

Toxicokinetics/toxicodynamics links bioavailability for assessing arsenic uptake and toxicity in three aquaculture species

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Abstract The purpose of this study was to link toxicokinetics/toxicodynamics (TK/TD) and bioavailability-based metal uptake kinetics to assess arsenic (As) uptake and bioaccumulation in three common farmed species of tilapia (*Oreochromis mossambicus*), milkfish (*Chanos chanos*), and freshwater clam (*Corbicula fluminea*). We developed a mechanistic framework by linking damage assessment model (DAM) and bioavailability-based Michaelis–Menten model for describing TK/TD and As uptake mechanisms. The proposed model was verified with published acute toxicity data. The estimated TK/TD parameters were used to simulate the relationship between bioavailable As uptake and susceptibility probability. The As toxicity was also evaluated based on a constructed elimination–recovery scheme. Absorption rate constants were estimated to be 0.025, 0.016, and 0.175 mL g⁻¹ h⁻¹ and As uptake rate constant estimates were 22.875, 63.125, and 788.318 ng g⁻¹ h⁻¹ for tilapia, milkfish, and freshwater clam, respectively. Here we showed that a potential trade-off between capacities of As elimination and damage recovery was found among three farmed species. Moreover, the susceptibility probability can also be estimated by the elimination–recovery relations. This study suggested that bioavailability-based uptake kinetics and TK/TD-based DAM could be integrated for assessing metal uptake and toxicity in aquatic organisms. This study is useful to quantitatively assess the complex environmental behavior of metal uptake and implicate to risk assessment of metals in aquaculture systems.

Keywords Arsenic · Bioavailability · Uptake · Ecotoxicology · Susceptibility · Fish · Shellfish

Introduction

Arsenic (As) is widespread in the environment from anthropogenic and natural processes. Environmental As is a hazardous trace element which exists in both organic and inorganic states. In the unpolluted surface water and groundwater, the average levels of As generally ranged from 1 to 10 µg L⁻¹, and freshwater varied typically from 0.15 to 0.45 µg L⁻¹ (Smedley and Kinniburgh 2002; Bissen and Frimmel 2003). In Taiwan, As contamination ranged from 10 to 1,820 µg L⁻¹ in the aquatic systems (Nordstrom 2002). Furthermore, As concentrations in fish (tilapia *Oreochromis mossambicus* and *Oreochromis* sp., milkfish *Chanos chanos*, Indo-Pacific tarpon *Megalops cyprinoids*, striped mullet *Mugil cephalus*, 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 (Lin et al. 2001; 2005a, b; Huang et al. 2003; Liao et al. 2003; Chen et al. 2004; Liu et al. 2005, 2007, 2008). Previous investigation indicated that As could accumulate in freshwater organism tissues, and humans who consume these As-contaminated tissues may pose health risk (Williams et al. 2006). It is known that As inhibits more than 200 enzymes and causes adverse effects leading to serious disorders such as blackfoot disease, skin lesions, and several cancers of the bladder, kidney, liver, and vasculature to human (Abernathy et al. 1999; Chen et al. 2005).

Based on the toxicological principles in the aquatic environment, the chemical toxicity depends on the internal (biological) and the external (geochemical) factors. The chemical bioavailability depends on the external elements

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such as pH, hardness, specific ions, and chemical reaction and toxicity influencing the mechanism of the bioavailability to aquaculture species (De Schampelaere and Janssen 2002). The major ions competition and complex effects on chemical species play important roles in affecting metal toxicity. On the other hand, the internal factors include organism-specific physiological makeup and acclimating capacities to their environment. Within species, body size and repair mechanism can affect organisms sustaining the chemical stresses.

A lot of physiological traits control the susceptibility to the metal stressors. The physiological processes include the uptake and elimination for bioaccumulation and detoxification, storage, and antioxidant physiology for the coping mechanism (Buchwalter et al. 2008). The biological mechanism that describes toxic chemical induced adverse response caused by their accumulation within the aquatic organisms, indicating that they could resist the toxicity invasion and compensates the health (Lee et al. 2002). However, As accumulation is dependent on membrane transport proteins of aquatic organisms, As binding to transport protein and mediate As uptake and arrive in the target site (Veltman et al. 2008). The As uptake capacity in the tissue would be the determinant factor to realize the As toxicity to aquaculture species. Recently, Lee et al. (2002) have developed damage assessment model (DAM) to describe the time course of median lethal concentration data for chemicals that acts through the reversible interaction between chemicals and receptors. In DAM, death occurs based on the cumulative damage which reaches a critical level.

In this study, we developed a mechanistic framework by linking DAM and As bioavailability uptake mechanisms to examine As toxicity on three aquaculture species of tilapia (*O. mossambicus*), milkfish (*C. chanos*), and freshwater clam (*Corbicula fluminea*) exposed to waterborne As. All of the study farmed species are traditional food fish and shellfish for people in Taiwan. Therefore, the tolerances of aquaculture species to As toxicity are needed to be estimated. Especially, the relationship between bioavailable As uptake and susceptibility with As toxicity. We considered geochemistry and biological mechanism to test the proposed model using published acute toxicity data and to predict median lethal concentrations (LC50) and susceptibility probability.

Materials and methods

Study data

The major information used in this study were taken from previous published data on the acute toxicity and exposure bioassays of waterborne As to tilapia, milkfish, and freshwater clam (Cheng 2003; Liao et al. 2004, 2008; Chou et al. 2006). The available data comprised an extensive range of physiological characterization and toxicity information such as As body burden and lethal toxicity, which permitted the present analysis to estimate the key physiological determinants. The time-dependent As body burden can be used to realize absorption, elimination, As uptake rate constants, whereas the time- and concentration-dependent mortality can be used to assess time-dependent median lethal concentration and further to realize the bioregulation mechanism between As stressor and aquaculture species.

