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Toxicokinetics/toxicodynamics with damage feedback improves risk assessment for tilapia and freshwater clam exposed to arsenic

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Abstract It has been proposed that irreversible responses of organisms exposed to contaminants are due to a systems-level feedback. Here we tested this hypothesis by reanalyzing the published data on toxicokinetics and survival probability based on a systems-level threshold damage model (TDM) incorporating with a positive damage feedback to explore the steady-state response and dynamic behavior of damage for tilapia and freshwater clam exposed to waterborne arsenic (As). We found that ultrasensitivity appeared in As–tilapia and freshwater clam systems with Hill coefficient $n \geq 4$, indicating that the positive damage feedback mechanism has been triggered. We confirmed that damage can trigger a positive feedback loop that together with As stressor increases irreversibility. This study also showed that TDM with positive feedback gave a much better predictability than that of TDM at As concentrations ranging from 100 to 500 mg l^{-1} for freshwater clam, whereas for tilapia, two models had nearly same performance on predictability. We suggested that mortality–time profile derived Hill coefficient could be used as a new risk indicator to assess the survival probability for species exposed to waterborne metals. We anticipated that the proposed toxicokinetics/toxicodynamics with a positive damage feedback may facilitate our understanding and manipulation of complex mechanisms of metal susceptibility among species and improve current risk assessment strategies.

Keywords Arsenic · Toxicokinetics · Toxicodynamics · Damage feedback · Ecotoxicology · Tilapia · Freshwater clam

Introduction

Generally, biological systems that are more sensitive to environmental stimulus are called ultrasensitivity in that ultrasensitive regulatory steps can be characterized by a Hill-type dose–response curve (Koshland et al. 1982). A positive cooperativity with the Hill coefficient $n > 1$ would be an example of ultrasensitivity (Koshland et al. 1982). A transient stimulus into a self-sustaining, irreversible response can be converted potentially by positive feedback loops. Positive feedback is often associated with uncontrolled processes. Positive feedback is defined as a set of regulatory steps that feeds the output signal back to the input. If signaling output activity increases, positive feedback will further increase input levels. A positive feedback loop with an ultrasensitive regulatory step can trigger a bistable switch.

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Recent studies reported that arsenic (As) concentrations in aquaculture waters ranged from 40 to 900 μg l^{-1}, whereas As levels in fish (tilapia *Oreochomis 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 to 23 μg g^{-1} dry wt, respectively, (Lin et al. 2001, 2005; Liao et al. 2003; Huang et al. 2003; Liu et al. 2006, 2007). Tilapia and freshwater clam are commercially important native species in Taiwan. These potentially risks on the health of tilapia and freshwater clam may result in severe economic losses nation-widely due to bans on harvesting of contaminated tilapia and freshwater clam and the need for costly monitoring programs.

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). Available experimental evidence (Lee et al. 2002a; Ashauer et al. 2007, 2010) indicates that a process-based DAM or referred to as threshold damage model (TDM), 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. 2002b; Reinert et al. 2002; Diamond et al. 2006; Ashauer et al. 2007) enhances our confidence in the estimates of the dynamic physiological response and recovery of aquaculture species to toxic metals. It is also recognized that irreversible responses are due to systems-level feedback and this systems-level view of irreversibility is supported by many experimental observations (Brandman and Meyer 2008).

The TDM 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. TDM 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. In TDM, 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.

Here we hypothesized that the damage with hazard can trigger a positive feedback loop that together with environmental metal stressors increases the irreversibility. The purpose of this study was to incorporate a positive feedback loop into a systems-level TDM to assess As susceptibility for tilapia and freshwater clam.

Materials and methods

Study data and settings

The previous published acute, chronic toxicity and toxicokinetic data (Tsai and Liao 2006a, b; Liao et al. 2009; Chen and Liao 2010) for two farmed species, tilapia (*O. mossambicus*) and freshwater clam (*C. fluminea*) exposed to As, give us the unique opportunity to examine the effects of positive damage feedback on As susceptibility. As 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 As 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 7 days. Our previous published As–tilapia and As–freshwater clam databases meet this principle.

Models

Ashauer et al. (2007, 2010) modified the DAM to develop a process-based TDM 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 TDM associated with the systems-level properties, damage response of organism and its environment can be described by three dynamic variables: the time-varying waterborne As concentration (the input), the internal damage (the bioaccumulation), and the survival (the output). Figure 1a illustrates the block diagram of continuous representation of systems-level TDM in tilapia.

A state–space representation can be used to describe the systems-level TDM (Fig. 1a),

$$\left\{\frac{dX(t)}{dt}\right\} = [A]\{X(t)\} + [B]\{u(t)\}, \tag{1}$$

where $\{X(t)\} = \{C_b(t),\ D(t),\ D_H(t)\}^T$ is the state variable vector in that $C_b(t)$ is the time-dependent As concentration in species (μg g^{-1} dry wt), $D(t)$ is the damage at time t (dimensionless), $D_H(t)$ is the cumulative damage with hazard (dimensionless), and t is the time in day; [A] is the state matrix characterizing the TDM with a form as

$$[A] = \begin{bmatrix} -k_2 & 0 & 0 \\ k_k & -k_r & 0 \\ 0 & 1 & 0 \end{bmatrix}, \tag{2}$$

in that k_2 is the depuration rate of As (d^{-1}), 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}); $[B]$ is the input matrix as

