Systems-level modeling the effects of arsenic exposure with sequential pulsed and fluctuating patterns for tilapia and freshwater clam

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Abstract

The purpose of this paper was to use quantitative systems-level approach employing biotic ligand model based threshold damage model to examine physiological responses of tilapia and freshwater clam to sequential pulsed and fluctuating arsenic concentrations. We tested present model and triggering mechanisms by carrying out a series of modeling experiments where we used periodic pulses and sine-wave as featured exposures. Our results indicate that changes in the dominant frequencies and pulse timing can shift the safe rate distributions for tilapia, but not for that of freshwater clam. We found that tilapia increase bioenergetic costs to maintain the acclimation during pulsed and sine-wave exposures. Our ability to predict the consequences of physiological variation under time-varying exposure patterns has also implications for optimizing species growing, cultivation strategies, and risk assessment in realistic situations.

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1. Introduction

Aquatic organisms are always exposed to temporal fluctuations of contaminants. Surface water runoff and precipitation-associated hydrologic dilution and dispersion, as well as degradation activity can generate pulsed exposures. Therefore, a simple time-invariant exposure pattern is unlikely to be the typical concentration encountered by aquatic organisms in their natural environment, which changes and fluctuates continuously in time. Traditional standard laboratory toxicity tests performing at constant exposure concentrations have made it possible to study and develop water quality criteria. Yet, such classical methods rather unspecifically investigate the toxicity on time-varying exposures. Due to this limitation, traditional toxicity tests have so far almost exclusively been carried out in assessing the effects of realistic time-varying exposures from measurements made under constant exposure conditions (Reinert et al., 2002). Thus, they have been restricted to the study of detoxification, elimination, and recovery mechanisms of organism during the periods between pulsed exposures. Furthermore, typical aquatic risk assessment can be improved if we are able to incorporate exposure timing and sequence for predicting the effects resulting from realistic exposures (Diamond et al., 2006; Ashauer et al., 2007a).

Recently, the relations between aquatic species exposed to fluctuating or pulsed contaminants and their responses are frequently studied (Reinert et al., 2002; Diamond et al., 2006; Ashauer et al., 2006, 2007a, b, c). Hickle et al. (1995) used the simple one-compartment first-order kinetics approach to develop a residue-based, pulse-exposure toxicokinetic model, providing a framework for designing studies involving complex exposure issues. Reinert et al. (2002) recommended that time-varying exposure testing and modeling should be considered if exposure profiles and chemical behavior present pulsed scenarios. Diamond et al. (2006) indicated that the effects of pulsed exposures of copper and zinc on aquatic organisms are much relied on the frequency, magnitude, duration of pulses, and the recovery period between events, suggesting a need to incorporate pulsed frequency into regulatory decision-making. Diamond et al. (2006) further indicated that recovery time was a significant factor affecting both fathead minnow and water flea survival for copper and zinc. They thus suggested that multiple with short (≤24 h) duration) pulsed exposures of metals will have deleterious effects if the organism is unable to eliminate the chemical rapidly enough to prevent a cumulative internal dose and toxic response from a second pulse. The adaptation mechanism also plays an important role for fish in response to a sequential pulsed exposure (Diamond et al., 2006).
Ashauer et al. (2007a, b, c) have recently developed a process-based threshold damage model based on damage assessment model (DAM) and DETox model to simulate the survival of the aquatic invertebrates after exposure to fluctuating and sequential pulses of pesticides. They suggested that the threshold damage model not only are capable of simulating the observed survival ($r^2$ ranging from 0.77 to 0.96) but also can be used to calculate the times that organisms require for recovering. They also implicated that the sequence where organisms are exposed to chemicals could matter just as important as the concentration and exposure duration.

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. Therefore, DAM-based threshold damage model associated with BLM provide a general mechanistic scheme for understanding the effects of long-term exposure patterns with sequential pulsed or fluctuating concentrations to aquatic organisms.

