusion and Gavis (1972) indicated that at equilibrium thermodynamics, As(V) dominants in oxidative environments of most surface water. Thus, As(V) being the most prevalent in most surface waters. A Monte Carlo technique was performed to generate 2.5- and 97.5-percentiles as the 95% confidence interval (CI) for all fitted models. Crystal Ball® software (Version 2000.2, Decisionerring, Inc., Denver, Colorado, USA) was used to implement the Monte Carlo simulation.

Results

Ecophysiological responses to arsenic in *C. fluminea*

A very rapid uptake fashion was found in *C. fluminea* exposed to arsenic over the course of uptake experiment (Fig. 2). The kinetic rate equation in Eq. 8 was fitted to uptake data to obtain the estimated uptake rate constant k_1 of 2.075 ± 0.442 ml g^{-1} d^{-1} (mean ± SE) and depuration rate constant k_2 of 0.73 ± 0.253 d^{-1} with $r^2 = 0.98$. On the other hand, a smooth depuration curve was found and the k_2 was estimated to be 0.1995 ± 0.022 d^{-1} with $r^2 = 0.78$ (Fig. 2). Using the value of k_2 in depuration phase determined above, the depuration half-life in the absence of uptake was estimated to be $\ln 2/k_2 = 3.474$ d. The equilibrium BCF can also be calculated to be 10.401 ml g^{-1}, indicating the potential arsenic accumulation capacity when

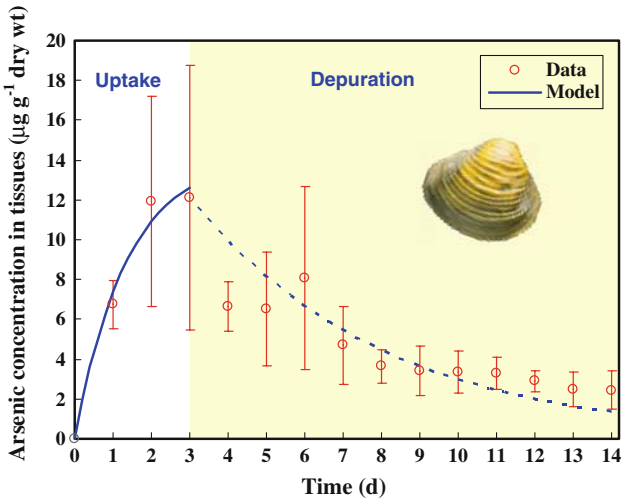

Fig. 2 Exposure experiment in the freshwater clam *C. fluminea* exposed to 5 mg l^{-1} arsenic for 3 days and another 11 days for depuration in clean water. Error bars are standard deviation from mean ($n = 5$)

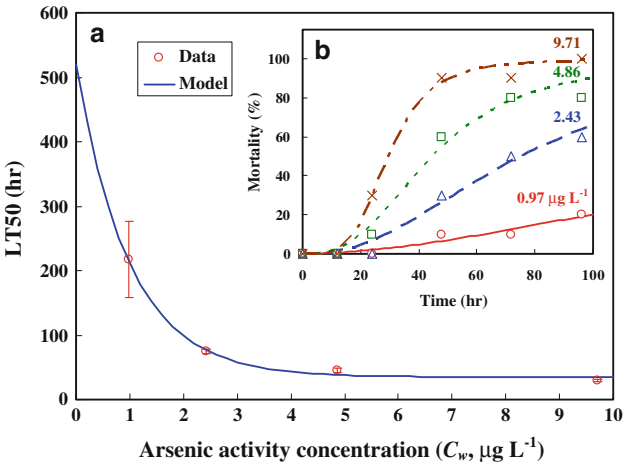

Fig. 3 **a** The best fitted regression to LT50(C_w) data (mean ± 95% CI) showing the relationship between LT50 and arsenic activity concentration. **b** Prediction of time-mortality profiles of *C. fluminea* exposed to As activity concentration ranging from 0.97 to 9.71 μg l^{-1} in that symbols indicate the measurements