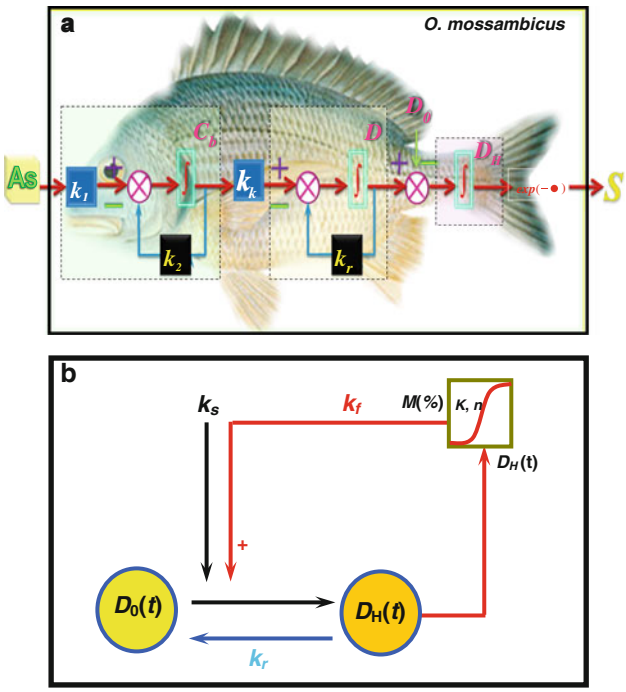

Fig. 1 **a** Block diagram showing the systems-level TDM applied in tilapia. **b** Schematic representation of the physiological damage mechanism with the positive damage feedback loop (See text for the symbol descriptions)

$$[B] = \begin{bmatrix} k_1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & -1 \end{bmatrix}, \tag{3}$$

in that k_1 is the uptake rate constant (ml g^{-1} d^{-1}); and $\{u\} = \{C_w, 0, D_0\}^T$ is the input vector in that C_w is the dissolved As concentration in the water (mg l^{-1}), and D_0 presents the threshold of damage (dimensionless).

Here a simple system is used to feature the positive damage feedback loop incorporating into the TDM (Fig. 1b). This system consists of a damage that can be reversibly converted between damage threshold (D_0) and damage with hazard (D_H) characterizing by damage recovery rate constant k_r. The process of threshold damage converting to damage with hazard is assumed to be regulated in two ways: (i) by an external stimulus of metal stressor k_s and (ii) by positive feedback with a Hill equation relationship between D_H generation and rate of generation of more D_H (Fig. 1b). Based on the essential features shown in Fig. 1b, a dynamic model of damage with hazard can be written as,

$$\frac{dD_H(t)}{dt} = k_s(D(t) - D_H(t)) + k_f\left(\frac{D_H^n(t)}{K^n + D_H^n(t)}\right) \times (D(t) - D_H(t)) - k_r D_H(t), \tag{4}$$

where $D = D_0 + D_H$ is the damage (−), k_f is the hazard feedback rate (d^{-1}), and K is the effective D_H for 50% response (D_H50) for the feedback as the function of D_H. A steady-state relationship between D_H and external stimulus of metal stressor k_s can be obtained by setting $dD_H(t)/dt = 0$ as

$$k_s = \frac{k_f(D_H^n D - D_H^{n+1}) - k_r(D_H^{n+1} + K^n D)}{D_H^{n+1} - D_H^n D + D_H K^n - D K^n}. \tag{5}$$

Therefore, for any given value of environmental stimulus of metal stressor, Eq. 4 can be used to calculate all of the possible steady-state value of D_H.

Data analysis and model parameterization

Uptake and depuration rate constants for tilapia were determined by fitting the integrated form of the kinetic rate equation to concentration data (Tsai and Liao 2006a) for constant As exposure, using nonlinear regression technique,

$$C_b(t) = C_b(t=0)e^{-k_2 t} + \frac{k_1}{k_2}C_w(1 - e^{-k_2 t}), \tag{6}$$

where $C_b(t = 0)$ is initial concentration of As in whole body of tilapia (μg g^{-1} dry wt).

The TDM-based relationship between survival probability $S(t)$ and hazard $D_H(t)$ (Lee et al. 2002a; Ashauer et al. 2007) as (Fig. 1b),

$$S(t) = e^{-D_H(t)}, \tag{7}$$

can be used to fit our published data (Tsai and Liao 2006a; Liao et al. 2009) of As concentration-specific time-dependent survival profiles to estimate killing rate constant k_k and recovery rate constant k_r for tilapia and freshwater clam varied with As concentration.

Here we employed no observed lethal concentration (NOLC) to estimate environmental stimulus rate of As stressor k_s. Therefore, concentration-specific environmental stimulus rate k_s can be calculated by

$$k_{s,i} = \frac{\left(\frac{C_{w,i} - NOLC}{NOLC}\right)}{\Delta t}, \tag{8}$$

where NOLC for tilapia and freshwater clam can be estimated from mortality–time data (Tsai and Liao 2006a; Liao et al. 2009), resulting in 1 mg l^{-1} for tilapia and 5 mg l^{-1} for freshwater clam.

To determine the strength of positive feedback rate (k_f) as the function of damage with feedback D_H, a cell-cycle concept was used. Pomerening et al. (2005) suggested that mitosis was originally controlled by an enzyme that produced a graded response; then, cooperativity or ultrasensitivity evolved, making the response more decisive; then, positive feedback was added. Wang et al. (2004) indicated that As-induced cytotoxicity could disturb cell cycle of ovary

cells of tilapia (TO-2 cells), demonstrating that arsenite in TO-2 cells induced accumulation of the second growth and mitotic phases (G_2/M) cells accompanied by a decrease in the proportion of first growth phase (G_1) cells. Based on the data from the effect of arsenite treatment on the cell-cycle progression of TO-2 cells (Wang et al. 2004), positive feedback rate (k_f) can be estimated approximately as the difference of ratio of cell populations between control and 5 μM arsenite-treated TO-2 cells over time after G_2/M phase cells increased. The resulting value was 0.87 d^{-1}. In this study, ranged values of 0.01–1 d^{-1} for k_f were used in the model implementation.

Practically, damage threshold D_0 can be determined by the published Hill dose–response model describing % mortality of tilapia versus waterborne As concentration based on 96-hr LC50 of 28.68 mg l^{-1} (95%CI, 15.98–47.38) (Tsai and Liao 2006a). We used NOLC = 1 mg l^{-1} with exposure time $t = 10$ day to calculate the damage threshold (D_0) via TDM for $D(t)$ (Ashauer et al. 2007).

$$D(t) = k_k \frac{k_1}{k_2} C_w \left(\frac{e^{-k_r t} - e^{-k_2 t}}{k_r - k_2} + \frac{1 - e^{-k_r t}}{k_r} \right), \quad (9)$$

resulting in $D_0 = 0.006$ for tilapia. Similarly, D_0 for freshwater clam was estimated to be 0.344 based on published Hill dose–response model describing % mortality of versus waterborne As concentration (Liao et al. 2008).

