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Hill coefficient-based stochastic switch-like signal directly governs damage-recovery dynamics in freshwater fish in response to pulse copper

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ABSTRACT

Growing evidence demonstrates that fluctuating metal stressors can have profound impact on the ecophysiological responses in aquatic species. However, how environmental stochasticity affects the complex damage-recovery dynamics in organisms remains difficult to predict. The objective of this paper was to investigate the stochastic behavior in the damage-recovery dynamics in tilapia in response to pulse waterborne copper (Cu). We developed a mathematical framework that allows discrimination between damage and recovery processes in tilapia exposed to designed pulse Cu scenarios. We built deterministic nonlinear models for the damage-recovery dynamics that produce response surfaces describing killing/recovery rate–Cu-pulse interval interactions. Here we showed that the stochastic switching behavior arose from competition among killing, recovery rates, and Cu pulse frequency. This competition resulted in an ultrasensitivity appeared in whole body, gills, muscle, liver, and kidney with Hill coefficients of $\geq$7, 4, 7, 5, and 5, respectively, at Cu 3 mg L^{-1}, dilution rate 0.05 h^{-1}, and pulse interval 72 h, indicating that a stochastic switch-like response was generated. We argue that the role of gill-associated Hill coefficient as a direct signal of the stochastic switch-like response in the damage-recovery dynamics in response to pulse metal stressor can serve as a sensitive indicator for risk detection in fluctuating environments. Our approach constitutes a general method to identify the stochastic switch-like response for aquatic species exposed to fluctuating metal stressors, which may help to predict and, eventually, expand our understanding of the damage-recovery dynamics. Finally, we implicate that Hill coefficient-based switch-like signal and its damage with hazard response can be linked in an information theoretic framework to handle environmental stochasticity.

1. Introduction

The association between aquatic species exposed to fluctuating or pulsed contaminants and their responses has been extensively studied in a wide variety of systems, demonstrating that fluctuating metal stressors can have profound impact on the ecophysiological responses (Meyer et al., 1995; Reinert et al., 2002; Diamond et al., 2006; Ashauer et al., 2007, 2010; Chen and Liao, 2012; Chen et al., 2012a,b). Recently developed model used to describe the survival process of aquatic species in response to fluctuating and sequential pulses of contaminants is a process-based threshold damage model (TDM) based on the damage assessment model (DAM) (Ashauer et al., 2007, 2010). They argued that the sequence where organisms are exposed to chemicals could matter as important as the concentration and exposure duration.

Chen et al. (2012b) have incorporated a positive damage feedback loop into a toxicokinetic/toxicodynamic (TK/TD)-based TDM to assess susceptibility for tilapia and freshwater clam in response to waterborne arsenic. They indicated that TDM with positive feedback frequently exhibited a switch-like behavior when exposure levels of metal stressor exceed certain thresholds. On the other hand, different metal stressors with specific Hill coefficients evoke different dynamic patterns of damage with hazard. Moreover, Hill coefficient that derived from a mortality-time profile is suggested as a risk indicator for assessing the survival probability for aquatic species exposed to waterborne metals.

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Chen et al. (2012b) further indicated that the positive damage feedback mechanism could be triggered at or above a Hill coefficient threshold of 4 for tilapia and freshwater clam in response to waterborne arsenic. The Hill coefficient derived from a Hill function-type dose–response profile is larger than 1 is referred to as ultrasensitivity (Koshland et al., 1982; Kim and Gelenbe, 2012).

The ultrasensitivity can be employed to the activating–deactivating damage systems. This general applicability across a range of metal concentrations for the damage response system and the interconvertibility between metal toxicity-induced damage and recovery time make the switch-like behavior an additional factor in ecological risk assessment strategy. An unresolved central question, however, is whether the observed switch-like behavior originates mainly from environmental stochasticity (Chen et al., 2015), or whether it stems from a deterministic process in the DAM that only appears to be random.

In this paper, we used freshwater tilapia *Oreochromis mossambicus*, an important food fish for the people of Taiwan, as the studied fish species. It is also one of the most abundantly invasive species in local freshwater and estuary ecosystems. Typically, tilapia growers in Taiwan used copper sulfate ($CuSO_4$) to exterminate phytoplankton for controlling skin lesions and gill disease (Carbonell and Tarazona, 1993; Chen and Lin, 2001; Chen et al., 2006). Consequently, Cu burdens in tilapia are relatively higher, ranging from 1.524 to 18 $\mu g\,g^{-1}$ dry wt (Lin et al., 2005).

The treatment of culture ponds with >1 $mg\,L^{-1}$ $CuSO_4$ is effective in killing algae and parasites (Banerjee et al., 1990; Boyd, 2005; Mischke and Wise, 2009; Miao et al., 2011). Grosell and Wood (2002) and De Boeck et al. (2007) indicated that high Cu burdens were likely to pose mortality risk for fish due to disruption of branchial ion regulation. Chen et al. (2012a,b) further revealed that pulse frequency or duration were more likely to alter survival probability and population growth in tilapia in response to waterborne Cu.