C. fluminea exposed to a given waterborne arsenic concentration.

A relationship between LT50 and exposure concentration was achieved followed short-term laboratory exposure to specific arsenic concentrations in the BLM scheme (Fig. 3a). Table 1 summarizes the affinity constants used in the BLM scheme for *C. fluminea*. The LT50 estimates were obtained by fitting the Hill equation to concentration-specific mortality data taking into accounting arsenic bioavailability (Fig. 3b). The results indicate that concentration-specific LT50 estimates were 216.96 ± 59.09, 75.12 ± 3.83, 44.88 ± 3.0, and 29.52 ± 1.26 h for exposure arsenic activity

concentrations 0.97, 2.43, 4.86, and 9.71 μg l^{-1}, respectively. The best-fitting model $LT50(C_w) = 34.75 + 484.87\exp(-C_w/0.99)$ ($r^2 = 0.99$, $p < 0.05$) well describes the relationship between LT50 and arsenic activity concentrations (Fig. 3a).

Dynamic linkage between bioavailability and bioaccumulation

Time-dependent fraction of the total number of arsenic binding sites occupied by arsenic at 50% effect ($f^{50\%}_{AsBL}(t)$), could be estimated by fitting Eq. 1 to LC50(t) data with known affinity constants (Table 1) and water chemistry data. Figure 4a shows the acute $f^{50\%}_{AsBL}(t)$ over time for *C. fluminea* in that predicted $f^{50\%}_{AsBL}(t)$ has the form as $f^{50\%}_{AsBL}(t) = 0.28 + 0.53\exp(-t/26)$ ($r^2 = 0.82$, $p < 0.05$).

Table 1 Input BLM affinity constants together with estimated bioaccumulation and BLM-based DAM parameters

BLM affinity constants	
log K_{MgBL} (M^{-1})	3.58[a]
log K_{HBL} (M^{-1})	5.40[a]
log K_{CaBL} (M^{-1})	LN(3.53, 1.03)[b]
log K_{NaBL} (M^{-1})	LN(3.09, 1.04)[b]
Bioaccumulation parameters	
k_1 (ml g^{-1} h^{-1})[c]	0.0865 ± 0.0184
k_2 (h^{-1})[c]	0.0083 ± 0.0009
BCF (ml g^{-1})	10.401
BLM-based DAM parameters	
$\sum K_{ionsBL}\{ions\}(-)$[d]	0.189
[a] (l g^{-1})[e]	9.51×10^4
$f^{50\%}_{AsBL}(\infty)$[e]	0.28
[BL^-] (μg g^{-1})[f]	0.109
$D_{L,50}/k_a$ (ng h g^{-1})[g]	1.197
k_r (h^{-1})[g]	17.42
k_k (g ng^{-1} h^{-1})[h]	0.579
BCF ({*ions*}, ∞)(ml g^{-1})[i]	4.487

[a] Adopted from Niyogi and Wood (2004)

[b] Adopted from Liao et al. (2007) in that LN(gm, gsd) represents lognormal distribution with a geometric mean and a geometric standard deviation

[c] Uptake rate constant and depuration rate constant estimated form Eq. 8 (mean ± SE)

[d] Parameter used in Eq. 1

[e] [a] and $f^{50\%}_{AsBL}(\infty)$ are estimated from Eq. 1 by fitting the acute toxicity data

[f] [BL^-] value is calculated as BCF/[a]

[g] $D_{L,50}/k_a$ and k_r are estimated from Eq. 6 by fitting the acute toxicity data

[h] k_k values are calculated as $\ln 2/(D_{L,50}/k_a)$

[i] Calculated from Eq. 7

On the other hand, the fitted $LC50_{AsBL}(t)$ profile ($r^2 = 0.95$, $p < 0.05$) estimated by Eqs. 1 and 6 was illustrated in Fig. 4b, by which two BLM-based DAM key parameters can be also estimated: killing rate $k_k = 0.58$ g ng^{-1} h^{-1} and recovery rate constant $k_r = 17.42$ h^{-1}. The predicted $f^{50\%}_{AsBL}(t)$shows a mild decreasing from nearly 0.81 initially and then slowly to a steady-state value of 0.28, whereas for $LC50_{AsBL}$, a dramatic decreasing from nearly 50 μg l^{-1} at the 5th hour to a steady-state value of 2 μg l^{-1} (Fig. 4a, b). Figure 4c shows the relationship between $LC50_{AsBL}(t)$ and $f^{50\%}_{AsBL}(t)$. A response surface is constructed to