To determine the toxicokinetic parameters, the tilapia exposure bioassay was using juvenile tilapia (mean body length=12.9±1.54 cm (mean±SD) and mean body weight=32.75±4.2 g wet weight) exposed to 1 mg L⁻¹ As, the milkfish exposure bioassay was using juvenile milkfish (mean body length=24.95 cm and mean body weight=237.82 g wet weight) exposed to 5 mg L⁻¹ As, and freshwater clam exposure bioassay was using adult freshwater clam (mean shell length=27.6±2.4 mm and mean body weight=6.19±0.86 g wet weight) exposed to 5 mg L⁻¹.

All of them were exposed in an uptake phase for 168 h. Afterwards, freshwater clams were transferred to clean water for 168 h to eliminate after the As uptake phase in the bioaccumulation bioassay. The acute toxicity bioassay of three aquaculture species were conducted to determine the 144-h LC50 values for tilapia and milkfish and 96-h LC50 for freshwater clam. The characteristics of water chemistry were presented in Table 1.

Geochemistry

To take into account the environmental factors and biological mechanisms, this study linked the geochem-

Table 1 Experimental conditions (mean ± SD) and available data in tilapia, milkfish, and freshwater clam acute toxicity bioassays

Farmed species	Temperature (°C)	pH	Water ionic concentration (mg L ⁻¹)			
			Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺
<i>O. mossambicus</i>	24.7±0.2	7.9±0.1	—	—	—	—
<i>C. chanos</i>	24.0±0.5	7.0±0.2	—	—	—	—
<i>C. fluminea</i>	24.3±1.3	8.0±0.1	24.8	1.0	4.9	2.7

istry condition and the absorption/As uptake mechanisms for improving the predictive ability of As toxicity to aquaculture species. Figure 1 illustrates the computational algorithm of this study.

In the geochemistry condition, sodium arsenite (NaAsO_2) stock solution is used in As-tilapia, As-milkfish, and As-freshwater clam bioassays (Cheng 2003; Liao et al. 2004, 2008). Wang et al. (2007) indicated that most of As in fish ponds of tilapia was in the form of As(V). Hence the oxidation and reduction of As needed to be considered. Here, the As states can be described as follows,



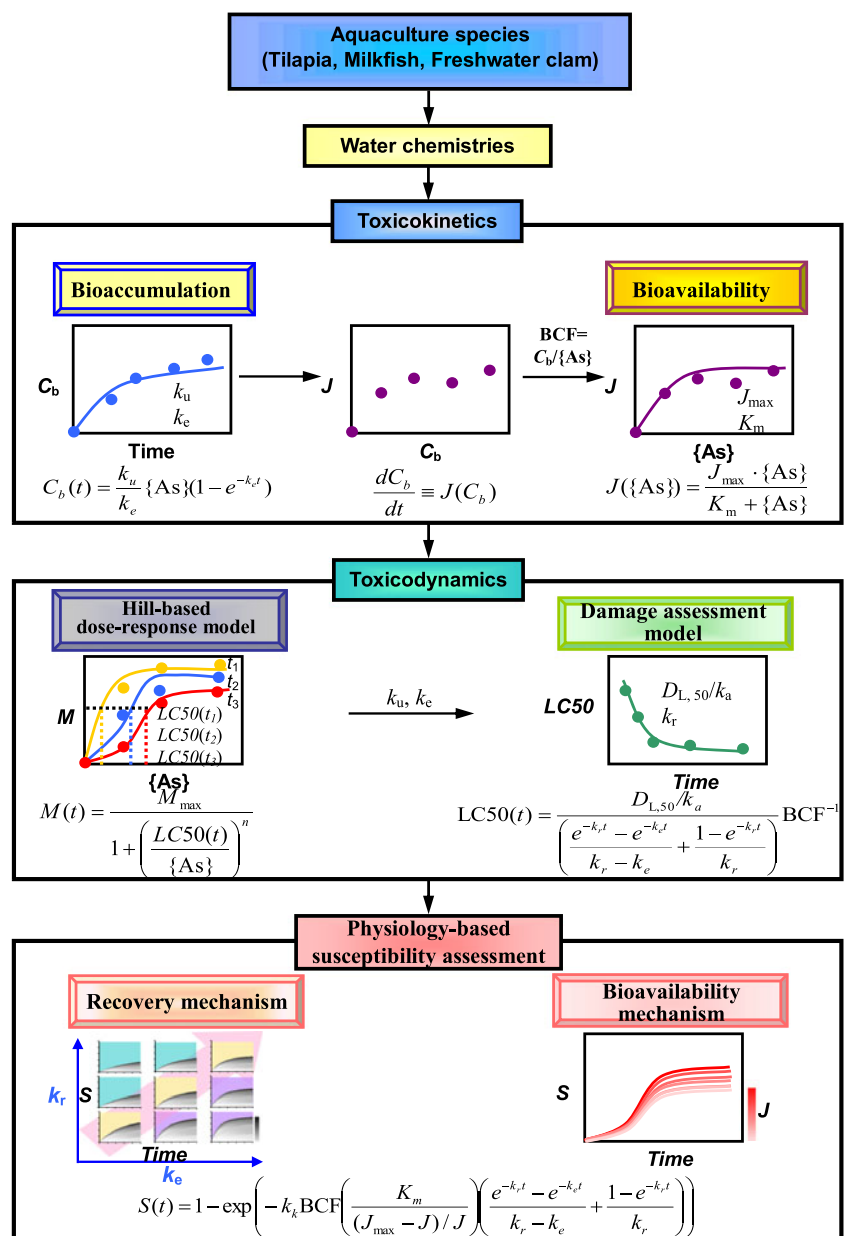
with O_2 ,



The effective chemical concentration was calculated by the principles of water chemistry. The theoretical expressions was based on the Debye–Huckel limiting law. The temperature, pH, measured ion concentration, and ionic strength were employed to calculate the activity coefficient to obtain the site-specific metallic ionic activity in the aquatic environment. The activity coefficient (γ) calculation can be described as follows (Stumm and Morgan 1981):

$$\gamma = 10^{-AZ^2\sqrt{I}} \quad (3)$$

Fig. 1 Schematic representation of computational algorithm to predict the susceptibility of tilapia, milkfish, and freshwater clam exposed to waterborne arsenic



in that

$$A = 1.82 \times 10^6 (PT_s)^{-3/2}, \quad (4)$$

$$I = \frac{1}{2} \sum_{i=1}^{i=i} C_i Z_i^2, \quad (5)$$

where A is a constant that depends on the water permittivity constant (P) and solution temperature, T_s (in Kelvin), where P is 78.3, I is the total metal ionic strength (in molar), C_i is the analytic ion concentration (in molar) of the each ion, and Z_i is the valence charge number of each ion in a solution.