Our goal was to investigate the stochastic switch-like behavior in the damage-recovery dynamics in tilapia in response to pulsed waterborne Cu. In this paper, we developed a mathematical framework that allows discrimination between damage and recovery processes in tilapia in response to the designed pulsed waterborne Cu settings. A TK/TD-based TDM with a Hill function-based positive damage feedback model was used to examine the complex mechanisms of pulse Cu susceptibility in tilapia. Finally, we built deterministic nonlinear models for the damage-recovery dynamics that produce response surfaces describing killing/recovery rate–Cu-pulse interval interactions.

Understanding this contribution to a dynamic response on a systems scale is essential both for depicting how aquatic species deploy regulatory processes to accomplish ecophysiological changes and for examining key damage-recovery dynamics controlling each process in response to fluctuating metal stressors.

2. Materials and methods

2.1. Study data

The TK data obtained from the sequential pulse Cu exposure experiments (Chen et al., 2015) and mortality data obtained from the Cu acute toxicity bioassays on tilapia population (Nussey et al., 1996) give us an opportunity for examining the switch-like response in the damage-recovery dynamics in tilapia to pulse Cu.

Briefly, Chen et al. (2015) conducted 10-d sequential pulse Cu exposure bioassay to determine pulse Cu accumulation, by using adult tilapia *O. mossambicus* to Cu concentrations by increasing from 100 to 300 $\mu g\,L^{-1}$. The pulse Cu exposure timings were occurred twice during the exposure periods at hours 24 and 144, respectively. The pulse exposure duration was carried out 6 h in each event. The water quality conditions were maintained at water temperature 28 °C with pH 7.8. The used toxicokinetic parameter estimates were summarized in supplementary Table S1.

To determine the time- and Cu-dependent mortality of tilapia, Nussey et al. (1996) conducted the 96-h toxicity experiments using following Cu concentrations of 0, 1.0, 1.7, 1.8, 2.0, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 3.0, 3.5, and 4.0 $mg\,L^{-1}$ to observe the survival fish in each exposure tank. The time intervals of mortality measurement were 2, 4, 8, 12, 16, 24, 32, 48, 72, and 96 h. The water quality conditions were also maintained at water temperature 28 °C and pH 7.8.

Table 1
Mechanistic expression of pulsed TK model and damage feedback TDM.

Bioaccumulation

$$\frac{dC(t)}{dt} = k_1 C_w(t) - k_2 C(t) \quad \text{(T1-1)}$$

Cumulative damage

$$\frac{dD(t)}{dt} = k_k C(t) - k_r D(t) \quad \text{(T1-2)}$$

Dynamics state of damage with hazard feedback loop

$$\frac{dD_H(t)}{dt} = k_s\,(D(t) - D_H(t)) + k_f \left(\frac{D_H^n(t)}{K^n + D_H^n(t)}\right) \cdot (D(t) - D_H(t)) - k_r D_H(t) \quad \text{(T1-3)}$$

Steady state of damage with hazard feedback loop

$$k_s = \frac{D_H^n - k_r K^n D_H - (k_f + k_r) D_H^{n+1}}{(D_H - D)(D_H^n + K^n)} \quad \text{(T1-4)}$$

Survival probability

$$S(t) = e^{-D_H(t)} \quad \text{(T1-5)}$$

Adopted from Chen et al. (2012a,b).
Symbol meanings: $C(t)$ is the time-dependent Cu burden ($\mu g\,g^{-1}$ dry wt), $D(t)$ is the time-dependent damage (−),$D_H(t)$ is the time-dependent damage with hazard, $S(t)$ is the time-dependent survival probability (−), k_1 is the uptake rate constant ($L\,g^{-1}\,h^{-1}$), k_2 is the elimination rate constant (h^{-1}), k_k is the killing rate constant ($g\,\mu g^{-1}\,h^{-1}$), k_r is the recovery rate constant (h^{-1}), k_s is the external stimulus of Cu stressor (h^{-1}), k_f is the hazard feedback rate constant (h^{-1}), K is the effective D_H for 50% response for the feedback (−), and n is the Hill coefficient (−).

2.2. Pulsed TK model and damage-feedback TDM

The TDM lays the foundations for predicting survival of aquatic organisms after exposed to sequential pulse and fluctuating patterns of chemical stressor. The dynamic equations for pulsed TK model and positive damage feedback TDM are listed in Table 1.

Briefly (Table 1), the first-order bioaccumulation model can be used to predict the body burden followed the exposure to sequential pulse Cu activities (Eq. (T1-1)). The time-dependent cumulative damage can be derived from the first-order damage accumulation model (Eq. (T1-2)). 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. Chen et al. (2012a,b) proposed a simple system used to describe the positive damage feedback loop incorporating with the TDM. The damage feedback system consists of the reversible damage that can be converted between damage threshold (D_0) and damage with hazard (D_H) by a damage recovery rate constant k_r.