The World Health Organization (NRC, 2001) considered arsenic is the top environmental chemical of concern. Arsenic also ranks first for understanding the effects of long-term exposure patterns with sequential pulsed or fluctuating concentrations to aquatic organisms. Arsenic also ranks first on the Agency for Toxic Substances and Disease Registry list of priority pollutants in the environment (http://www.astdr.cdc.gov/cerca/05list.html). Previous investigation indicated that there is a strong correlation between arsenic concentration in groundwater with 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.astdr.cdc.gov/cerca/05list.html). Previous investigation indicated that there is a strong correlation between arsenic concentration in groundwater and blackfoot disease (BFD) of residents in southwestern coastal areas of Taiwan (Chen et al., 2005). Lin et al. (2001, 2005), Liao et al. (2003), Huang et al. (2003), and Liu et al. (2006, 2007) have conducted a long-term investigation during 1998–2007 in BFD-endemic areas of Taiwan. They reported that arsenic concentrations in aquaculture waters ranged from 40 to 900 μg L$^{-1}$, whereas arsenic levels in fish (tilapia Oreochromis mossambicus, milkfish Chanos chanos, and large-scale mullet Liza macrolepis) and shellfish (hard clam Meretrix lusoria, freshwater clam Corbicula fluminea, and oyster Crassostrea gigas) ranged from 1 to 350 and 4–23 μg g$^{-1}$ dry wt, respectively. Recently, the phenomenon of die fluctuations in the concentrations of arsenic and other heavy metals in rivers and stream have been reported extensively (Gammons et al., 2005, 2007; Parker et al., 2007). Many factors could affect die changes in water chemistry characteristics in arsenic-rich aquatic ecosystems. Gammons et al. (2007) indicated that pH- and temperature-dependent sorption of arsenic onto hydrous metal oxides or biofilms on the streambed are the dominant factors that control die fluctuations in water chemistry in an arsenic-rich stream.

Because of the combinatorial nature of DAM-based threshold damage model, it may be necessary to use a systems approach to understand fluctuation-driven impact on the species response. Hence, the purpose of this paper was to use a quantitative systems-level approach employing threshold damage model associated with BLM to examine the physiological responses of organism to fluctuating or sequential pulsed arsenic concentrations. 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 responses of organism to more realistic complex exposure patterns.

Farming of tilapia and freshwater clam are the most popular aquaculture types in the BFD-endemic areas because of their high market value to Taiwan’s aquaculture industry (http://www.fa.gov.tw). In this study, farmed tilapia and freshwater clam were used as indicators together with previous published toxicity and toxicokinetic data for examining the physiological responses to time-varying arsenic exposures.

2. Materials and methods

2.1. Arsenic toxicity and toxicokinetic data

The previous published acute, chronic toxicity and toxicokinetic data (Tsai and Liao, 2006; Liao et al., 2008) for two farmed species, tilapia (O. mossambicus) and freshwater clam (C. fluminea) exposed to arsenic, give us the unique opportunity to examine the effects of long-term arsenic exposure with sequential pulsed or fluctuating patterns. Arsenic was chosen for practical and theoretical reasons, with the availability of reasonable amounts of suitable information as the primary consideration. Generally, as prerequisites for data suitability, exposure and whole-body arsenic burdens measured by accepted analytical techniques were required. In light of this aspect, exposure data were considered to be acceptable only when whole-body burden data were available and when the exposure duration was at least 14 days. Our previous published arsenic-tilapia and arsenic-freshwater clam databases meet this principle. The pervious published arsenic toxicity and toxicokinetic data for tilapia and freshwater clam are listed in Table 1.

2.2. BLM-based bioaccumulation model

The basic hypothesis of the BLM is that the degree of toxic effect is determined by the fraction bound to the site of action (i.e., biotic ligand). The relationship between the observed effect and the fraction of occupied biotic ligand is not altered by the physicochemistry of the bulk water (Pagenkopf, 1983; Heijerick et al., 2002; De Schamphelaere and Janssen, 2002). The crucial factors include the activity of the free metal ion, the complexation capacity of the biotic ligand, the concentration of unoccupied biotic ligand sites, the affinity (stability) constant for metal ion binding to biotic ligand sites at equilibrium, and the concentrations of all cations that compete with the metal for binding sites on the biotic ligand (Fig. 1A) (De Schamphelaere and Janssen, 2002). Our previous study (Liao et al., 2004) based on the organ-specific dose–response relationships indicated that gill is a more sensitive organ than liver, intestine, and stomach when tilapia exposure to arsenic.

At equilibrium thermodynamics, arsenate (As(V)) dominants in oxidative environments of most surface water. As(V) being the most prevalent in most surface waters (Ferguson and Gavis, 1972). Here the BLM is used to predict the degree of As(V) binding at the site of action causing toxicity in aquaculture species. Thus, based on BLM scheme (De Schamphelaere and Janssen, 2002), the effect concentration for XX response over time (ECX(t)) can be expressed in terms of the time course fraction of the total number of As(V) binding sites occupied by As(V) at XX effect (i.e., $f_{XX}(t)$).