To determine concentration-specific K value, we first fitted three-parameter Hill equation of

$$M(t) = \frac{M_{\max}}{1 + \left(\frac{LT50}{t}\right)^n}, \quad (10)$$

to published concentration-specific mortality–time curves, where $M_{\max}$ is maximum mortality (%) and LT50 is the 50% mortality lethal time (d), to obtain concentration-specific $M_{\max}$, LT50, and n values. We then incorporated estimated rate constant values of k_1, k_2, k_k, k_r, with LT50 estimates into Eq. 9 to calculate D(LT50). Finally, K value can be obtained by following the relationship of $D(\text{LT50}) - D_0 = D_H(\text{LT50}) \equiv K$ varied with waterborne As concentrations.

Figure 2 depicts the framework and computational algorithm of this study.

Uncertainty analysis and simulation scheme

TableCurve 2D (Version 5.0) and 3D (Version 4.0) (AISN Software Inc., Mapleton, OR, USA) packages were used to perform all curve fittings. 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. Mathematica® (Version 5.1, Wolfram Research Inc., Champaign, IL, USA) was used to perform all simulations of TDM and positive damage feedback dynamics.

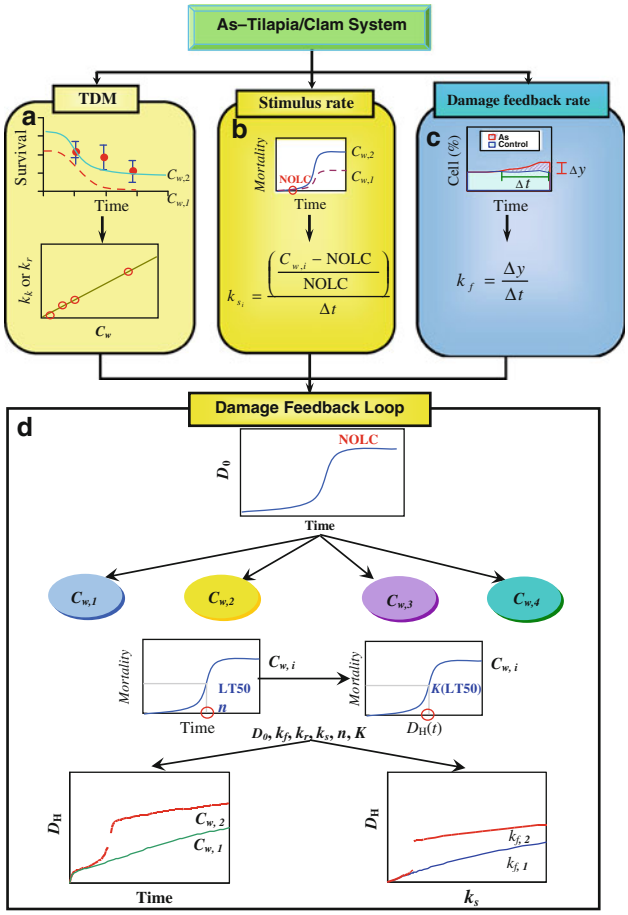

Fig. 2 A conceptual algorithm showing the determinant estimations and analytical method to predict the damage dynamic behaviors of tilapia and freshwater clam

Results

Parameter estimations

The kinetic rate equation in Eq. 6 was fitted to As–tilapia uptake data to obtain the estimated uptake rate constant k_1 of 0.481 ± 0.072 ml g^{-1} d^{-1} (mean ± SE) and depuration rate constant k_2 of 0.164 ± 0.063 d^{-1} with $r^2 = 0.98$ (Fig. 3a). Table 1 summarizes the toxicokinetic data for tilapia and freshwater clam, indicating a BCF = 2.932 for tilapia and a BCF = 2.842 for freshwater clam. The TDM was best fitted to survival probability-time profile data of tilapia ($r^2 = 0.44$–0.99) and freshwater clam ($r^2 = 0.71$–0.99) to obtain concentration-specific values of killing rate constant (k_k) and recovery rate constant (k_r) (Fig. 3b, c). A polynomial model was also used to associate the relationships between killing rate constant (k_k)/recovery rate constant (k_r) and As concentration for model simulation purpose, indicating a good correlation ($r^2 = 0.70$–0.98) (Fig. 4a–d).