The process of threshold damage converting to reversible damage with hazard is regulated in two pathways (Eq. (T1-3)): (i) by an external stimulus of Cu stressor k_s and (ii) by a positive feedback with a Hill equation relationship between D_H generation and rate of generation of mode D_H. A steady-state relationship between D_H and external stimulus of metal stressor k_s can be obtained by setting $dD_H(t)/dt = 0$ (Eq. (T1-4)). The survival probability can be derived directly from the TDM and is given by an exponential function of damage with hazard (Eq. (T1-5)).

2.3. Switch-like responses and Cu residence time

To explore the interaction between tissue damage and recovery rates, we calculated residence time of Cu in tissue. Mathematically, the residence time of Cu in each tissue can be defined as

$$\bar{t} = \frac{\int_0^t tC(t)dt}{\int_0^t C(t)dt}, \quad (1)$$

where $\bar{t}$ is the tissue-specific Cu residence time (h) and $C(t)$ is the tissue-specific Cu burden profile ($\mu g\, g^{-1}$).

For a constant waterborne Cu concentration C_w, the tissue-specific Cu burden profile can be calculated based on a one-compartment TK model with known uptake and elimination rate constants of k_1 ($L\, g^{-1}\, d^{-1}$) and k_2 (d^{-1}) as $C(t) = BCF_{ss}C_w(1 - e^{-k_2 t})$ where $BCF_{ss} = k_1/k_2$ is the steady-state bioconcentration factor.

Therefore, in light of the TK-based Cu burden profile, Cu residence time in a specific target organ or tissue can be expressed solely as a function of k_2 followed by Eq. (1) as

$$\bar{t} = \frac{1}{2k_2}\left[\frac{(k_2 t)^2 + 2(k_2 t e^{-k_2 t} + e^{-k_2 t} - 1)}{k_2 t + e^{-k_2 t} - 1}\right]. \quad (2)$$

On the other hand, $\bar{t}$ can also be calculated in a time-dependent pulse waterborne Cu scheme as

$$C_w(t) = C_0 + C_1\left(\sum_{i=1}^{2n-1} U(t - t_i) - \sum_{j=2}^{2n} U(t - t_j)\right), \quad (3)$$

where C_0 is the initial Cu concentration ($\mu g\, L^{-1}$), C_1 is the pulse Cu concentration ($\mu g\, L^{-1}$), and $U(t–t_i)$ is the unit step function with the pulse timing of t_i at the ith-h.

Generally, the cooperative and competitive aspects of an interesting dose–response can be modeled dynamically by a homogeneous analytical approach represented by the Hill kinetics. The Hill kinetics-based dose–response relationship is generally defined as

$$y(t, C_w) = M_{min} + \frac{M_{max} - M_{min}}{1 + (LT50/t)^n}, \quad (4)$$

where M_{min} and M_{max} are the minimum and maximum mortality responses at specific Cu exposure concentration (–), t is the response time, LT50 is the median lethal time (h), and n is the Hill coefficient.

Here we rewrite Eq. (4) to capture the impact of Hill coefficient on the switch-like behaviors in terms of mortality and damage (D) as

$$n = \frac{\log(M_{max} - M(t)/M(t) - M_{min})}{\log(K(LT50)/D(t))}. \quad (5)$$

where $M(t)$ is the time-dependent mortality (–) in that mortality can be refined as $M(t) = 1 - S(t) = 1 - e^{-D(t)-D_0}$.

2.4. Hill-based killing and recovery rates modeling

From a viewpoint of pharmacokinetics, the saturable killing rate can be commonly characterized by a Hill function (Bourne, 1995). Therefore, the average killing and recovery rate constants in the proposed TK/TD-based TDM with damage feedback (see Table 1) subjected to a pulse Cu can then be derived by integrating Hill-based killing/recovery rate functions with respective to time over a pulse interval as

$$\overline{k_k} = \frac{1}{\tau}\int_0^\tau k_{k,min} + \frac{(k_{k,max} - k_{k,min})}{1 + (k_k C_w 50/C_w(t))^{n_k}} dt, \quad (6)$$

$$\overline{k_r} = \frac{1}{\tau}\int_0^\tau \frac{k_{r,max}}{1 + (C_w(t)/k_r C_w 50)^{n_r}} dt, \quad (7)$$

where $\overline{k_k}$ is the average killing rate constant ($g\, \mu g^{-1}\, h^{-1}$), $\overline{k_r}$ is the average recovery rate constant (h^{-1}), τ is the pulse interval (h), $k_{k,max}$ and $k_{r,max}$ are the maximum killing and recovery rates, respectively; $k_k C_w 50$ and $k_r C_w 50$ are the Cu concentration that induced the median killing and recovery rates ($mg\, L^{-1}$), and n_k and n_r are the Hill coefficients with respect to the average killing and recovery rates, respectively.