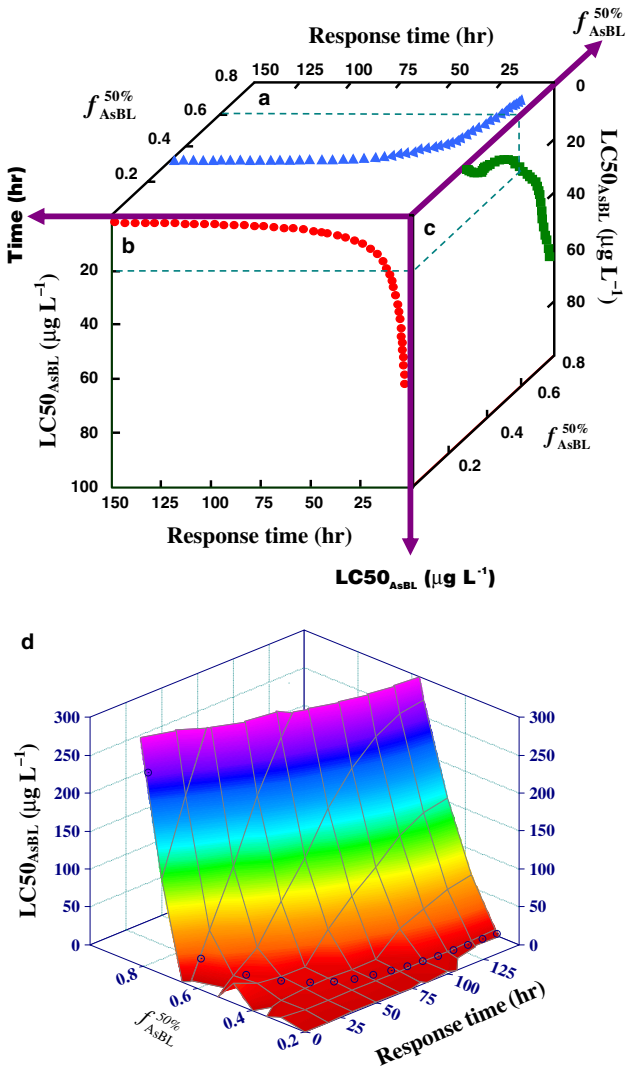

Fig. 4 **a** Relationships between predicted fraction of the total number of As binding sites occupied by arsenic at 50% mortality ($f^{50\%}_{AsBL}$) and response time (t). **b** Fitting proposed BLM-based DAM LC50 equation to published experiment LC50(t) data for freshwater clam. **c** Quantitative relationship between $LC50_{AsBL}(t)$ and $f^{50\%}_{AsBL}(t)$. **d** A response surface showing the relationships among $f^{50\%}_{AsBL}$, $LC50_{AsBL}$, and response time (t)

demonstrate the relationship between $LC50_{AsBL}(t)$, $f_{AsBL}^{50\%}(t)$, and response time, indicating a strong correlation between $LC50_{AsBL}(t)$ and $f_{AsBL}^{50\%}(t)$ (Fig. 4d).

Moreover, the concentrations of unoccupied biotic ligand site, $[BL^-]$, can also be calculated from the relationship of $[a] = BCF[BL^-]^{-1}$ with a fitted $[a]$ value ($[a] = 9.51 \times 10^4$ l g^{-1}), resulting in $[BL^-] = 0.109$ μg g^{-1}. The affinity parameter ($\sum K_{ionsBL}\{ions\}$) in BLM scheme-based $LC50(t)$ model (Eq. 1), can be calculated to be 0.189. Taken together, the estimated essential bioaccumulation and BLM-based DAM parameters are summarized in Table 1.