Bioaccumulation model and bioavailable As uptake

First-order bioaccumulation model can be used to predict the organism's body burden following exposure to As concentrations. Absorption and elimination rate constants were determined by fitting the following integrated form of the kinetic rate equation to bioaccumulation data for As exposure,

$$C_b(t) = C_b(t=0)e^{-k_e t} + \frac{k_u}{k_e} \{As\} (1 - e^{-k_e t}), \quad (6)$$

where $C_b(t=0)$ is initial dependent concentration of As in aquaculture species tissue (in microgram per gram dry wt), k_u is the organism absorption rate constant (in milliliters per gram per hour), k_e is the elimination rate constant (in per hour), and $\{As\}$ is the As activity in the tank (in milligrams per liter). As concentration can be converted into As activity by multiplying activity coefficient in Eq. 3.

In light of the As-freshwater clam study, the accumulative capacity of freshwater clam was influenced by the As concentration dilution in the water phase (Liao et al. 2008). Hence, the bioaccumulation equation for freshwater clam could be written as,

$$C_b(t) = C_b(t=0)e^{-k_e t} + \frac{k_u}{k_e} \{As\} e^{-k_0 t} (1 - e^{-k_e t}) \quad (7)$$

where k_0 is the dilution rate of waterborne As to freshwater clam (in per hour).

Metal uptake processes of aquaculture species rely on the diffusive mass transfer from water through the gill, then metal binds to the transport protein on the biological membrane, and eventually the metal will distribute throughout the body. Different geochemical conditions result in the effective metal uptake capacity of aquaculture species. The relationship between body burden and metal uptake rate can be described as,

$$\frac{dC_b}{dt} \equiv J(C_b), \quad (8)$$

where J is the As uptake rate constant (in micrograms per gram per hour).

The relationship between As activity $\{As\}$ and As uptake rate constant (J) can also be obtained by a steady-state BCF definition as the $k_u/k_e \equiv C_b/\{As\}$. Hence, the best equation to predict the steady-state metal uptake could be described by Michaelis–Menten kinetics,

$$J(\{As\}) = \frac{J_{\max} \cdot \{As\}}{K_m + \{As\}} \quad (9)$$

where $J_{\max}$ is the maximum As uptake rate (in micrograms per gram per hour) and K_m is the affinity constant (in milligrams per liter).

Toxicodynamic model

To estimate the median lethal concentration (LC50), we reanalyzed the dose–response data from acute toxicity bioassay of three aquaculture species. The relationship between As activity and mortality could be described by a Hill-based dose–response function. Thus, the mortality in aquaculture species in response to As activity can be obtained as,

$$M(t) = \frac{M_{\max}}{1 + \left(\frac{LC50(t)}{\{As\}} \right)^n}, \quad (10)$$

where $M(t)$ is the time-dependent mortality (in percent response) based on As activity (in milligrams per liter) at any given time t , $M_{\max}$ is the time-specific maximum mortality (in percent), and n is a Hill coefficient which is a measure of cooperativity or a slop factor.

In light of a biologically DAM, the relationships between chemical tissue residues and accumulation-induced organism damage can be described by a first-order damage accumulation model and a first-order bioaccumulation model (Lee et al. 2002). Based on DAM, the damage-based LC50 can be derived as,

$$LC50(t) = \frac{D_{L,50}/k_a}{\left(\frac{e^{-k_r t} - e^{-k_e t}}{k_r - k_e} + \frac{1 - e^{-k_r t}}{k_r} \right)} BCF^{-1}, \quad (11)$$

where k_a is the damage accumulation rate (in grams per microgram per hour), $D_{L,50}/k_a$ is a coefficient which reflects the compound equivalent toxic damage level required for median lethal (in micrograms per hour per gram), k_e is the depuration rate (in per hour), k_r is the damage recovery rate constant (in per hour), and BCF is the bioconcentration factor (in milliliters per gram).

In the DAM scheme, the accumulation-induced organism damage is proportional to body chemical concentration, whereas the damage recovery is proportional to the cumulative damage that can be expressed as,

$$D(t) = k_a \text{BCF}\{\text{As}\} \left(\frac{e^{-k_r t} - e^{-k_e t}}{k_r - k_e} + \frac{1 - e^{-k_r t}}{k_r} \right). \quad (12)$$

According to Eq. 2, the cumulative hazard is proportional to the cumulative damage level. Thus, the time-dependent susceptibility probability can be expressed as the exponential of cumulative hazard,

$$S(t) = 1 - \exp \left(-k_3 \cdot k_a \text{BCF}\{\text{As}\} \left(\frac{e^{-k_r t} - e^{-k_e t}}{k_r - k_e} + \frac{1 - e^{-k_r t}}{k_r} \right) \right), \quad (13)$$

where $S(t)$ is the probability to susceptibility and the cumulative hazard can be refined as $H(t) = k_3 D(t)$ where $k_k = k_3 k_a$ is the killing rate that describes the relationships between cumulative damage and hazard level.