Table 1

<table>
<thead>
<tr>
<th>Toxicity data</th>
<th>Freshwater clam</th>
<th>Tilapia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC50 (μg L$^{-1}$)</td>
<td>28d-EC50 (96 h-EC50)</td>
<td>1.54 (1.28–1.80)$^b$</td>
</tr>
<tr>
<td>Toxicokinetic data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_1$ (h$^{-1}$)</td>
<td>0.0714 ± 0.279$^a$</td>
<td>0.016 ± 0.002$^a$</td>
</tr>
<tr>
<td>$k_2$ (h$^{-1}$)</td>
<td>0.0163 ± 0.073$^a$</td>
<td>0.0032 ± 0.0006$^b$</td>
</tr>
<tr>
<td>BCF (mL g$^{-1}$)</td>
<td>4.38</td>
<td>5</td>
</tr>
<tr>
<td>Water chemistry characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.96 ± 0.14</td>
<td>7.75 ± 0.02</td>
</tr>
<tr>
<td>Temp (°C)</td>
<td>24.26 ± 1.26</td>
<td>26.7 ± 0.24</td>
</tr>
<tr>
<td>Ca$^{2+}$ (mg L$^{-1}$)</td>
<td>24.8</td>
<td>NA$^a$</td>
</tr>
<tr>
<td>Mg$^{2+}$ (mg L$^{-1}$)</td>
<td>1.0</td>
<td>NA</td>
</tr>
<tr>
<td>Na$^+$ (mg L$^{-1}$)</td>
<td>4.9</td>
<td>NA</td>
</tr>
<tr>
<td>K$^+$ (mg L$^{-1}$)</td>
<td>2.7</td>
<td>NA</td>
</tr>
<tr>
<td>Cl$^{-}$ (mg L$^{-1}$)</td>
<td>7.6</td>
<td>NA</td>
</tr>
<tr>
<td>NH$_4$ (mg L$^{-1}$)</td>
<td>0.26</td>
<td>NA</td>
</tr>
<tr>
<td>NO$_3$ (mg L$^{-1}$)</td>
<td>0.318</td>
<td>NA</td>
</tr>
<tr>
<td>Affinity constants (M$^{-1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>log $K_{N_{H_{4}}}$</td>
<td>Acute toxicity</td>
<td>Chronic toxicity$^d$</td>
</tr>
<tr>
<td>log $K_{N_{A}}$</td>
<td>3.58$^b$</td>
<td>11.11</td>
</tr>
<tr>
<td>log $K_{N_{O}}$</td>
<td>5.40$^c$</td>
<td>LN(6.36, 1.06)</td>
</tr>
<tr>
<td>log $K_{C_{l}}$</td>
<td>LN(3.53, 1.03)$^a$</td>
<td>LN(3.40, 1.08)</td>
</tr>
<tr>
<td>log $K_{N_{O}}$</td>
<td>LN(3.09, 1.04)$^a$</td>
<td>LN(2.57, 1.17)</td>
</tr>
</tbody>
</table>

$^a$ Adopted from Liao et al. (2008). 
$^b$ Adopted from Tsai and Liao (2006). 
$^c$ NA – Not available. 
$^d$ Adopted from Niyogi and Wood (2004). 
$^e$ Adopted from Liu et al. (2007) in that LN(gm, gsd) represents lognormal distribution with a geometric mean and a geometric standard deviation. 
$^f$ Adopted from Chen et al. (2009).
where $\sum K_{\text{inorg}}(\text{ions}) - K_{\text{inorg}}(\text{Ca}^{2+}) + K_{\text{inorg}}(\text{Mg}^{2+}) + K_{\text{inorg}}(\text{Na}^{+}) + K_{\text{inorg}}(\text{H}^+)$. Thus, the commonly employed one-compartmental toxicokinetic model can be modified by the BLM concept for linking the relationship between $\text{BCF}(\text{ions})$ and $f^{\text{X}}_{\text{AsBL}}(t)$.