The relationships between time-profiles of $BCF(\{ions\}, t)$ and $f_{AsBL}^{50\%}(t)$ in that $f_{AsBL}^{50\%}(t)$, $BCF(\{ions\}, t)$, and $BCF(\{ions\}, t) - f_{AsBL}^{50\%}(t)$ profiles reveal an interesting interaction in this study (Fig. 5). They are predicted with the input of BLM parameters (i.e., $\sum K_{ionsBL}\{ions\}$and $[a]$), predicted $f_{AsBL}^{50\%}(t)$, and DAM parameters (i.e., $D_{L,50}/k_a$ and k_r) (Eq. 7). Given the related BLM and DAM parameters, the response time-specific lognormal probability distributions of $BCF(\{ions\},t)$ and $f_{AsBL}^{50\%}(t)$can be estimated (Fig. 5a, b). A joint distribution of response time-specific arsenic bioavailability and bioaccumulation therefore can be determined (Fig. 5c). A steady-state relationship can be achieved and can be described quantitatively by Eq. 7 as $BCF(\{ions\}, \infty) = 1.75((1 - f_{AsBL}^{50\%}(\infty))/f_{AsBL}^{50\%}(\infty))$ (Fig. 5c). It reveals a negative association between $BCF(\{ions\},\infty)$ and $f_{AsBL}^{50\%}(\infty)$, indicating that *C. fluminea* with higher $f_{AsBL}^{50\%}(\infty)$ gives a lower capacity to accumulate bioavailable arsenic.

Dynamics of survival in $k_2 - k_r$ regimes

In this study, we linked bioaccumulation parameter of k_2 and DAM parameter of recovery rate constant k_r to better understand the potential biological consequences of accumulated arsenic in *C. fluminea*. Using our exposure assay, k_2 was determined to be 8.3×10^{-3} h^{-1} and k_r can be calculated to be 17.42 h^{-1}. Here we used a $k_2 - k_r$ regime to explore the dynamics of survival in *C. fluminea* (Fig. 6). Specifically, the 50% survival time (ST50) increased from 93.3 to 102.93 h with increasing k_2 (0.0067–0.0103 h^{-1}) and k_r (14.57–20.57 h^{-1}) (Fig. 6d–f followed the shaded arrow). No compensation relationship was plausibly found in the $k_2 - k_r$ regimes, i.e., increasing k_r may not compensate for lower k_2, and vice versa.

A positive linear relationship was found between the ability to eliminate arsenic from *C. fluminea* and the damage repair capability followed the best-fitting model $k_2 = -0.032 + 0.0023k_r$ ($r^2 = 0.97$, $p < 0.05$) (Fig. 6j). The findings reveal that once k_2 is determined experimentally, the value of k_r can be calculated by the above fitted linear model or by measuring the survival probability. Note, however, that the recovery time (t_r) can also be estimated from the recovery rate constant estimate as $t_r = 1/k_r$.