The relationship between As uptake rate and the susceptibility probability can be determined by combining Eqs. 9 and 13 and can be rearranged as,

$$S(t) = 1 - \exp \left(-k_3 \cdot k_a \text{BCF} \left(\frac{K_m}{(J_{\max} - J)/J} \right) \left(\frac{e^{-k_r t} - e^{-k_e t}}{k_r - k_e} + \frac{1 - e^{-k_r t}}{k_r} \right) \right). \quad (14)$$

We employed the TableCurve 2D (Version5, AISN Software, Mapleton, OR) to optimal fit the published data to obtain optimal statistical models. A Windemere Humic Aqueous Model (WHAM) Version 6 (WHAM VI, Centre for Ecology & Hydrology, Lancaster, UK) was used to perform the calculation of equilibrium chemical species. The default inorganic As form in WHAM is arsenate As(V). The Crystal Ball® software (Version 2000.2, Decisionerring, Inc., Denver, CO, USA) was employed to implement Monte Carlo (MC) simulation. A MC technique was performed to obtain the 2.5th- and 97.5th percentiles as the 95 % confidence interval for toxicological and physiological responses of farmed species to As(V) exposures. It showed that 10,000 iterations are sufficient to ensure the results.

Results and discussion

Bioavailable toxicokinetic parameters

Figure 2 depicts that the As accumulation in three aquaculture species was best described by the first-order bioaccumulation model ($r^2=0.83-0.98$) (Eq. 6). The rapid accumulation fashions were found in tilapia and milkfish

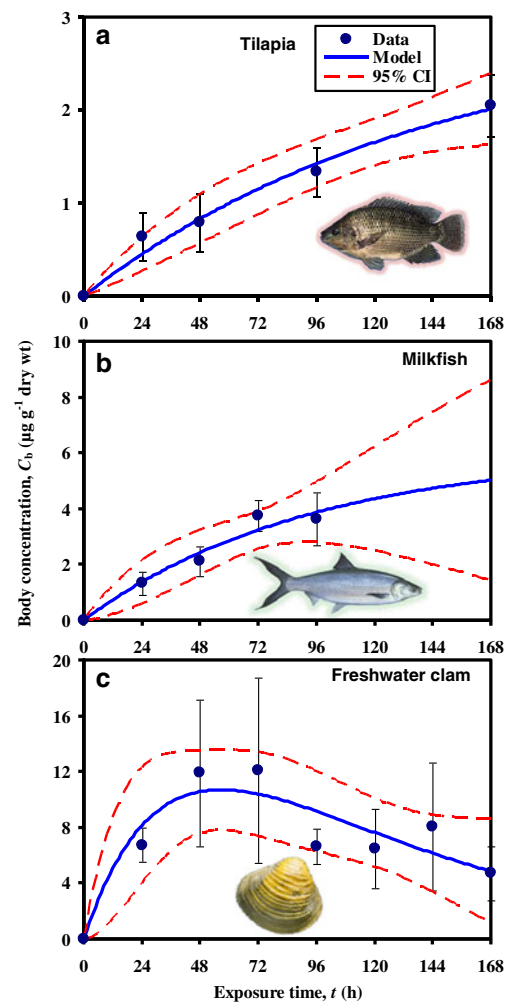


Fig. 2 The best-fit regression curves of As burden to **a** tilapia; **b** milkfish; and **c** freshwater clam from first-order bioaccumulation model. Errors bar are standard error from mean

exposed to 1 and 5 mg L⁻¹ As in the course of 168 h uptake phase (Fig. 2a, b). The toxicokinetic rate equation in Eq. 6 was fitted to As accumulation data of tilapia and milkfish to obtain the absorption rate constant (k_u) of 0.025 ± 0.004 mL g⁻¹ h⁻¹ (mean $\pm$ SE) and 0.016 ± 0.004 mL g⁻¹ h⁻¹ and elimination rate constant (k_e) of 0.007 ± 0.003 and 0.014 ± 0.052 h⁻¹, respectively. The other kinetic rate equation with As dilution rate in Eq. 7 was fitted to accumulation data of freshwater clam, resulting in k_u of 0.175 ± 0.068 mL g⁻¹ h⁻¹ and k_e of 0.14 ± 0.052 h⁻¹ in uptake phase (Fig. 2c).

We used the Michaelis–Menten kinetics in Eq. 9 to fit the As uptake rate of three aquaculture species (Fig. 3) to determine the maximum As uptake rate constant ($J_{\max}$) and the affinity constant (K_m). The resulting $J_{\max}$ and K_m estimates were 22.875 ± 10.090 ng g⁻¹ h⁻¹ and 0.041 ± 0.138 mg L⁻¹ for tilapia ($r^2=0.71$), 63.125 ± 25.756 ng g⁻¹ h⁻¹ and 0.516 ± 0.880 mg L⁻¹ for milkfish ($r^2=0.78$), and

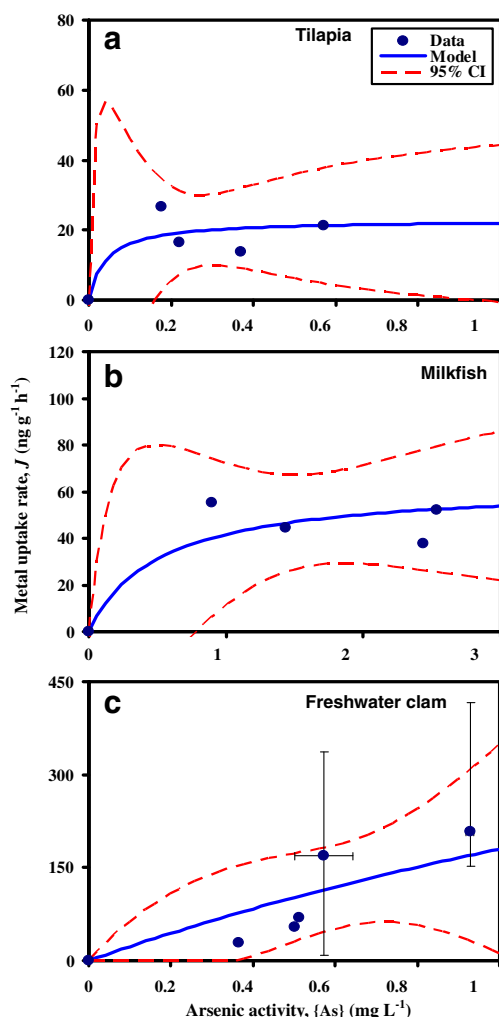


Fig. 3 Optimal fit of Michaelis–Menten kinetic model to estimated As uptake rate in **a** tilapia; **b** milkfish; and **c** freshwater clam

$788.318 \pm 2918 \text{ ng g}^{-1} \text{ h}^{-1}$ and $3.378 \pm 14.974 \text{ mg L}^{-1}$ for freshwater clam ($r^2=0.70$).