\[
\text{ECX}(t) = \frac{f^{\text{X}}_{\text{AsBL}}(t) \cdot (1 + \sum K_{\text{inorg}}(\text{ions}))}{(1 - f^{\text{X}}_{\text{AsBL}}(t))} \times \text{[time-dependent factor]} \times \text{[time-dependent factor of damage]} \times \text{[time-dependent factor of bioavailability]}
\]

\[
\text{BCF}(\text{ions}), t) = \frac{f^{\text{X}}_{\text{AsBL}}(t) \cdot (1 + \sum K_{\text{inorg}}(\text{ions}))}{(1 - f^{\text{X}}_{\text{AsBL}}(t))}
\]

\[
\text{As(V)} = \frac{\text{ECX}(t)}{\text{As(V)}_{\text{inorg}} + K_{\text{inorg}}(\text{ions})}
\]

The primary focus of the present study is on the systems-level viewpoint of the organism damage response. Insights into the threshold damage model associated with the systems-level properties, damage response of organism and its environment can be described by three dynamic variables: the time-varying water As(V) concentration (the input), the internal damage (the bioaccumulation), and the hazard (the output).

2.3. General model for internal damage kinetics

Ashauer et al. (2007a, b, c) modified the DAM to develop a process-based threshold damage model that laid the foundations for predicting survival of aquatic organisms after exposure to sequential pulsed and fluctuating patterns. The primary focus of the present study is on the systems-level viewpoint of the organism damage response. Insights into the threshold damage model associated with the systems-level properties, damage response of organism and its environment can be described by three dynamic variables: the time-varying water As(V) concentration (the input), the internal damage (the bioaccumulation), and the hazard (the output). Fig. 1B illustrates the block diagram of continuous representation of systems-level threshold damage model.

First, first-order bioaccumulation model can be used to predict the organism body burden followed the exposure to sequential pulsed As(V) concentrations (Fig. 1C),

\[
\frac{dX(t)}{dt} = k_1 \cdot (\text{As(V)}_{\text{inorg}}(t) - k_2 X(t))
\]

where $X(t)$ is the body burden of [As(V)] at time $t$ (pmol g$^{-1}$ d$^{-1}$). $D_{\text{As}}(t)$ is the BCF of As(V) to organism considering the competition of cations [ions] at time $t$. In view of Eq. (2), BCF [ions], $t$ has the form as (Tsai et al., 2009),

\[
\text{ECX}(t) = \frac{f^{\text{X}}_{\text{AsBL}}(t) \cdot (1 + \sum K_{\text{inorg}}(\text{ions}))}{(1 - f^{\text{X}}_{\text{AsBL}}(t))} \times \text{[time-dependent factor]} \times \text{[time-dependent factor of damage]} \times \text{[time-dependent factor of bioavailability]}
\]

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

\[
\text{BCF}(\text{ions}), t) = \frac{f^{\text{X}}_{\text{AsBL}}(t) \cdot (1 + \sum K_{\text{inorg}}(\text{ions}))}{(1 - f^{\text{X}}_{\text{AsBL}}(t))}
\]

\[
\text{As(V)} = \frac{\text{ECX}(t)}{\text{As(V)}_{\text{inorg}} + K_{\text{inorg}}(\text{ions})}
\]
killing rate constant in Eq. (6) is the proportionality factor describing the relation between the cumulative damage and hazard, whereas 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 (Ashauer et al., 2007b). Thus, the recovery rate constant can be used to estimate the recovery time of the organisms from internal damage.

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 injury at a give time t (Fig. 1B),

\[
\frac{dH(t)}{dt} = D(t) - D_0
\]

where \(H(t)\) is the cumulative hazard (dimensionless) and \(D_0\) presents the threshold of damage (dimensionless).

2.4. Physiological response models

In the physiological effect of tilapia, the mode of action on feeding decrease was more correlated significantly with tilapia growth inhibition than maintenance and food consumption (Tsai and Liao, 2006). An ontogenetic-based West growth model can be used to describe feeding decrease in tilapia growth inhibition (Tsai and Liao, 2006),

\[
W(t) = \left( \frac{W_{\text{max}}S(t)}{1 - (1 - \left( \frac{W_{\text{max}}S(t)}{A} \right)^{0.05})^{1.4}} \right) \exp \left( -\frac{At}{4(W_{\text{max}}S(t))^{1.4}} \right)^{1.4}.
\]

where \(W(t)\) is the time-dependent body weight (g), constant 0.05 g is the body weight at birth of tilapia in uncontaminated water (www.fishbase.org/home.htm), \(A\) is a species-specific growth coefficient (0.023 g^{1/4} d^{-1}), \(W_{\text{max}}\) is the ultimate body weight of tilapia under the contaminated water adverse effect in that \(W_{\text{max}}=1130\ g\), and \(S(t)\) is a time-dependent safe probability that links the health of organism to body burdens and cumulative hazard \(H(t)\). Thus, \(S(t)\) 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),

\[
S(t) = e^{-H(t)}\quad (9)
\]