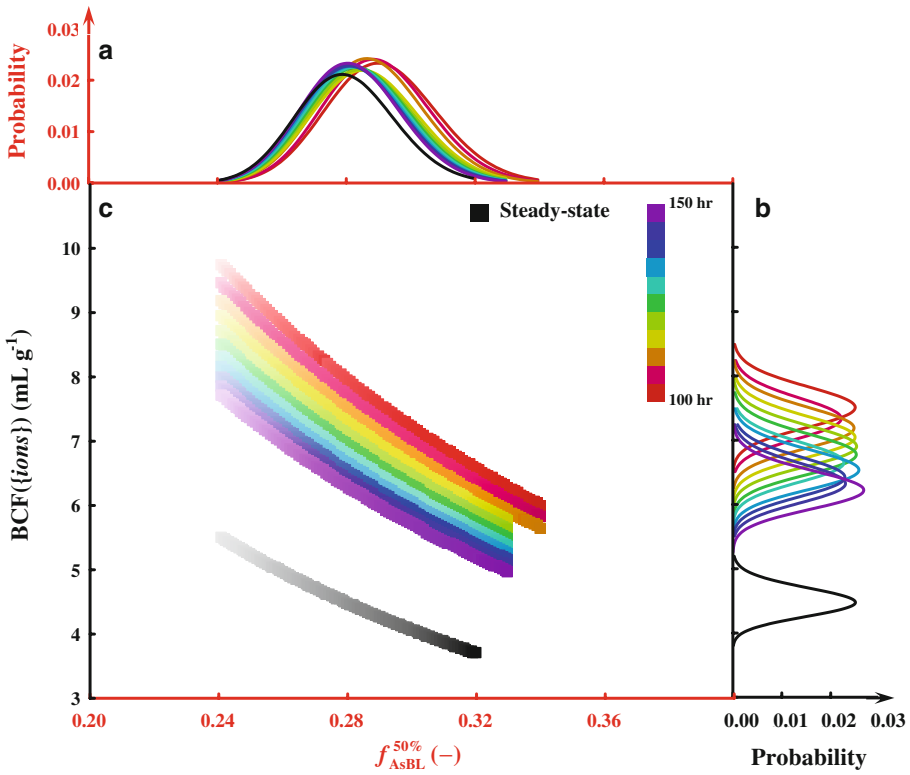

Fig. 5 **a** The lognormal probability distributions of $f_{AsBL}^{50\%}$ varied with response times 100–150 h. **b** The lognormal probability distributions of $BCF(\{ions\})$ varied with response times 100–150 h. **c** The joint distributions showing the quantitative relationships between $BCF(\{ions\})$ and$f_{AsBL}^{50\%}$ varied with response times 100–150 h together with steady-state distribution