Acute toxicodynamic parameters

The optimal fits of DAM model to $LC50(t)$ data of tilapia, milkfish, and freshwater clam associated with the input toxicokinetic parameters and experimental data of water chemistry characteristics shown in Tables 1 and 2 are shown in Fig. 4. The $LC50(t)$ estimates were obtained by Hill-based dose–response function (Eq. 10). The results showed that $LC50(t)$ of tilapia were 33, 22.08, 18, 15, 11, and 9.81 mg L^{-1} at the response times of 24–144 h ($r^2=0.82$ –0.89), $LC50(t)$ of milkfish were 11.2, 7.28, 7, and 5.89 mg L^{-1} at the response times of 2–72 h ($r^2=0.86$ –0.95), and $LC50(t)$

of freshwater clam were 11.2, 7.28, 7, and 5.89 mg L^{-1} at the response times of 12–96 h ($r^2=0.99$).

The estimated DAM model-specific parameters of different aquaculture species are listed in Table 2. The results revealed that damage-based model was capable of describing the $LC50(t)$. The coefficient that reflects the compound equivalent toxic damage level required for median lethal $D_{L,50}/k_a$ was calculated to be $2.104 \pm 0.133 \text{ } \mu\text{g h g}^{-1}$ and recovery rate constant k_r of $7.875 \pm 0.500 \text{ h}^{-1}$ for tilapia ($r^2=0.87$), $D_{L,50}/k_a$ of $0.143 \pm 0.019 \text{ } \mu\text{g h g}^{-1}$ and k_r of $4.692 \pm 0.674 \text{ h}^{-1}$ for milkfish ($r^2=0.59$), and $D_{L,50}/k_a$ of $9.279 \pm 0.648 \text{ } \mu\text{g h g}^{-1}$ and k_r of $8.299 \pm 0.584 \text{ h}^{-1}$ for freshwater clam ($r^2=0.94$).

Physiology-based susceptibility assessment

We employed DAM to describe and predict the time-dependent susceptibility probability with the capacity of As uptake rate constant (J) for three aquaculture species in 168 h duration (Fig. 5). The input parameters are estimated from available data sets (Table 2). In predicted susceptibility probability of freshwater clam, $S(t)=0.097$ was lower than that of tilapia (0.104) and milkfish (0.901) when $J=22 \text{ ng g}^{-1} \text{ h}^{-1}$ at $t=168 \text{ h}$. Results can be explained by the estimated killing rate (k_k) and recovery rate (k_r) (Table 2). It reveals that $k_k=0.075 \text{ g ng}^{-1} \text{ h}^{-1}$ of freshwater clam is much more lower than $0.329 \text{ g ng}^{-1} \text{ h}^{-1}$ of tilapia and $4.847 \text{ g ng}^{-1} \text{ h}^{-1}$ of milkfish, whereas 8.299 h^{-1} of the k_r is more higher than that 7.875 h^{-1} of tilapia and 4.692 h^{-1} of milkfish.

Elimination and recovery give the direct contact to decrease bioaccumulation and coping mechanism for organism, respectively. To understand the mechanism underlying the As tolerance of aquaculture species, the extent to which these physiological traits interactions are correlated is still unknown. Thus, increasing the information of elimination–recovery interaction may provide additional perspectives on this sophistication of aquaculture species response to As stressors. In this study, we coupled elimination rate constant (k_e) with recovery rate constant (k_r) to assess the potential ecophysiological regulation of As susceptibility in tilapia, milkfish, and freshwater clam. Based on the previous exposure bioassays, k_e estimates ranged from 0.001 to 0.025, 0.0006 to 0.05, and 1.75×10^{-5} to 0.09 h^{-1} and k_r estimates ranged from 5.765 to 10.491, 2.009 to 9.419, and 6.051 to 11.116 h^{-1} for tilapia, milkfish, and freshwater clam, respectively. Here we used a k_e – k_r regime to describe the dynamics of susceptibility by employing the DAM for waterborne As concentrations ranging from 0 to

Table 2 Parameter estimates for toxicokinetic/toxicodynamic models applied to the As accumulation and mortality data of tilapia, milkfish, and freshwater clam (see text for the symbol meanings)

	Tilapia	Milkfish	Freshwater clam
{As} (mg L ⁻¹)	0.805	4.033	2.974
Bioaccumulation parameters			
k_u (mL g ⁻¹ h ⁻¹)	0.025±0.004 ^a	0.016±0.004	0.175±0.068
k_e (h ⁻¹)	0.007±0.003	0.011±0.008	0.014±0.052
k_0 (h ⁻¹)	—	—	0.012±0.016
BCF (mL g ⁻¹)	3.57	1.45	12.5
r^2	0.98	0.96	0.83
p value	<0.01	<0.05	<0.01
Bioavailable metal uptake parameters			
$J_{\max}$ (ng g ⁻¹ h ⁻¹)	22.875±10.090	63.125±25.756	788.318±2918
K_m (mg L ⁻¹)	0.042±0.138	0.516±0.880	3.378±14.974
r^2	0.71	0.78	0.7
p value	0.07	<0.05	<0.01
DAM parameters			
$D_{L,50}/k_a$ (ng h g ⁻¹)	2.104±0.133	0.143±0.019	9.279±0.648
k_r (h ⁻¹)	7.875±0.500	4.692±0.674	8.299±0.584
k_k (g ng ⁻¹ h ⁻¹)	0.329	4.847	0.075
r^2	0.87	0.59	0.94
p value	<0.01	<0.05	<0.01

^aMean ± SE

6 mg L⁻¹ in tilapia, milkfish, and freshwater clam (Figs. 6, 7, and 8).