In this study, we considered the dilution rate of Cu concentration along with the pulse interval. Thus, the equation used to describe time-dependent diluted Cu concentration has the form as

$$C_w(t) = C_0 \cdot e^{-k_0 t} + \sum_n U(t - N \cdot \tau) \cdot C_0 \cdot e^{-k_0(t - N\cdot\tau)}, \quad (8)$$

where k_0 is the dilution rate (h^{-1}) and N is the pulse interval (h).

Hence, we designed six initial exposure concentrations (C_0: 0.5, 1, 2, 3, 4, and 5 $mg\, L^{-1}$), ten pulse interval (τ: 2, 4, 8, 12, 30, 48, 60, 80, 120, and 240 h) and three dilution rates (k_0: 0.05, 0.1, and 0.5 h^{-1}) to describe the fluctuating exposure to Cu. Thus, Eqs. (6)–(8) can be used to construct the relationships among Cu concentration, pulse interval, and average killing/recovery rates.

In doing so, the average killing rate constant ($\overline{k_k}$) can be characterized by the threshold of Cu concentration and pulse interval at 50%, describing by a nonlinear function as,

$$\overline{k_k}(C_w, \tau) = k_{k,min} + (k_{k,max} - k_{k,min}) \cdot \left(\frac{1}{1 + (k_k C_w 50/C_w)^{n_c}}\right) \cdot \left(\frac{1}{1 + (\tau/k_k \tau 50)^{n_\tau}}\right), \quad (9)$$

where $k_k C_w 50$ and $k_k \tau 50$ are the Cu concentration ($mg\, L^{-1}$) or pulse interval (h) that induced 50% killing rates, respectively.

On the other hand, a similar form of nonlinear function can be used to describe the relationships among average recovery rate constant ($\overline{k_r}$), Cu concentration, and pulse interval as

$$\overline{k_r}(C_w, \tau) = k_{r,max} \cdot \left(\frac{1}{1 + (C_w/k_r C_w 50)^{n_c}}\right) \cdot \left(\frac{1}{1 + (k_r \tau 50/\tau)^{n_\tau}}\right), \quad (10)$$

where $k_r C_w 50$ and $k_r \tau 50$ are the Cu concentration or pulse interval that induced 50% recovery rates, respectively.

2.5. 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, Decisioneering, Inc., Denver, CO, 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 under pulsed Cu exposures and positive damage feedback dynamics. To estimate uncertainty in the residence time calculations, we propagated the upper and lower uncertainty in the elimination rate constant estimates.

The study framework that covers Hill coefficient-based stochastic switch-like signal in the damage-recovery dynamics in tilapia in response to pulse Cu is illustrated in supplementary Fig. S1.