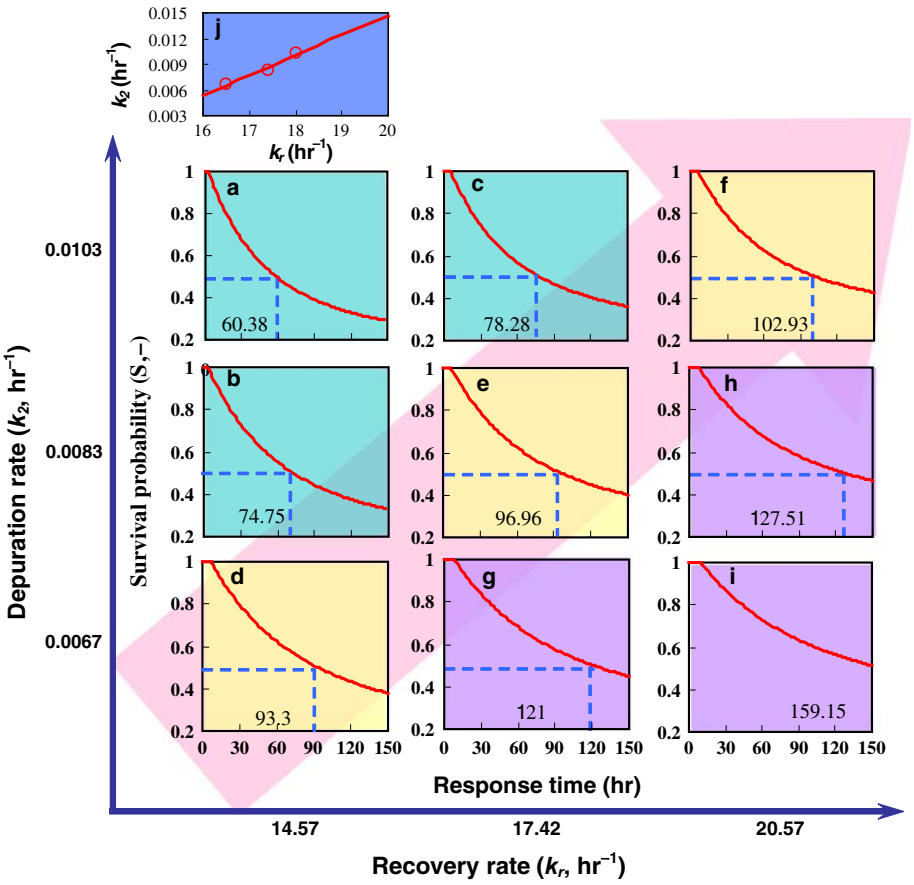

Fig. 6 **a–i** Dynamics of survival probability distributions in the recovery rate constant (k_r) and depuration rate constant (k_2) regimes. **j** The fitted linear model of the relationship between the recovery rate and depuration rate constants

Discussion

BLM-based DAM

To better understand the processes driving internal damage change and assess their potential impact on ecophysiological response after arsenic exposures, a processs-based DAM in the BLM scheme was used to examine the effects dynamically. Understanding the processes of exposure on aquaculture species response as a dynamical system will require viewing it on several levels, including bioavailability, bioaccumulation, internal damage, and cumulative hazard and survival probability, in individual species over time. The present approach is a step in this direction and can be enhanced by existing ecotoxicological modeling methods and relevant experimental data. The present approach provides a window into ecotoxicological scheme and opens the way for understanding how *C. fluminea* show ecophysiological responses to arsenic exposure.

Thus, a BLM-based DAM, formulated by understanding of inherent interactions between metal stressors and receptors and metal regulations of organisms, can be used to quantify time-dependent toxicity of metal bioavailability incorporating the co-influence of metal and damage accumulation under a broad range of metal stressor-driven environment. The BLM-based time-varying DAM containing nonlinearities can predict survival dynamics of *C. fluminea* after arsenic exposures. A key question is whether these predictions result from the present simple model actually provide fundamental insight into the dynamics of species responses.

Thus, we anticipated that the model predictions can be coupled quantitatively with the essential experiments where direct determinations of toxicokientic rate constants and manipulation of environmental conditions can be achieved. Well-performed metal exposure experiments and related ecotoxicological models (e.g., process-based DAM) in simulating survival after metal exposures in particular are capturing the essence of dynamics of several model systems, for example, freshwater invertebrate (*Gammarus pulex*) (Ashauer et al. 2007a, b, c), aquatic insect (Buchwalter et al. 2008), and freshwater snails (*Lymnaea stagnalis*) (Croteau and Luoma 2009).

Although the present used models are costly to parameterize, the mechanisms can be applied to a broad range of organisms that share general life-history features, such as feeding-dependent growth rates of aquaculture species dictated by allometric scaling of energy acquisition and expenditure. Using these confrontations between theory and experiment, the important linkages between ecophysiology

and life-history traits can be found that explain dynamics of organism responses in metal stressor-driven environments (Buchwalter et al. 2008).

Ecophysiological responses implications

In this study, survival dynamics of *C. fluminea* exposed to arsenic are determined by 2 key parameters: the capacity to eliminate body arsenic burden (k_2) and the reversible ability to recover the damage (k_r). The k_2 can be estimated experimentally. However, direct measurement of k_r in process-based DAM is difficult, if not impossible, because it not much known underlying the mechanism of damage recovery. Once k_2 is estimated, however, the value of k_r can be calculated by BLM-based DAM algorithm presented in this study. We then used a BLM-based DAM to analyze how the survival response characterizing by ST50 changes depending on these parameters. Our results indicate that changes in depuration and recovery rate constants may shift the dynamics of survival distributions. In general, depuration rate constant k_2 alone was not necessarily a strong predictor in BLM-based DAM mechanism. Recovery rate constant in the feedback loop (Fig. 1e) also reveals the importance of the whole mechanism.