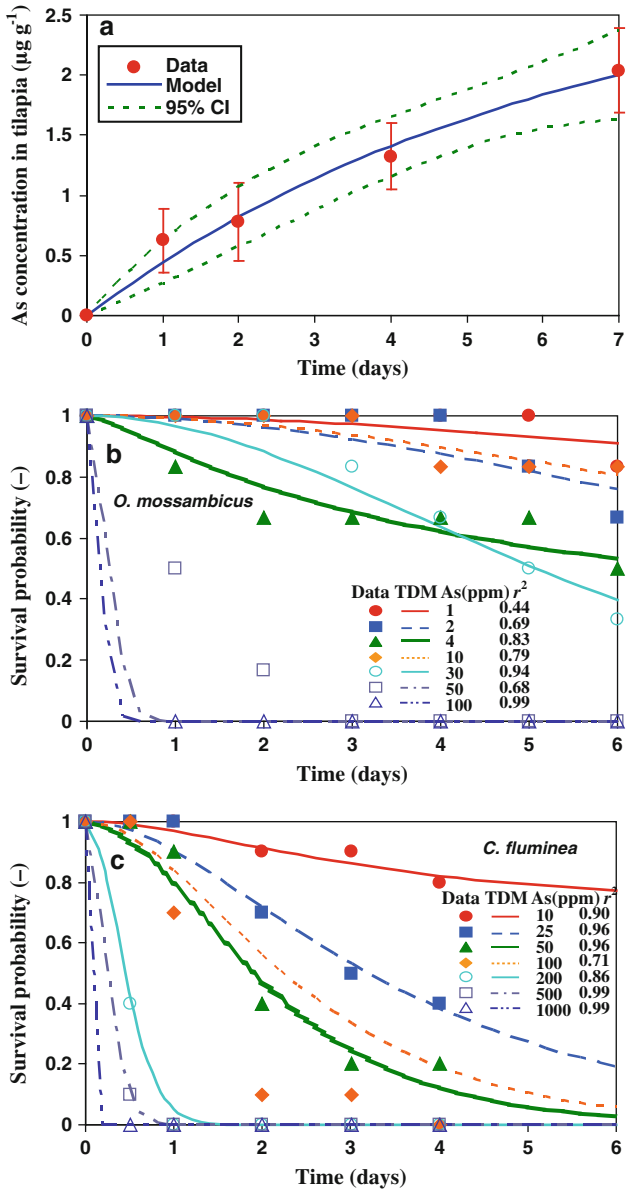

Fig. 3 **a** Fitting kinetic rate equation to published experimental exposure data in tilapia exposed to 1 mg l^{-1} As. Fitting TDM-based survival probability equation to survival probability data to estimate k_k and k_r parameters in **b** As–tilapia system and **c** As–freshwater clam system

Table 2 summarizes the parameter estimates including rate constants of TDM k_k and k_r together with positive damage feedback model parameters of K, n, and k_s varied with exposed As concentrations ranging from 2 to 50 mg l^{-1} for tilapia and 10–500 mg l^{-1} for freshwater clam.

Positive damage feedback behavior

We solved Eq. 5 numerically to understand the relationship between environmental As stimulus (k_s) and steady-state

Table 1 Toxicokinetic data (mean ± SE) used in the proposal model for two species exposed to As

Parameter	Tilapia[a]	Freshwater clam[b]
k_1 (ml g^{-1} d^{-1})	0.481 ± 0.072	2.075 ± 0.442
k_2 (d^{-1})	0.164 ± 0.063	0.730 ± 0.253
BCF (ml g^{-1})	2.932	2.842

[a] Estimated from Eq. 6 with $r^2 = 0.98$

[b] Adopted from Chen and Liao (2010)

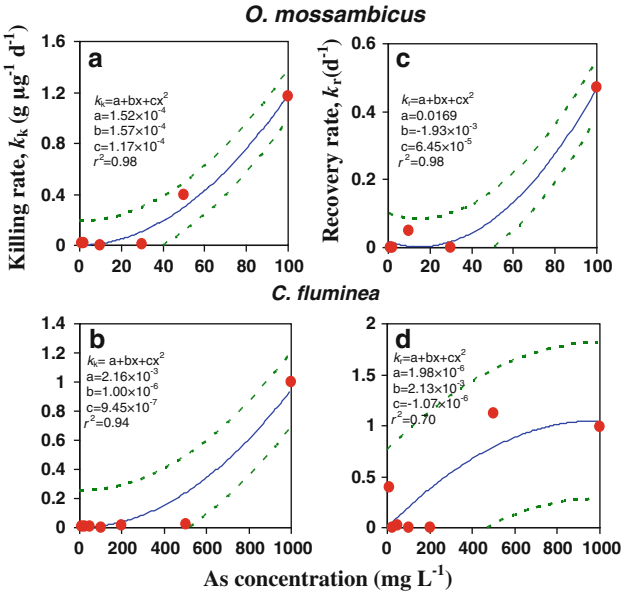

Fig. 4 A relationship between the killing rate constant and As exposure concentration in **a** tilapia and **b** freshwater clam. A relationship between the recovery rate constant and As exposure concentration in **c** tilapia and **d** freshwater clam

response of damage with hazard (D_H) varied with the strength of feedback rate (k_f) ranging from 0.01 to 1 d^{-1} for tilapia exposed to waterborne As 2–50 mg l^{-1} (Fig. 5a–d) and for freshwater clam exposed to 10–500 mg l^{-1} As (Fig. 5e–j). A no-feedback behavior ($k_f = 0$) was also included for comparison.

Generally, the no-feedback behavior is monostable and the D_H–k_s profile is a smooth shape. In the As–tilapia system (Fig. 5a–d), at a specific exposure concentration with a specific $n \leq 4$, as the strength of feedback rate k_f increases, the D_H–k_s profile also experienced a monostable and a nearly smooth fashion (Fig. 5b). When $n > 3$ (Fig. 5a, b, d), as the strength of feedback rate k_f increases, the D_H–k_s profile experienced a sigmoidal fashion with an initial point at $D_H = 0$, indicating the positive feedback damage loop reflected cooperativity or ultrasensitivity.

On the other hand, in the As–freshwater clam system (Fig. 5e–j), when $n < 4$, as the strength of feedback rate k_f increases, the D_H–k_s profile experienced a monostable and

Table 2 Summary of parameter estimates used in the model implementation for tilapia and freshwater clam exposed to As (see text for symbol meaning)

Tilapia						
NOLC (mg l^{-1})	1					
D_0 (–)[a]	0.006					
C_w (mg l^{-1})	2	4		30		50
k_k (g μg^{-1} d^{-1})[b]	0.001	0.027		0.110		0.3
k_r (d^{-1})[b]	0.013	0.010		0.017		0.082
k_s (d^{-1})[c]	0.1	0.3		2.9		4.9
$D(t = 10$ day) (–)[d]	0.0269	0.154		46.889		172.68
R_{max} (%)[e]	100	100		100		100
LT50 (d)[e]	6.66	9.12		4.93		1.01
n (–)[e]	7	1		4		3
K (–)[f]	0.0079	0.125		7.906		2.756
Freshwater clam						
NOLC (mg l^{-1})	5					
D_0 (–)[a]	0.344					
C_w (mg l^{-1})	10	25	50	100	200	500
k_k (g μg^{-1} d^{-1})[b]	0.002	0.0028	0.005	0.0117	0.04	0.239
k_r (d^{-1})[b]	0.021	0.0524	0.103	0.201	0.381	0.792
k_s (d^{-1})[c]	0.1	0.4	0.9	1.9	3.9	9.9
$D(t = 10$ d) (–)[d]	0.683	1.853	5.073	19	82.814	634.38
R_{max} (%)[e]	100	100	100	100	100	100
LT50 (d)[e]	9.04	3.13	1.87	1.23	0.49	0.34
n (–)[e]	2	2	3	4	20	6
K (–)[f]	0.258	0.036	0.166	0.852	1.114	10.014