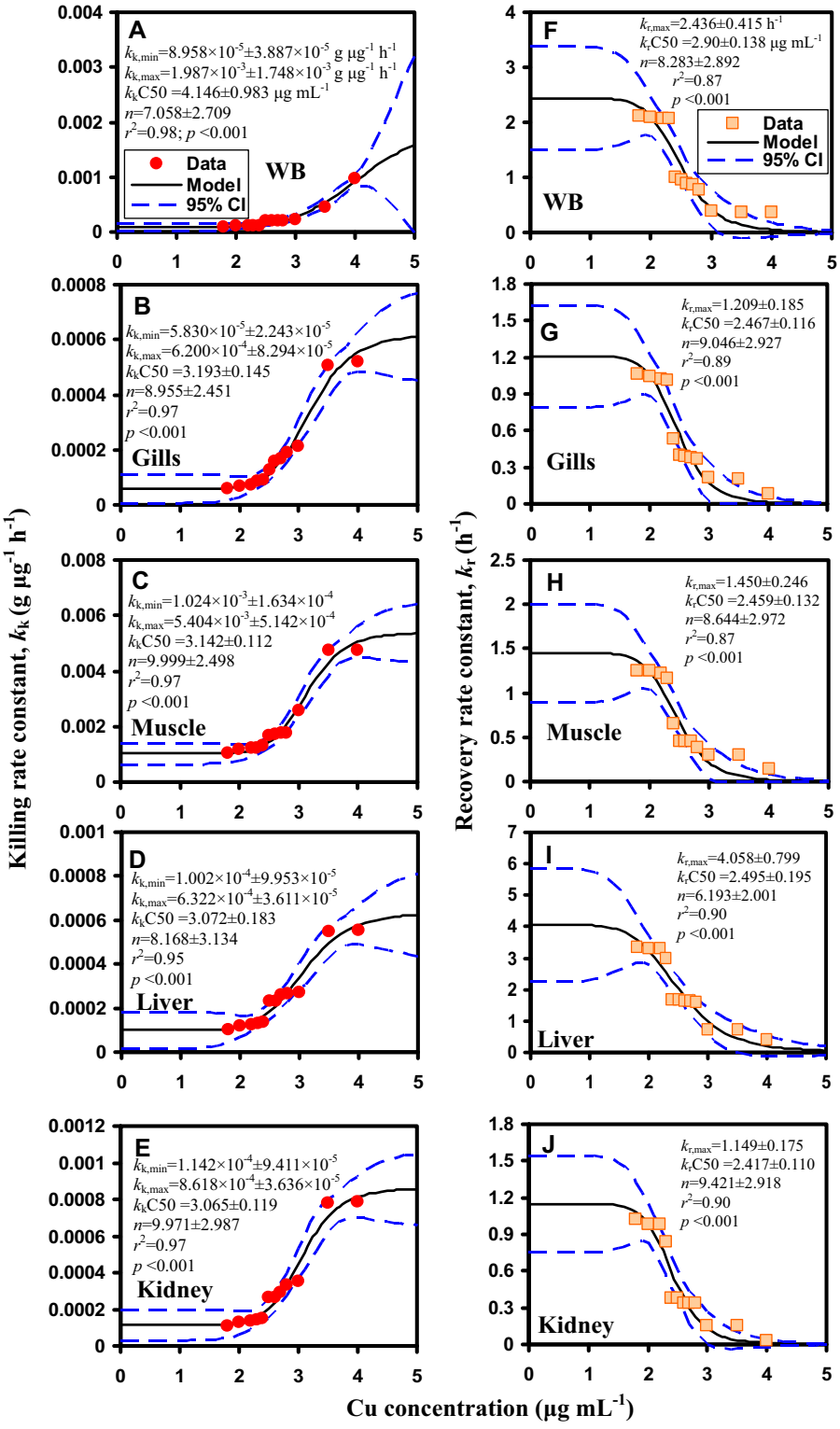

Fig. 1. Relationship between killing rate constant and Cu concentration in (A) whole body, (B) gills, (C) muscle, (D) liver, and (E) kidney of tilapia. Relationship between recovery rate constant and Cu concentration in (F) whole body, (G) gills, (H) muscle, (I) liver, and (J) kidney of tilapia.

3. Results

3.1. Parameterization

Our results indicated that the Hill model provided the best fit for mortality data of tilapia with high r^2 values (0.84–0.99, $p < 0.005$ or 0.001) with the fitted Hill coefficients (n) ranged from 2.2 to 9.45, revealing ultrasensitivity (see supplementary Table S2). The average LT50 estimates are 235, 234.36, 228.31, 179.98, 98.07, 90.83, 88.57, 84.10, 81.52, 47.02, 30.28, and 24.95 h with respect to Cu exposure concentrations of 1.8, 2, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 3, 3.5, and 4 mg L^{-1}, respectively (Table S2).

The survival function of TDM $S(t) = e^{-D(t)-D_0}$ was optimally fitted to survival probability-time profile data of target tissues

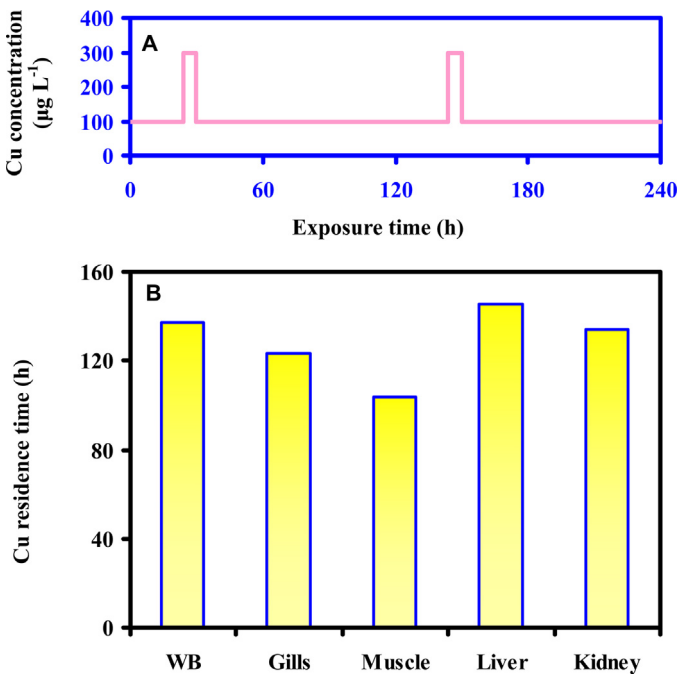

Fig. 2. (A) Pulse Cu exposure concentration pattern induced (B) the tissue-specific mean Cu residence times.