Valve daily activity was used as the physiological response for freshwater clam. The 14-day continuous observation of individual valve opening under the status of free-range burrowing behavior was analyzed to obtain the variation of valve daily activities from sixteen freshwater clams (Liao et al., 2009). The valve daily activity data were based on the mean proportion of valve opening of sixteen clams at hourly intervals during a 14-day observed duration (Liao et al., 2009). This study employed the BLM-based together with Hill model-based valve closure model to predict the valve daily activity in freshwater clam in response to As(V) (Liao et al., 2009),

\[
\psi(t, [\text{As(V)}(t)]) = \psi(t, 0) + R(t, [\text{As(V)}(t)]) \times (1 - \psi(t, 0)),
\]

where \(\psi(t, [\text{As(V)}(t)])\) is the valve daily closure activity in the present of time-varying As(V) at time t, \(\psi(t, 0)\) is the valve daily closure activity at time t in the absence of arsenic and is given by (Liao et al., 2005),

\[
\psi(t, 0) = 1 - \left( \frac{26 + 3.3 \times \sin \left( \frac{2\pi(t + 0.99)}{t} \right)}{t} \right),
\]

with estimates of a base line B = 0.59, an amplitude A = 0.13, a phase \(\phi = 0.034\), and a daily period \(\tau = 21.32\) h (95% CI: 20.58–22.05 h). \(R(t, [\text{As(V)}(t)])\) in Eq. (10) is the valve closure response (% response) over time based on As(V) activity and can be expressed as a Hill model,

\[
R(t, [\text{As(V)}(t)]) = \frac{R_{\text{max}}(t)}{1 + \left( \frac{EC_{50}(t)}{[\text{As(V)}(t)]} \right)^{n(t)}}
\]

where \(R_{\text{max}}(t)\) is the response time-specific maximum response (%), and \(n(t)\) is a response time-dependent Hill coefficient.

2.5. Statistical analyses and simulation scheme

TableCurve 2D (Version 5, ASN Software Inc., Mapleton, OR, USA) was performed to fit the published data to obtain the optimal fitted statistical models. 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 As(V), Crystal Ball® software (Version 2000.2, Decisioneering, Inc., Denver, Colorado, USA) was used to implement Monte Carlo simulation to obtain 2.5th- and 97.5th-percentiles as the 95% confidence interval (CI). Mathematica® (Version 5.1, Wolfram Research Inc., Champaign, IL, USA) was used to perform all simulations of the toxicological and physiological responses of farmed species to time-varying As(V) exposures.

3. Results

3.1. Bioavailability and bioaccumulation

Table 1 summarizes the water chemistry characteristics and affinity constants used in the BLM scheme for tilapia and freshwater clam. Time-dependent fraction of the total number of As(V) binding sites occupied by As(V) at 50% effect \(f_{\text{BLsA}}\) (t), could be estimated by fitting Eq. (1) to published EC50(t) data of tilapia from Tsai and Liao (2006) as well as LC50(t) data of freshwater clam from Liao et al. (2008), respectively (Fig. 2A, C), with known affinity constants and water chemistry data (Table 1). Fig. 2B shows the chronic \(f_{\text{BLsA}}\) (t) over time for tilapia in that predicted \(f_{\text{BLsA}}\) (t) has the form as \(f_{\text{BLsA}}(t) = 0.1 + 0.31\exp(-t/100)\) (\(r^2 = 0.91\)). Fig. 2D shows the predicted acute \(f_{\text{BLsA}}(t) = 0.28 + 0.53\exp(-t/26)\) for freshwater clam (\(r^2 = 0.88\)).