[a] Estimated from Eq. 9 with NOLC = 1 and 5 mg l^{-1}, respectively, for tilapia and freshwater clam and $t = 10$ day

[b] Estimated based on TDM fitted to survival-time data (Fig. 3b, c)

[c] Estimated from Eq. 8

[d] Calculated from Eq. 9

[e] Estimated based on Eq. 10 to mortality–time data with $r^2 = 0.81$–0.99 for tilapia and $r^2 = 0.93$–0.99 for freshwater clam

[f] Calculated based on $D(\text{LT50}) - D_0 = D_H(\text{LT50}) \equiv K$

a smooth fashion with the initial point not at $D_H = 0$ (Fig. 5e–g). When $n \geq 4$ (Fig. 5 h–j), as the strength of feedback rate k_f increases, the D_H–k_s profile experienced a sigmoidal fashion with an initial point at $D_H = 0$.

This sigmoidicity appeared in As–tilapia and As–freshwater clam systems for $n \geq 4$ allows us to estimate the environmental As stimulus rate (k_s) that is needed to activate a positive damage feedback mechanism (Table 3). Table 3 indicates that k_s needed to activate a positive damage feedback for tilapia exposed to waterborne As at 2 and 30 mg l^{-1} were estimated to be 0.0012–0.0028 and 0.0002–0.0012 d^{-1}, respectively. Meanwhile, for freshwater clam, k_s needed to activate a positive damage feedback were 0.0009–0.0081 d^{-1} that were decreased with increasing of k_f for exposed As concentration at 100–500 mg l^{-1}, respectively.

Figure 6 demonstrates the dynamic behavior of damage with hazard (Eq. 4) for tilapia and freshwater clam exposed to waterborne As varied with feedback strength (k_f), respectively. Table 4 gives the time that is needed to activate the positive damage feedback mechanism for tilapia and freshwater clam varied with As concentration and feedback strength. We found that the dynamic response behavior of $D_H(t)$ depended more on As concentration (C_w) than on Hill coefficient (n). Thus, it is suggested that the As–tilapia and As–freshwater clam systems should stay close to the steady-state k_s–D_H profile where the steady-state k_s–D_H relationship (Fig. 5) defined the dynamic behavior of $D_H(t)$ (Fig. 6).

Survival probability assessment and validation

To assess the survival probability for tilapia and freshwater exposed to waterborne As, we linked mortality–time data and positive feedback-based TDM to better understand the potential biological response. Here we used a k_f–k_s scheme to explore the survival probability based on varied mortality–time curves reflecting the effective 50% D_H (K) and Hill coefficient (n) exposed to different As concentrations

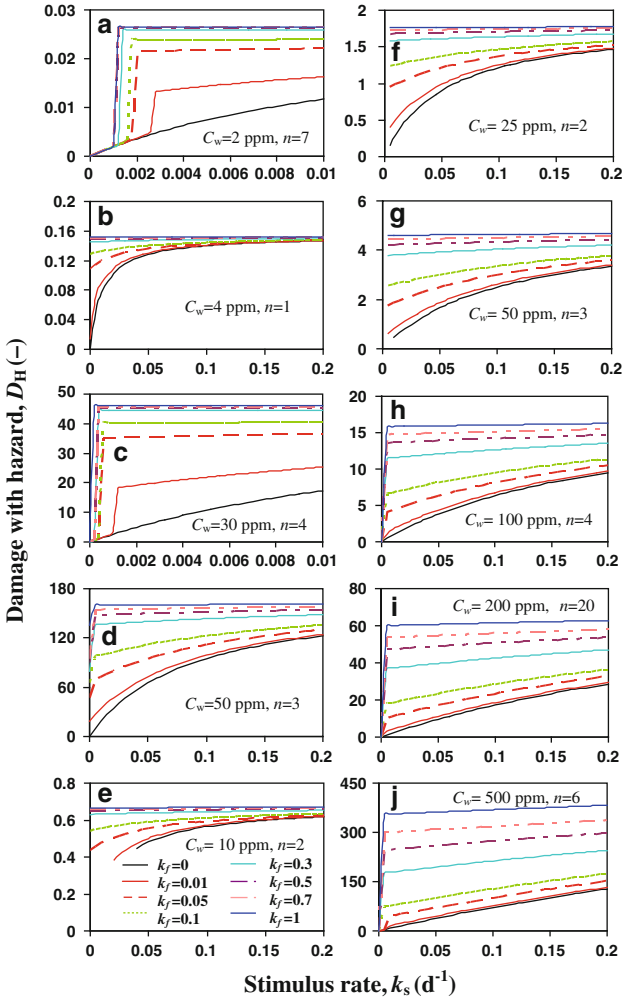

Fig. 5 **a–d** The steady-state damage with hazard responses underlying environmental As stimulus and strength of feedback for tilapia and **e–j** freshwater clam exposed to waterborne As of 2–50 mg l^{-1} and 10–500 mg l^{-1}, respectively