The results indicated that the maximum susceptibility probability at 168 h increased from 0.081 to 0.598 with increasing k_e (0.001–0.025 h⁻¹) and decreasing k_r (5.765–10.491 h⁻¹) when tilapia is exposed to 6 mg L⁻¹ As concentration (Fig. 6a–c, follow the shaded arrow). Similarly, the maximum susceptibility probability of milkfish and freshwater clam increased from 0.297 to 0.999 and 0.0009 to 0.433 with increasing k_e and decreasing k_r when waterborne As is at 6 mg L⁻¹, respectively (Figs. 7a–c and 8a–c). Figures 6, 7, and 8 show that the arrow represents the relationship between k_e and k_r . These results indicated that a negative linear relationship was found between the capacity to eliminate As and the damage repair faculty compensation in three aquaculture species. The correlation was easily found in the k_e – k_r regimes, i.e., increasing k_r may compensate for lower k_e .

As toxicity assessment with toxicological parameters

The model simulation results indicated that freshwater clam is more insensitive than that of tilapia and milkfish to As toxicity, due in part to the water chemistry characteristics of freshwater clam which is more abundant than that of the other two aquaculture systems.

Furthermore, critical parameters such as absorption (k_u), elimination (k_e), metal uptake (J), killing (k_k), and damage recovery (k_r) rate constants play the key roles in determining the susceptibility of tilapia, milkfish, and freshwater clam exposed to waterborne As. For bioconcentration and bioavailable metal uptake parameters, the low susceptibility was found in freshwater clam, even though the k_u and $J_{\max}$ capacities of freshwater clam are higher than the two fish (Table 2). However, there is a finding to realize that the capacity of aquatic organism to absorb As solution from water cannot represent the true As intake in the internal tissue and toxicity. The results met this principle. Even though the absorption rate (0.025 mL g⁻¹ h⁻¹) of tilapia was higher than that of milkfish (0.016 mL g⁻¹ h⁻¹), yet the As uptake capacity of milkfish was higher than that of tilapia.

Moreover, to determine the As toxicity for three aquaculture species, damage-based parameters could be quite obviously revealed that the As toxic effect on freshwater clam was more adverse than that of tilapia and milkfish. Moreover, freshwater clam had a higher k_r (8.299 h⁻¹) than that of tilapia (7.875 h⁻¹) and freshwater clam (4.692 h⁻¹). Besides, k_k of freshwater clam were less than the others. Based on the results, we suggested that the parameters of DAM could be used to well characterize the As toxic effect on aquaculture species.

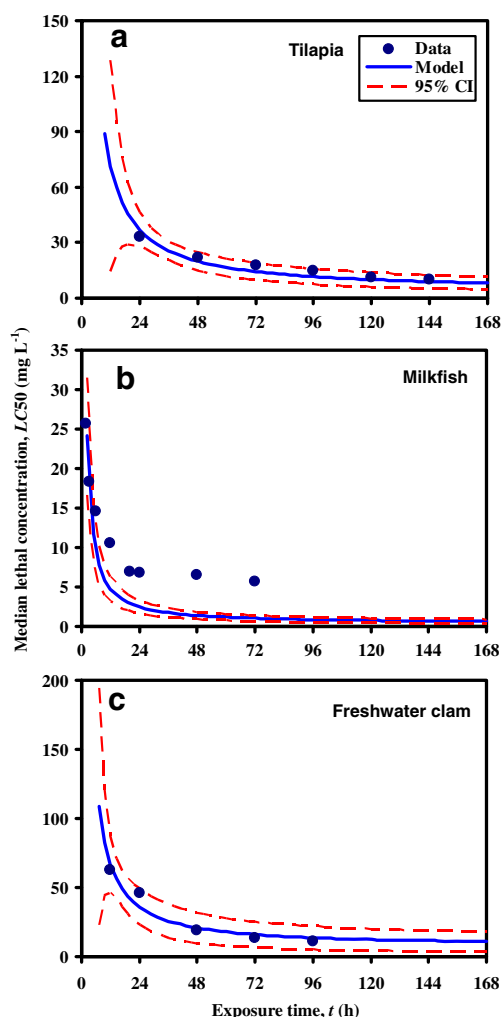


Fig. 4 Optimal fit of DAM to the LC50(*t*) data and in **a** tilapia; **b** milkfish; and **c** freshwater clam

Site-specific water chemistries affect the metal bioavailability and toxicity by considering both metal speciation (affected by pH, formation of organic and inorganic complexes) and competition between the major cations and the metal for binding to biotic ligand on the organism. Niyogi and Wood (2004) assumed that biotic ligand can transport protein. Metal would bind to transport protein, which transport across the cell membrane, then accumulate in the cells and tissues. Moreover, Croteau and Luoma (2007) assumed that whole body tissue concentration could be used to represent the membrane transport processes. There are no different assumptions to realize the tissue-specific metal uptake from the transport protein mechanism. Hence, the amount of the transport proteins would determine how many metals uptake in aquatic organisms. It is

important to integrate bioavailability and bioaccumulation kinetics into a mechanistic framework for arsenic uptake in tilapia.

Geochemistry influences

Lin et al. (2004) indicated that calcium and magnesium play important roles in bridging the complexation of arsenate with humic substance. As expected, high cations concentration strongly reduced As bioavailability. In addition, to describe and predict toxicity dynamics,