in tilapia (r^2 = 0.73–0.99 in whole body, r^2 = 0.72–0.98 in gills, r^2 = 0.72–0.99 in muscle, r^2 = 0.63–0.99 in liver, and r^2 = 0.76–0.99 in kidney) to estimate damage threshold (D_0), concentration-dependent killing (k_k) and recovery (k_r) rate constants (see supplementary Table S3). The D_0 estimates were 0.00212, 0.0017, 0.0248, 0.0254, and 0.0133 in whole body, gills, muscle, liver, and kidney, respectively. As Cu concentration increases, the killing rates increases, whereas the smaller recovery rates, the lower Cu concentration. The resulted k_k estimates ranged from 8.958×10^{-5}–9.625×10^{-4}, 5.833×10^{-5}–5.213×10^{-4}, 1.042×10^{-3}–4.754×10^{-3}, 1.042×10^{-4}–5.542×10^{-4}, and 1.083×10^{-4}–7.833×10^{-4} g μg^{-1} h^{-1}, whereas k_r estimates ranged from 2.124 to 0.371, 1.068–0.08, 1.250–0.144, 3.333–0.388, and 1.020–0.028 h^{-1}, respectively, for whole body, gills, muscle, liver, and kidney (Table S3).

The 4- and 3-parameter Hill models were also used to associate the relationships between killing rate constant (k_k)/recovery rate constant (k_r) and Cu burden of target tissue on the model simulation experiments. The results indicated that a good correlation (r^2 = 0.87–0.98) was found (Fig. 1). The tissue/organ-specific maximum killing rate ($k_{k,max}$) and recovery rate constants ($k_{r,max}$), the Cu concentration that induced the median killing (k_kC_w50) and recovery (k_rC_w50) rates can also be estimated (Fig. 1).

3.2. Cu residence time in target organs

Based on the sequential pulse Cu exposure pattern (Fig. 2a), the Cu residence time function (Eq. (1)) linking the first-order bioaccumulation model (Eq. (T1-1)) and the sequential pulse Cu pattern equation (Eq. (3)), the Cu residence times in the whole body, gills, muscle, liver, and kidney can be estimated (Fig. 2b). The whole body and kidney experience the nearly same Cu residence times of 137 and 134 h, respectively. Liver shows the highest Cu residence time in tilapia of 145 h, indicating that liver plays a principle site to store Cu in response to fluctuating environments. The Cu residence time of target organ followed the order of liver > whole body > kidney > gills > muscle (Fig. 2b).

3.3. Interactions among killing/recovery kinetics, Cu, and pulse interval

We found that as Cu concentration increased, the average killing rate increased, whereas the average killing rate decreased with increasing time of pulse interval (Fig. 3). Eq. (9) provides the best fit for relationships among average killing rate, Cu concentration, dilution rate, and pulse interval of each tissue with all the fitted Hill coefficients revealing ultrasensitivity (r^2 = 0.83–0.99) (Fig. 3).

Overall, k_kC_w50 estimates of the whole body, gill, muscle, liver, and kidney increased with increasing k_0, ranged from 0.932 to 4.192, 0.845–3.243, 0.846–3.237, 0.829–3.117, and 0.840–3.168 mg L^{-1}, respectively. However, $k_k\tau50$s of the whole body, gill, muscle, liver, and kidney increased with decreasing k_0, ranging from 2.401 to 14.888, 3.320–18.851, 3.397–19.149, 3.344–19.510, and 3.425–19.585 h, respectively (supplementary Table S4).

On the other hand, the average recovery rate increases with decreasing Cu concentration, whereas the average killing rate increases with increasing time of dosing interval (Fig. 4). The situations of the relationships between k_rC_w50 and k_0 or between $k_r\tau50$ and k_0 have the similar fashion with those of between k_kC_w50 and k_0 and between $k_k\tau50$ and k_0 (Fig. 4). The estimated k_rC_w50 of the whole body, gill, muscle, liver, and kidney, ranged from 6.625 to 9.196, 6.284–9.716, 6.281–9.780, 6.388–9.942, and 6.223–9.817 mg L^{-1}, respectively, whereas $k_r\tau50$ of the whole body, gill, muscle, liver, and kidney, ranged from 1.584 to 10.383, 1.779–11.653, 1.780–11.682, 1.736–11.527, and 1.806–11.842 h, respectively (supplementary Table S5).

3.4. Hill coefficient and stochastic switch-like behavior

We constructed the relationships among time (t), damage (D), and Hill coefficient (n) by Eq. (7). Before 32 h, there are various kinds of curves, such as positive, negative, and fluctuating in all tissues and whole body (Fig. 5a–c, e–g, i–k, m–o, q–s). However, most tissues present the positive relationships between n and D after 24 h, besides whole body and kidney (Fig. 5d, h, l, p, t). We found that the times approaching steady-state (t_{ss}) with respect to the positive relationships between n and D of whole body, gill, muscle, liver, and kidney are 32, 8, 20, 20, and 32 h, respectively.