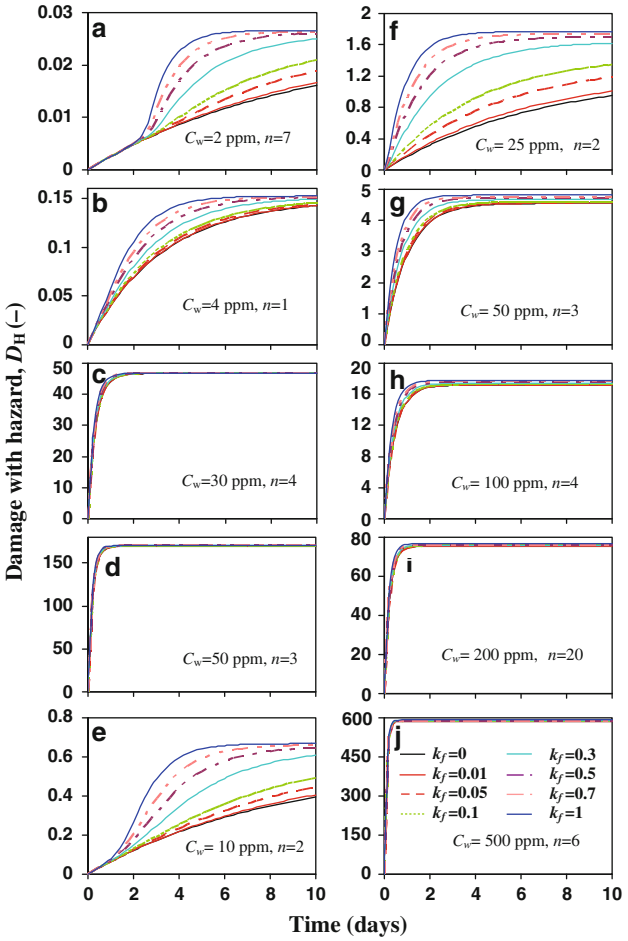

Fig. 6 **a–d** The dynamic damage with hazard behaviors varied with strength of feedback for tilapia and **e–j** freshwater clam exposed to waterborne As of 2–50 mg l^{-1} and 10–500 mg l^{-1}, respectively

Table 3 Environmental As stimulus rate (k_s, d^{-1}) needed to activate damage feedback mechanism for tilapia and freshwater clam varied with As concentration (C_w, mg l^{-1}) with a specific Hill coefficient (n) and feedback strength (k_f, d^{-1})

k_f								
C_w	n	0.01	0.05	0.1	0.3	0.5	0.7	1
Tilapia								
2	7	0.0028	0.0020	0.0018	0.0014	0.0012	0.0012	0.0012
4	1	–	–	–	–	–	–	–
30	4	0.0012	0.0006	0.0006	0.0004	0.0004	0.0004	0.0002
50	3	–	–	–	–	–	–	–
Freshwater clam								
10	2	–	–	–	–	–	–	–
25	2	–	–	–	–	–	–	–
50	3	–	–	–	–	–	–	–
100	4	0.0045	0.0025	0.0020	0.0014	0.0012	0.0011	0.0009
200	20	0.0041	0.0038	0.0036	0.0034	0.0033	0.0033	0.0032
500	6	0.0081	0.0057	0.0049	0.0039	0.0036	0.0033	0.0031

Table 4 Time (d) needed to activate positive damage feedback mechanism for tilapia and freshwater clam varied with As concentration (C_w, mg l^{-1}) and feedback strength (k_f, d^{-1})

k_f							
C_w	0.01	0.05	0.1	0.3	0.5	0.7	1
Tilapia							
2	–	3.65	3.09	2.5	2.29	2.16	2.04
4	–	–	1.55	0.31	0.17	0.12	0.08
30	–	–	–	0.24	0.1	0.08	0.07
50	–	–	–	0.04	0.01	<0.01	<0.01
Freshwater clam							
10	–	2.67	1.64	0.88	0.67	0.56	0.46
25	0.43	0.11	0.08	0.05	0.04	0.03	0.03
50	–	–	0.07	0.03	0.03	0.02	0.02
100	–	–	–	0.03	0.03	0.02	0.02
200	–	–	–	0.01	<0.01	<0.01	<0.01
500	–	–	–	–	–	<0.01	<0.01

(Figs. 7, 8). When tilapia exposed to 2 and 30 mg l^{-1} As, the survival probability for 10-day exposure decreased promptly with increasing of k_f and k_s due in part to the mortality–time curves exhibited cooperativity ($n \geq 4$) (Fig. 7a, b, c). On the other hand, when tilapia exposed to As concentration of 4 mg l^{-1}, the survival probability decreased relative slowly as k_f and k_s increased for the mortality–time curve did not exhibit cooperativity ($n = 1$) (Fig. 7a, d).

In the As–freshwater clam system, when mortality–time curve did not exhibit cooperativity at the exposed As concentrations of 10 and 50 mg l^{-1} ($n < 4$), the survival probabilities of freshwater clam decreased gradually with increasing of k_f and k_s (Fig. 8a–c). Yet, when freshwater clam exposed to a higher As concentration of 100 mg l^{-1}, the survival probability decreased promptly as k_f and k_s increased because the mortality–time curve exhibited cooperativity ($n = 4$) (Fig. 8a, d).

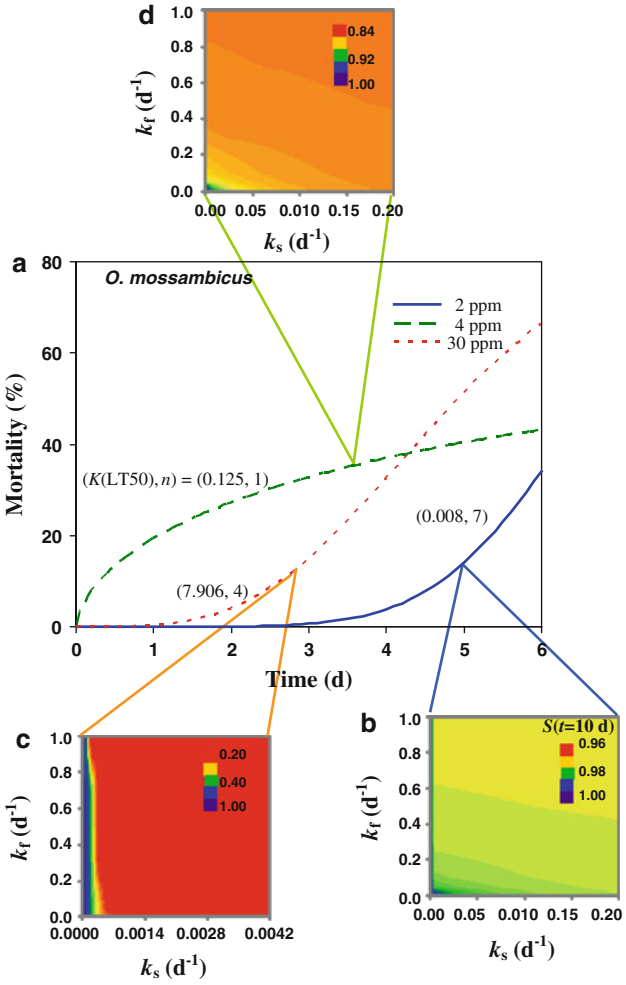

Fig. 7 **a** Time–mortality profiles of tilapia revealed the 50% D_H (i.e., K) and Hill coefficient (n) exposed to 2, 4 and 30 mg l^{-1}. **b–d** The k_f and k_s scheme to reflect the survival probability of tilapia exposed to 2, 4 and 30 mg l^{-1} for 10 days based on effective K and n parameters