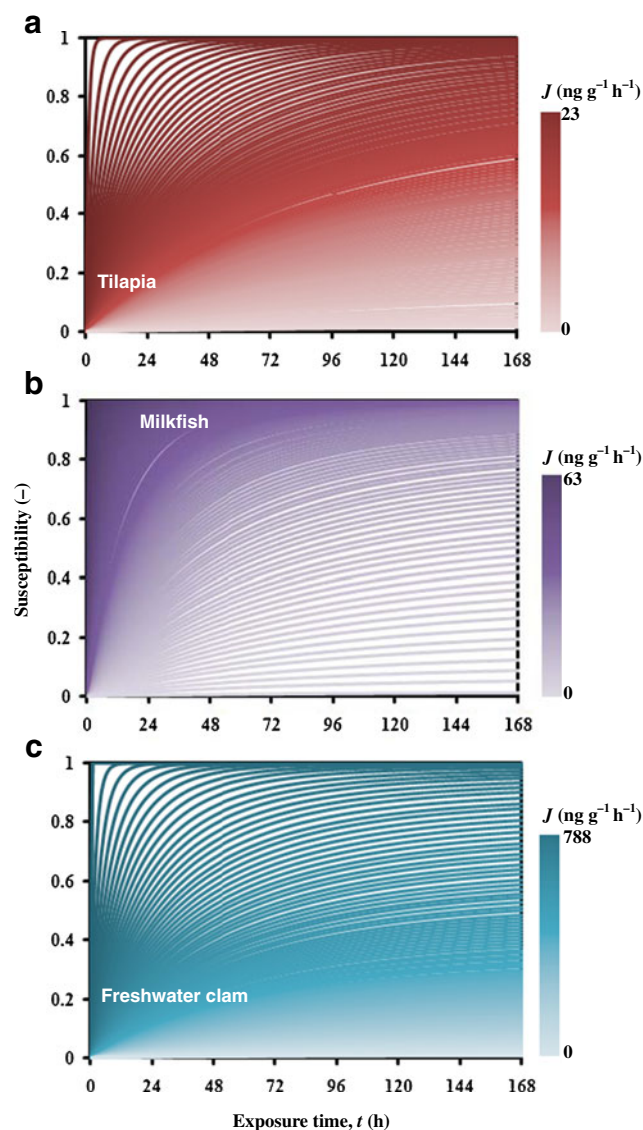


Fig. 5 Simulation of dynamics susceptibility probability as function of As uptake rate 0–maximum for **a** tilapia; **b** milkfish; and **c** freshwater clam

Fig. 6 Predicted dynamics of susceptibility probability performed by DAM in the elimination rate constant (k_e) and recovery rate constant (k_r) of tilapia

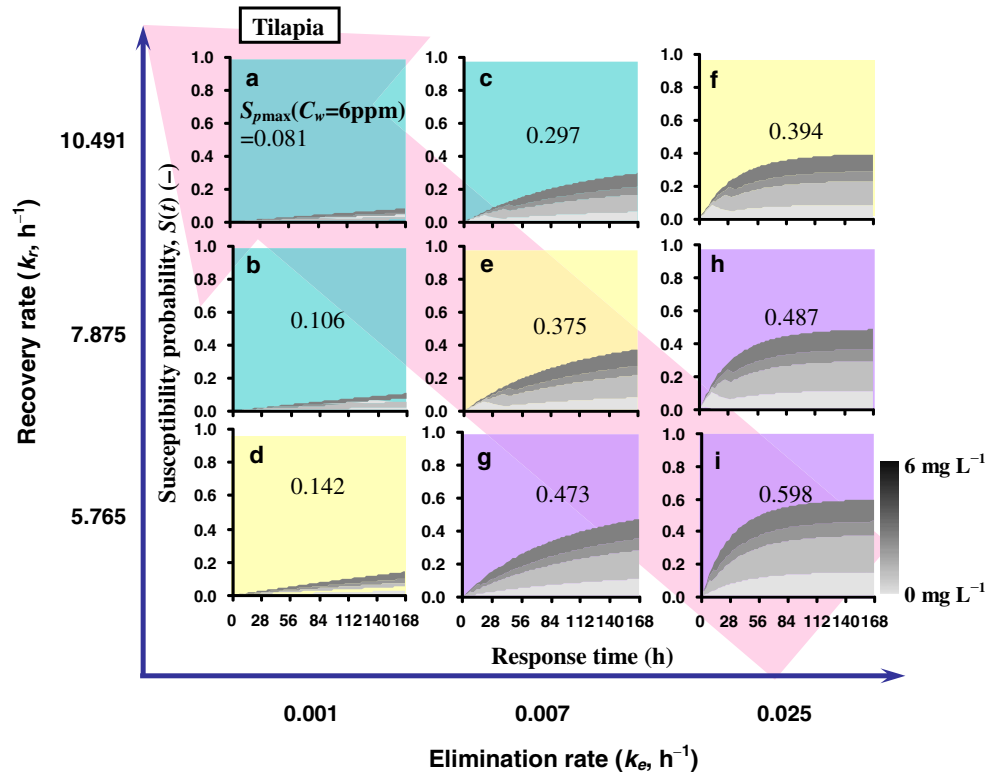


Fig. 7 Predicted dynamics of susceptibility probability performed by DAM in the elimination rate constant (k_e) and recovery rate constant (k_r) of milkfish