Fig. 6 shows the dynamic behaviors of damage with hazard (D_H) varied with the strengths of feedback rate (k_f) ranging from 0 to 1 h^{-1} for tilapia exposed to fluctuating Cu concentrations (Fig. 6a–c). The initial exposure concentrations (C_0) are 2, 3, and 4 mg L^{-1} and the pulse interval (τ) is 72 h. Generally, a non-feedback behavior (k_f = 0) is monostable with a smooth shape curve. We found the D_H–t profile experienced a monostable and a nearly smooth fashion in Fig. 6d, g, and j, with the n less than 4. However, when $n \geq 4$ (Fig. 6e, f, h, i, k–r), as the strength of feedback rate k_f increases, the D_H–t profile presents a bistable fashion or a switch-like behavior, indicating that the positive damage feedback loop generates the ultrasensitivity. We also found that Hill coefficient of n = 4 with respect to gill was the threshold value to trigger the damage feedback mechanism under the same exposure schemes.

We solved Eq. (T1-4) (Table 1) numerically to understand the relationships between environmental Cu stimulus (k_s) and the steady-state response of damage with hazard (D_H) varied with k_f for tilapia in response to fluctuating Cu concentrations. The k_s needed to activate a positive damage feedback for tilapia tissues decreased by increasing of k_f (Fig. 7). Compared with D_H–t profile, we found that the steady-state D_H–k_s profile was more likely to pose the stochastic switch-like response. The results also indicated that the gill-associated Hill coefficient with a threshold value of n = 4 with t_{ss} = 8 h that was necessary to generate the stochastic switch-like behavior.

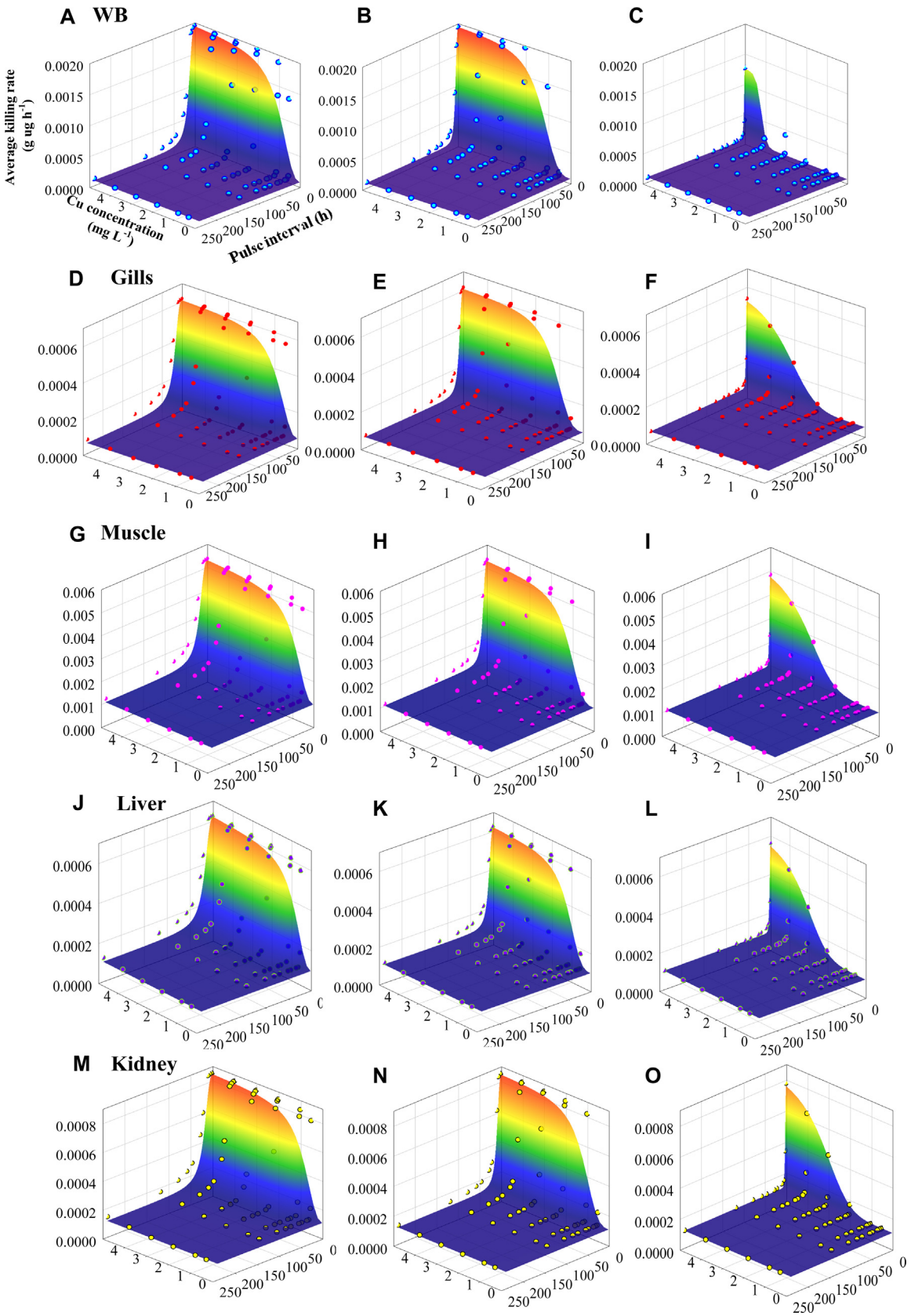

Fig. 3. Response surface describing the relationship among average killing rate, Cu concentration, and pulse interval varied with different dilution rates k_0: 0.05 (first column), 0.1 (second column), and 0.5 h^{-1} (third column) of (A)–(C) whole body, (D)–(F) gills, (G)–(I) muscle, (J)–(L) liver, and (M)–(O) kidney.