In this study, we found a linearly positive correlation between bioaccumulation parameter k_2 and DAM parameter k_r. Yet, a potential tradeoff between the ability to eliminate arsenic and the ability to recover the damage is not found. We suggested that an ecophysiological significance of *C. fluminea* exposed to arsenic can be revealed by a $k_2 - k_r$ parameter regime varied with different specific values. The combined use of exposure experiments and mathematical calculation allowed us to determine fundamental similarity in the ecotoxicological response mechanisms of aquaculture species exposed to metal stressor. It would be of interest to investigate similar parameters related to detoxicfication rate constants (Croteau and Luoma 2009), which may open up additional perspectives on these sophistication of aquaculture species response to environmental metal-stressors.

The gill is the first interaction site with the trace metal which has a finite interaction capacity. The BLM suggests that the fraction of the biotic ligand sites occupied by As(V) ions, f_{AsBL}, could provide an approximation of the As(V) concentration at target sites and toxicity. The fraction of the biotic ligand sites occupied by arsenic increased representing the organism accumulates more As(V) toxicities. In this study, we quantified the relationship between BCF($\{ions\}$)comprising the water chemistry influences and $f_{\mathrm{AsBL}}^{50\%}$. Where $f_{\mathrm{AsBL}}^{50\%}$ is related to the As(V) binding proportion, representing the ability induces a 50% effect when organism are exposed to a toxicant. Therefore, the low value of the As(V) binding proportion in biotic ligands at 50% effect represents a relative higher toxicity. Moreover, the toxicity can increase with the increasing of BCF($\{ions\}$) and decreasing of $f_{\mathrm{AsBL}}^{50\%}$ values. Therefore, the BCF($\{ions\}$) and $f_{\mathrm{AsBL}}^{50\%}$ relations reveal a negative association.

Given the direct contact with ambient water, gills are proposed to be the first and most important targets of bivalves exposed to waterborne metals (Jorgensen 1996). Several studies also indicated that the major route of uptake for the chemicals that concentrate in bivalves was across the gill epithelium (Dietz and Byrne 1990; Zheng and Dietz 1998). Hence, responses of bivalve to metals were mediated by physiological regulation mechanisms at the gill (Zheng and Dietz 1998). Gill regulation is a time-dependent acclimation characterizing by both how quickly the acclimation is activated to prevent further effects and how long the acclimation stays in place during nonstressed conditions to put in action when the next exposure comes.

Given that both physiological parameters of aquatic organisms and geochemistry parameters of ambient water are considered, this approach is of potential utility to develop and refine the ambient water quality criteria (Paquin et al. 2002; Niyogi and Wood 2004). Hence, the integration between BLM and DAM can further be used to describe metal-gill binding interactions and to predict metal toxicities to aquatic organisms in the field situations. Although the current model was employed for freshwater clam, the underlying principle of linking metal bioavailability and internal damage accumulation to environmental sensitivity caused by metal exposures is broadly applicable across aquaculture species. Our ability to predict the consequences of ecophysiological response has also implications for optimizing species growing, cultivation strategies, and risk assessment in realistic situations.

Biomonitoring implications

In the future study, we may integrate BLM-based DAM with freshwater clam valve movement for biomonitoring purpose. This idea is to seek an integrated approach describing the dynamic link between valve daily activity and arsenic bioavailability associated with bioaccumulation that predicts the clam gill arsenic binding capacity and soft tissue arsenic burden for risk prediction. It thus would be useful to link valve daily activity in *C. fluminea* for arsenic bioavailability and bioaccumulation directly. It suggests 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 (Liao et al. 2007; Jou 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 aquatic contaminants. 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 promised as a biological response to contaminants because it is relatively easy and inexpensive to monitor, mirroring responses at ecologically relevant concentrations. 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. 2007).

This study may also assist government to evaluate the biomagnification of arsenic in food chains and detect harmful concentrations threshold in high-trophic level organisms including humans. It hoped that BLM-based DAM incorporating with valve daily system can offer a convenient and useful tool to detect accurately free ion activity of arsenic to account for arsenic bioavailability and bioaccumulation potential of *C. fluminea*. This present method may also set a path toward more mechanistic understanding as well. This research may also provide mechanistic insights into the development of biomonitoring organisms such as *C. fluminea* mimicking metal bioaccumulation in a real situation.

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