The concentrations of unoccupied biotic ligand site, [BL−], can also be calculated from the relationship of \([a] = \text{BCF}[\text{BL}−]^{-1}\) with...
Table 2
Parameter estimates for BLM-based threshold damage model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{ionBL}$</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>[a] $\times 10^5$</td>
<td>1.32</td>
<td>M$^{-1}$</td>
</tr>
<tr>
<td>f$^{50%}_{AsBL}$</td>
<td>1.56</td>
<td>(1.0 $\times 10^{-3}$ - 1.78)</td>
</tr>
<tr>
<td>D$_{ionBL}$/k$_i$</td>
<td>73.87</td>
<td>(6.0 $\times 10^{-4}$ - 2.27)</td>
</tr>
<tr>
<td>k$_i$</td>
<td>0.44</td>
<td>(g pmol$^{-1}$ h$^{-1}$)</td>
</tr>
<tr>
<td>BCF(ions)</td>
<td>3.30</td>
<td>(mL g$^{-1}$)</td>
</tr>
</tbody>
</table>

- Parameter used in Eq. (1).
- $K_{ionBL}$ is the estimated from Table 2.
- $d$ is calculated from Eq. (3).
As(V)(t) = C_0 + C_1(\delta(t - T_1) + \delta(t - T_2)) with T_1 = 0.5 and T_2 = 25 days (Fig. 4C, J) varying with C_0 = 0.1 and C_1 = 4.4 and 8.9 nM, respectively. As expected, higher pulsed concentrations increase the cumulative hazard (Fig. 4F, M) and, hence, decrease the median safe time (ST50) (Fig. 4G, N). However, it is noteworthy that our results show that at same exposure level, cluster pulsed exposures (Fig. 4C, B) have shorter ST50 than those of less cluster pulsed (Fig. 4N), indicating the pulse timing and sequence where tilapia are exposed to As(V) could be mattered.

We used As(V) concentration varying sinusoidally over a range of periods \(T\) (\(As(V)(t) = 0.082 + 0.081\sin(2\pi t/T + 2.8)\)) to examine the underlying dynamic responses in response to these periodic patterns (Temp = 26.4 °C, pH = 7.13). The sine-wave exposure pattern was chosen so that input As(V) loading would mimic the cycling changes in arsenic concentrations observed in the arsenic-rich stream and pond systems (Gammons et al., 2007). Tilapia respond to sine-wave was simulated with \(T = 5\) days (Fig. 5A–F) and 30 days (Fig. 5G–L), respectively. The results indicate that tilapia in response to greater periodic change (i.e., low-frequency) experience much longer ST50 than that of smaller periodic (i.e., high-frequency) fluctuation (Fig. 5F, L).

Similarly, we investigated the effects of sequential pulsed and periodic sine-wave fluctuation on dynamic response of freshwater clam (Fig. 6). As(V) exposure concentrations ranged from 3 to 30 mg L\(^{-1}\) at water chemistry condition of Temp = 25 °C and pH = 8. The damage threshold is estimated to be \(D_0 = 0.032\) (EC5 = 24 \(\mu\)g L\(^{-1}\)) based on a Hill dose-response model describing the relationship between valve closing response and waterborne...
arsenic concentration at response time of 300 min with EC50 of 0.35 mg L⁻¹ (95% CI, 0.30–0.40) (Liao et al., 2009). From the results taken at two sequential pulsed patterns (Fig. 6A–G), it can be readily seen that unlike tilapia, the profiles of safe rate for freshwater clam are at a similar fashion (Fig. 6G). Note, however, that freshwater clam in response to periodic sine-wave patterns (Fig. 6H–N) reveals the similar response of tilapia in that ST50 is significantly longer at low-frequency pattern (Fig. 6N).

3.3. Physiological response and recovery time estimation

In view of Eq. (8), plots of dimensionless mass ratio \( r(t) = W(t)/\langle W_{\text{max}} \rangle_e g(t) \), for tilapia followed period-specific sequential pulsed and sine-wave As(V) exposures reveal that cluster sequential pulses and high-frequency sine-wave patterns can increase tilapia energy acquisition and expenditure to overcome externally fluctuation-driven environments (Fig. 7). For freshwater clam, on the other hand, the period-specific pulsed and sine-wave As(V) exposure affect insignificantly the valve closure behavior (Fig. 8). The results demonstrate clearly that the nature of the response was different between the two farmed species in response to sequential pulsed and fluctuating As(V) exposure.