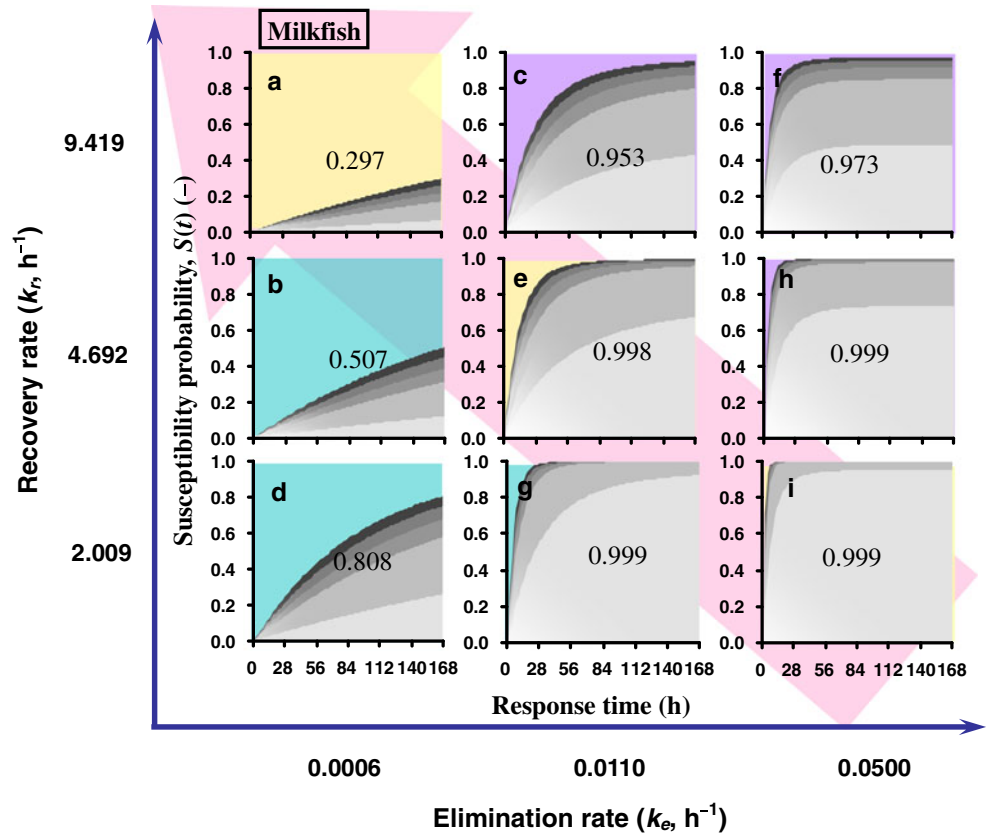
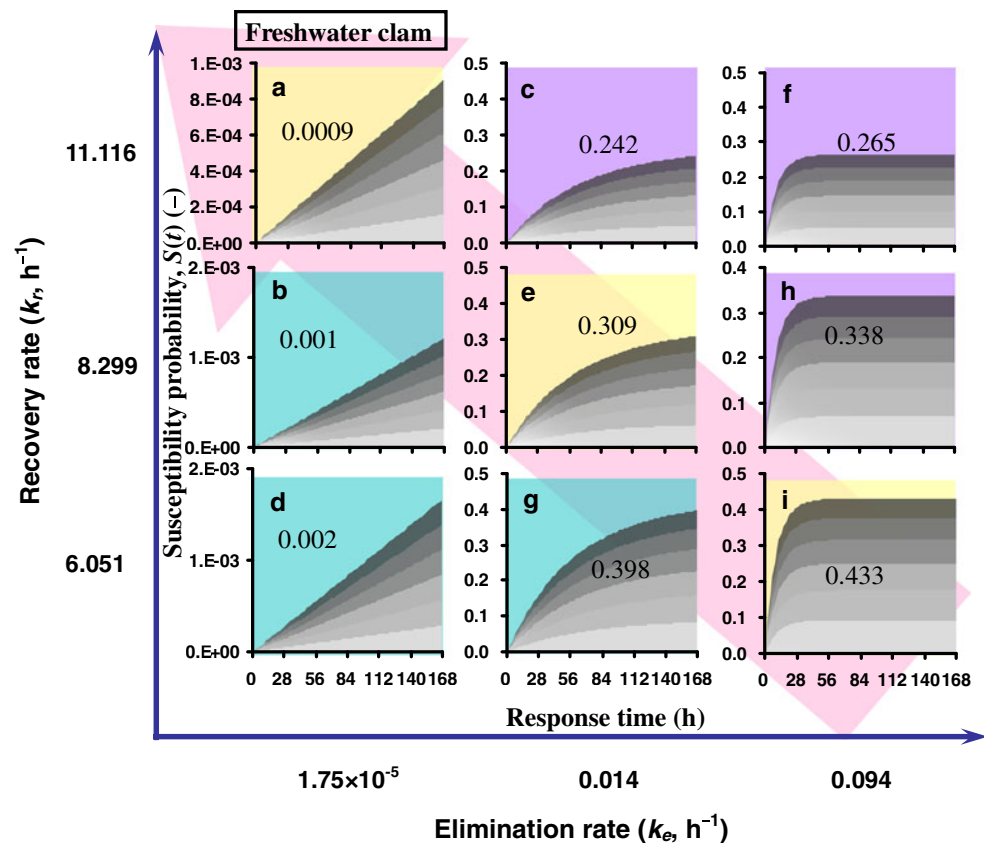


Fig. 8 Predicted dynamics of susceptibility probability performed by DAM in the elimination rate constant (k_e) and recovery rate constant (k_r) of freshwater clam



three different aquaculture species experiments should be conducted at the same setting at the same exposure level, duration, and environmental condition. The mechanisms of As toxicity to tilapia, milkfish, and freshwater clam are still not investigated thoroughly. The well-known geochemical models, e.g., biotic ligand model, did not have and a priori estimate of As toxicity to aquaculture species. To assess metal stress for aquaculture species, the specific parameter of geochemical model is very sensitive in estimating the affinity constants (Niyogi and Wood 2004).

The chemical bioavailability was greatly influenced by field condition, and the biological effect will be extremely swayed with chemical. Even though all of toxic responses result from the time course of accumulation and amount of chemical delivered to the site of toxic action, the environmental condition still plays a major role in metal regulation. Thus, not a single model can be omitted. The capacities of these ions could reduce the greater toxicity for As. It can be related to the specific toxic mode of action of the ions (De Schamphelaere and Janssen 2002). There still exists limited information on how these ions affect As toxicity to these aquaculture species. Previous As toxicity studies have focused on exposure duration, concentration,

and route of exposure (Bears et al. 2006). Nevertheless, it is also dependent on the physiological factors such as transport proteins to mediate As uptake in target organs and detoxification mechanisms (Buchwalter et al. 2008).

Conclusions

This study suggests that bioavailable metal uptake linking DAM is capable of predicting the metal toxicity for aquaculture species exposed to waterborne As activity. A highly significant correlation was found regarding bioaccumulation data in toxicokinetic model. Nevertheless, we also found that the parameters estimated from bioavailable metal uptake-based DAM can reasonably explain the differences of As toxicity for three aquaculture species. Furthermore, this study found that the capacity of aquatic organism absorbing As solution from water cannot represent the true As intake in the internal tissue and toxicity. Therefore, this study suggests that bioavailable metal uptake linking DAM could be used for the application of new knowledge on environmental risk assessment. It is especially useful in the preliminary assessment of acute toxicity of metals since it is influenced by both geochemical and biological mechanisms.

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