Discussion

Positive feedback-based TDM

In this study, we used a simple mechanistic model for systems-level damage response process, we show that incorporation of the positive feedback as a function of damage with hazard poses a significant effect on the species coping process in response to environmental metal stressors. The response can be accounted for by the intrinsic ultrasensitivity of the metal toxicity-induced damage–recovery interaction and a positive feedback loop where the interaction is embedded. The present study is a

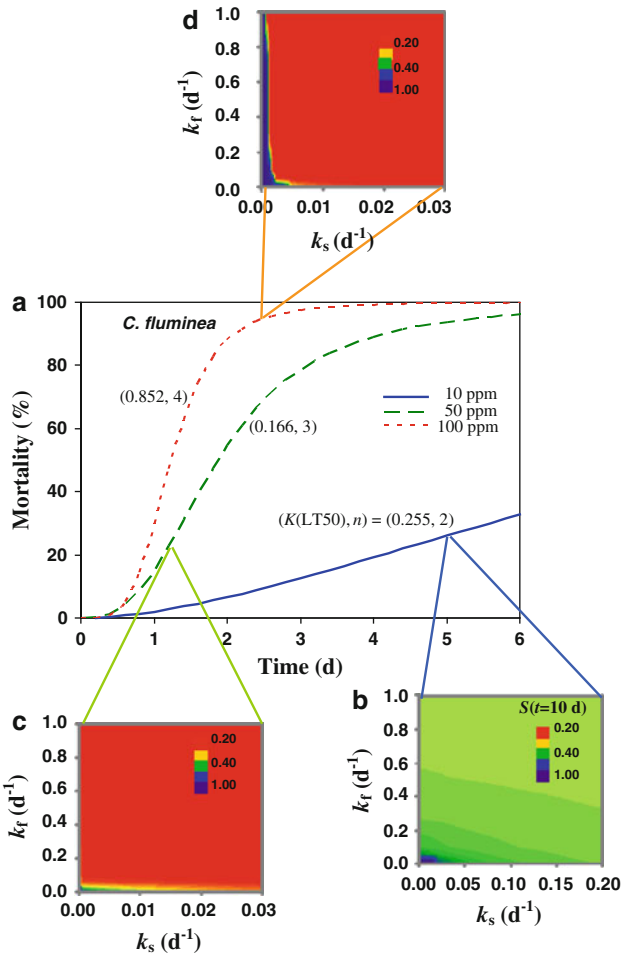

Fig. 8 **a** Time–mortality profiles of freshwater clam revealed the 50% D_H (i.e., K) and Hill coefficient (n) exposed to 10, 50 and 100 mg l^{-1}. **b–d** The k_f and k_s scheme to reflect the survival probability of freshwater clam exposed to 10, 50 and 100 mg l^{-1} for 10 days based on the effective K and n parameters

practical attempt to understand the role of feedback mechanism in organism exposed to As stresses. The mechanisms composed of both natural and biological elements could be beneficial to characterize the dynamics features of biological system-levels and to modulate the signal of damage response.

Most of studies proposed aquatic toxicology models to assume that increasing body residue, critical target occupation, and metabolites in the live organism were strongly associated with damage response and mortality inducing (Lee et al. 2002a, b; Lee and Landrum 2006; Ashauer et al. 2007; Jager et al. 2011). Such as critical body residue model, critical area under the curve model, DAM, multi-component DAM, and TDM. However, the dynamics of damage or mortality not only focus on body residue function but also bioavailability, biotransformation, and damage/repair mechanisms on a cellular scale. The lethal effect was overestimated based on the body residue, since these models assumed that the body residue in the live organism was similar with that of dead animals. Due to the body residue in dead organism is difficult to measure. Otherwise, most other modeling approaches assume some toxic effect function without any biological or mechanistic justification (Bedaux and Kooijman 1994).

Traditional TDM only focus on the uptake, elimination, killing, and recovery capacities to describe the abnormal physiological reaction that may exist some dubious problem to query this reasonable prediction. This study present positive feedback-based TDM to describe that specific chemical exposure blocked enzyme and synthesis of new enzyme induce cytotoxic in tilapia. Since different toxicants have typical toxic effects in applied the cells scale with different doses and exposure periods. Positive feedback-based TDM can provide the physiological and biological mechanisms and to clearly describe that the abnormal cell-cycle regulation affect the damage promotion in tilapia. To compare the predictability between positive feedback-based TDM and traditional TDM, a validation between previous experimental data and predicted values was performed. The results show that the positive feedback-based TDM gives a much better predictability than that of TDM at As exposure concentrations ranging from 100 to 500 mg l^{-1} for freshwater clam, whereas for tilapia, two models had the same predictability (Fig. 9).