The contrast in physiological response suggests that during the pulsed As(V) exposure, the recovery time that affects species survival may feature different fashions. The recovery time (\( t_R \)) can be estimated from recovery rate constant estimates (i.e., \( t_R = 1/k_r \)) that obtained by fitting the first-order damage model (Eq. (6)) to (1 – safe rate)-time distribution in each sequential pulsed and sine-wave As(V) exposures. Our results show that damage recovery times of nearly 5.26–6.25 days and 0.18–0.38 days for tilapia exposed to cluster and less cluster sequential pulsed As(V) exposures, respectively; whereas for freshwater clam, the recovery times were estimated to be 1.15–1.45 days for two sequential pulsed patterns (Table 3). For period-specific sine-wave As(V) exposures, on the other hand, recovery times increased with the increasing of period: 8.20 and 16.67 days for tilapia and 2.70 and 14.29 days for freshwater clam after sine-wave exposures with \( T = 5 \) and 30 days, respectively (Table 3).

4. Discussion

4.1. Systems-level based mechanistic model

To better understand the processes driving internal damage change and assess their potential impact on physiological response
after pulsed and fluctuating As(V) exposures, a BLM-based threshold damage model was used to examine the effects dynamically. We tested the present model and triggering mechanisms by carrying out a series of modeling experiments in which we used periodic pulses and sine-wave as featured exposures and run the model forward in time and compared the outputs.

Understanding the processes of pulsed 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 safe rates, in individual species over time. The present approach is a step in this direction and can be enhanced by existing ecotoxicological modeling methods and assays. The present approach provides a window into ecotoxicological scheme and opens the way for understanding how aquaculture species show different responses to sequential pulsed and fluctuating metal exposure patterns.

Thus, a BLM-based threshold damage model, formulated by understanding of inherent interactions between chemicals and receptors and internal regulations of organisms, can be used to quantify time-dependent toxicity of chemical bioavailability incorporating the co-influence of chemical and damage accumulation under a broad range of fluctuation-driven environments. The BLM-based time-varying threshold damage model containing nonlinearities can predict survival dynamics of aquaculture species after sequential pulsed and fluctuating As(V) 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 well-studied experimental systems where direct measurements of rates and manipulation of environmental conditions can be achieved. Pulsed metal exposure experiments

Fig. 6. Simulations of the sequential pulsed As(V) exposure with 5 days pulse period, and 0.5 and 25 days pulsed period (left) and the sine-wave As(V) exposure with 5 days and 30 days period (right) for freshwater clam. (A, H) The body burdens simulated by Eq. (4). (B, C, I, J) The sequential pulsed As(V) concentrations range from 0.1 to 4.5 nM and 0.1–9 nM, and sine-wave As(V) concentration ranged from 0.001 to 0.163 nM (D, K) The time course of the damage. (E, L) The hazard rate. (F and M) The cumulative hazard. (G, N) The safe probabilities that calculated by Eqs. (6), (7), and (9).
and related ecotoxicological models (e.g., process-based threshold damage model) in simulating survival after sequential pulsed exposure in particular are capturing the essence of dynamics of several model systems, for example, freshwater invertebrate (Gammarus pulex) (Ashauer et al., 2007a, b, c), fathead minnow (Pimephales promelas) (Hickle et al., 1995; Diamond et al., 2006), and water flea (Daphnia magna) (Diamond et al., 2006).

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 tilapia dictated by alloometric scaling of energy acquisition and expenditure. More importantly, the relevant biological features captured by the model enables us to look beyond the behaviors for their internal coping mechanisms (Buchwalter et al., 1997). However, freshwater clam are filter-feeder animals, they could extend siphon from their valve to filtrate organic matter for uptake within few minutes. When they exposed to the pollutant stressors, they could endure long-lasting periods within closed valve to protect themselves. Hence, freshwater clam is less sensitive than that of tilapia to arsenic.

Fig. 7. A plot of the dimensionless mass ratio ($r(t)=W(t)/W_{max}(S(t))$) for tilapia with the sequential pulsed (A, B) and sine-wave As(V) patterns (C, D).

4.2. Impact of pulsed exposure on aquaculture species response

Our results indicate that changes in the dominant frequencies and timing of As(V) pulses may shift the safe rate distributions for tilapia, but not for that of freshwater clam. The physiological differences in adaptation mechanisms between tilapia and freshwater calm may explain the difference in response between the two species to pulsed and sine-wave As(V) exposures (Beyers et al., 1999). Recovery rate constant in the feedback loop (Fig. 1C) reveals the importance of this mechanism. Owing to direct contact with ambient water, gills are proposed to be the first and most important targets of fish/shellfish exposed to waterborne metals (Wong and Wong, 2000; Tao et al., 2000). Several studies also indicated that the major route of uptake for metals that concentrate in fish/shellfish is across the gill epithelium (Jorgensen, 1996; Pelgrom et al., 1997). However, freshwater clam are filter-feeder animals, they could extend siphon from their valve to filtrate organic matter for uptake within few minutes. When they exposed to the pollutant stressors, they could endure long-lasting periods within closed valve to protect themselves. Hence, freshwater clam is less sensitive than that of tilapia to arsenic.

Hence, responses of fish/shellfish to metals are mediated by physiological regulation mechanisms at the gill (Zhou et al., 2005). 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 non-stressed conditions to put in action when the next pulse comes (Diamond et al., 2006). Our results indicate that tilapia increase bioenergetic costs to maintain the acclimation during pulsed and sine-wave As(V) exposures.

The BLM, derived from the gill surface interaction model and the free ion activity model, has been widely used to predict the toxicological effects of metals on aquatic organisms in the last decade (Niyogi and Wood, 2004; Bielmyer et al., 2007). In the BLM, the metal toxicity is resulted from the free metal ions reacting with the binding sites at the site of action for aquatic organisms. The surface membrane of the gill, considered a negatively charged ligand, is widely recognized as the biotic ligand of fish (De Schamphelaere and Janssen, 2002; Paquin et al., 2002; Morgan and Wood, 2004). In practice, the BLM has been successfully applied to predict both acute and chronic toxicity of metals on aquatic organisms (De Schamphelaere and Janssen, 2002; Schwartz and Vigneault, 2007).

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 toxicology can further be used to describe metal-gill binding interactions and to predict pulsed metal toxicities to aquatic organisms in the field situations undergoing pulsed and fluctuating exposures.

Typical assessment methods do not incorporate pulse timing and sequence, which are critical in assessing realistic situations (Reinert et al., 2002). Our results show that pulse timing and period have potential impact on the physiological responses of aquaculture species. Diamond et al. (2006) indicated that pulse timing matters in metal exposures, suggesting that there is a need to incorporate frequency (e.g., number of pulses and recovery time between pulses) into regulatory decision-making and risk assessment protocol. Ashauer et al. (2007a) further pointed out that pulsed toxicity tests together with threshold damage model-based toxicokinetic parameters could be used in risk assessment to improve biomonitoring systems that estimate quantitatively the effects based on real-world exposure patterns.

Typically, environmental forcing controls the timing of process transition in organisms, creating a critical window of actual sensitivity to pulsed and fluctuating toxicity that may be common for seasonally regulated life-history traits in aquaculture species. Although the current model was employed for tilapia and...
freshwater clam, the underlying principle of linking metal bioavailability and internal damage accumulation to environmental sensitivity caused by pulsed and fluctuating exposures is broadly applicable across aquaculture species. Our ability to predict the consequences of physiological variation under time-varying exposure patterns has also implications for optimizing species growing, cultivation strategies, and risk assessment in realistic situations.

4.3. Implications

Ashauer et al. (2006, 2007a, b, c) have provided an excellent research work on organisms exposed to fluctuating and sequential pulses of contaminants. This present paper is inspired by their work. Mathematical methods of the kind developed by them can provide a new language in which to articulate the ecotoxicological processes involving pulsed and fluctuating exposure. It is not inconceivable that this conclusion might be better suited for understanding aquaculture species exposed to fluctuating and pulsed metals. Understanding the existing published experimental evidence (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 time of aquaculture species to sequential pulsed and fluctuating exposures. Further, an important of this study is that physiological and recovery time estimates of the direct pulsed and fluctuating effects must, at least partly, rely on ontogenetic and damage assessment models and that pure estimates from experiments are currently not possible.

A major difficulty in studying ecotoxicological modeling from a systems perspective has been the lack of information regarding timing and sequence in which organisms are exposed to chemicals.

Fig. 8. The valve closure response of freshwater clam in response to sequential pulsed (A) and sine-wave As(V) patterns (B, C).
We have studied physiological responses of aquaculture species to pulsed and fluctuating As(V) exposures by using a systems-level based minimal model that captures the essential features of the underlying internal damage mechanisms. The model produces results that are in quantitative agreement with the existing experimental data for sequential pulsed exposure patterns (Ashauer et al., 2007a, b). The agreements with this diverse set of experiments validate the model and its assumptions. Our ability to predict the consequences of physiological variation under time-varying exposure patterns has also implications for optimizing species growing, cultivation strategies, and risk assessment in realistic situations.


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