Our results show that the ultrasensitivity appeared both in As–tilapia and As–freshwater clam systems to be Hill coefficient $n \geq 4$. These kind of ultrasensitive stimuli-response profiles behave more like a switch than a Michaelis–Menten (M–M) type system ($n = 1$) does, i.e., the response to small concentration is minimal, but once

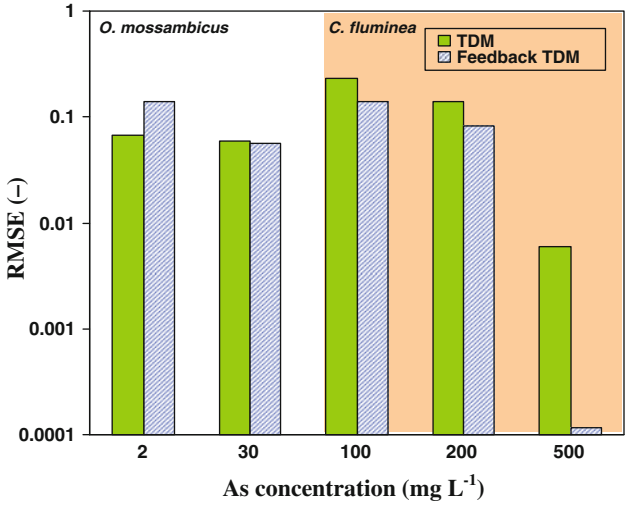

Fig. 9 Root–mean–squared–error (RMSE) between TDM and positive feedback-based TDM in predicted survival probability of tilapia and freshwater clam under As exposure

the system begins to respond, it switches from off to on over a narrower range of metal concentrations than does a M–M type system (Koshland et al. 1982). Tsai and Liao (2006a) also revealed that Hill model can be used to describe the dose–response relationships between mortality and As concentration for tilapia *O. mossambicus* with a positive cooperativity of fitted Hill coefficient $n = 4.07$. On the other hand, the Hill model can also be used to describe the As concentration–valve closing response relationships varied with different response times of 15–300 min in freshwater clam *C. fluminea* with fitted Hill coefficients ranging from 1.63 to 3.67, indicating positive cooperativity (Liao et al. 2009).

We have elucidated the mathematical basis for the damage response at the systems-level TDM incorporating a positive feedback loop. Our theoretical framework shows that with the appropriate kinetic parameters, various systems can display ultrasensitive responses, whereas the same system of reactions exhibited subsensitive responses with other set of kinetic parameters. Our results implicate that the feedback becomes strong enough to maintain the system in the on state even when the stimulus is decreased to zero. At this point, the system may be able to convert a transient stimulus into an essentially irreversible response.

Our mathematical simulations revealed that the damage with hazard could trigger a positive feedback loop that together with environmental metal stressors increases the irreversibility (Novak et al. 2007). We used the model to make predictions of survival probability and guide the risk assessment framework that provides unique mechanistic insights into the farmed (aquatic) species–metal stressor systems.

Decisive factor of metal toxicity-induced damage response

Feedback loops shape systems-level-based damage process model. Feedback has distinct role in shaping physiological responses in damage–recovery scheme of farmed species in response to environmental metal stressors. We suggest that mortality–time profile derived Hill coefficient could be used as a new risk indicator to assess the survival probability for tilapia and freshwater clam exposed to waterborne As. Our study indicates that ultrasensitive (switch-like) responses to metal stressors could be characterized by the Hill coefficient (Koshland et al. 1982).

The presence of positive feedback in ecophysiological response system in aquatic species can cause a discontinuous shift of states of damage with hazard response of species in response to small environmental changes of metal stressor. We suggest that recovery and detoxification processes may buffer and prevent positive feedback, which would contribute to high survival probability (Buchwalter et al. 2008). Furthermore, the results strongly imply that the traditional view of ecotoxicological models should be changed to a systems-level damage model, by incorporating the positive feedback loop.

A regulator triggers state change. Near the threshold point, a small change in one parameter, such as regulator concentration or recovery duration, causes switching of the responding components. Such responses called ultrasensitive. Cooperativity yields ultrasensitivity. Positive feedback loops play an important role in producing switching behavior and are considered necessary for bistability (Brandman and Meyer 2008; Chang et al. 2010). Introducing cooperativity would increase the sensitivity even further, depending on the kinetic parameters. These simulations show that the ultrasensitivity mechanism can apply to both the activating and deactivating damage systems. This general applicability across a range of concentrations for the damage response system and the interconvertibility between metal toxicity-induced damage and recovery time make the switch-like mechanism an additional factor in ecological risk assessment strategy.

Implications on realistic field risk assessment

A mathematical analysis revealed that a system-level TDM with elements of opposite regulatory activity (strength of positive feedback and recovery) constitutes a minimal requirement for describing susceptibility in aquatic organisms in response to metal toxicity. This mechanism underlying the positive feedback-based systems-level TDM could represent an additional essential element facilitating the traditional environmental risk assessment. We suggest that Hill coefficient can be seen as a new early warning signal in field risk assessment strategy.

Analytical and simulations indicate that positive feedback-based TDM can appropriately address the damage response dynamics. Our findings indicate that positive feedback as a function of damage is an important mechanism that can explain patterns of species survival probability exposed varied metal concentrations followed a specific mortality–time profile depicting by the strength of feedback—external stimuli scheme. Our study may provide some insights into the carry-over toxicity concept (Ashauer et al. 2010). Generally, carry-over toxicity occurs when organisms exposed to an environmental toxicant survive but carry some damage resulting in reduced fitness (Ashauer et al. 2010).

Recent progress in linking the positive feedback loop to a systems-level TDM, combined with a quantitative kinetic model of damage with hazard such as the one presented here, puts us on the road to a new insight into the environmental risk assessment for aquatic species exposed to waterborne metal stressors (Jager et al. 2006; Curis et al.

2009). Our theoretical work shows how linked positive feedback loop may produce the robust switch-like responses required in ecophysiological response systems that regulate the organism recovery and survive when exposed to an environmental toxicant. Therefore, this study shows a significantly improved systems-level TDM.

Coping with variations in environmental metal stressors is crucial for maintaining optimal damage/hazard–recovery function in aquatic organisms in response to metal toxicity (Buchwalter et al. 2008). Different organisms have different levels of sensitivity to such variations, and need for effective compensation mechanism arises when organisms cannot tolerate these alterations. We anticipate that this present proposed positive feedback-based systems-level toxicological model could be incorporated into ecotoxicology and risk assessment of chemicals to improve current risk assessment methods.

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