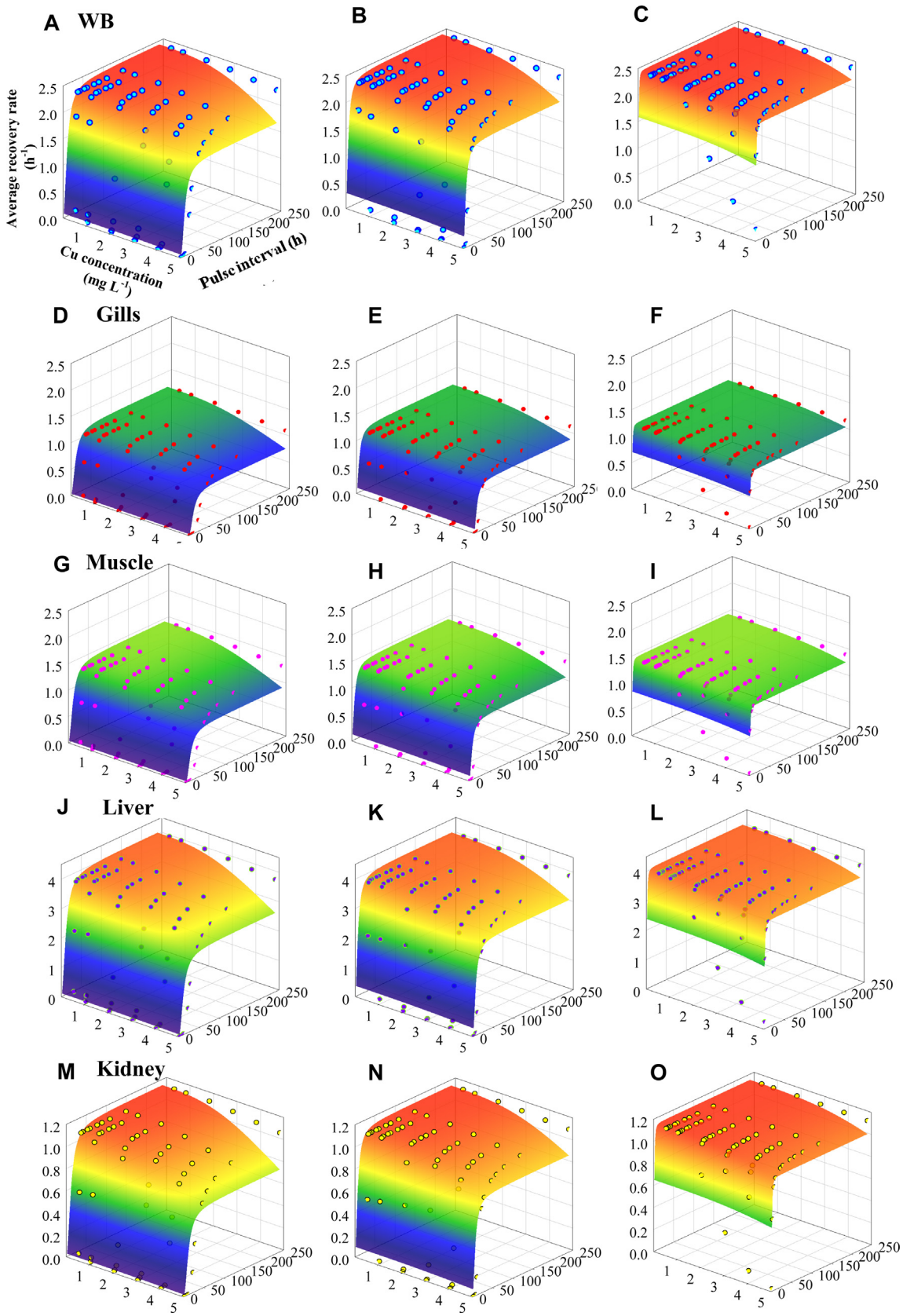

Fig. 4. Response surface describing the relationship among average recovery rate, Cu concentration, and pulse interval varied with different dilution rates k_0: 0.05 (first column), 0.1 (second column), and 0.5 h^{-1} (third column) of (A)–(C) whole body, (D)–(F) gills, (G)–(I) muscle, (J)–(L) liver, and (M)–(O) kidney.

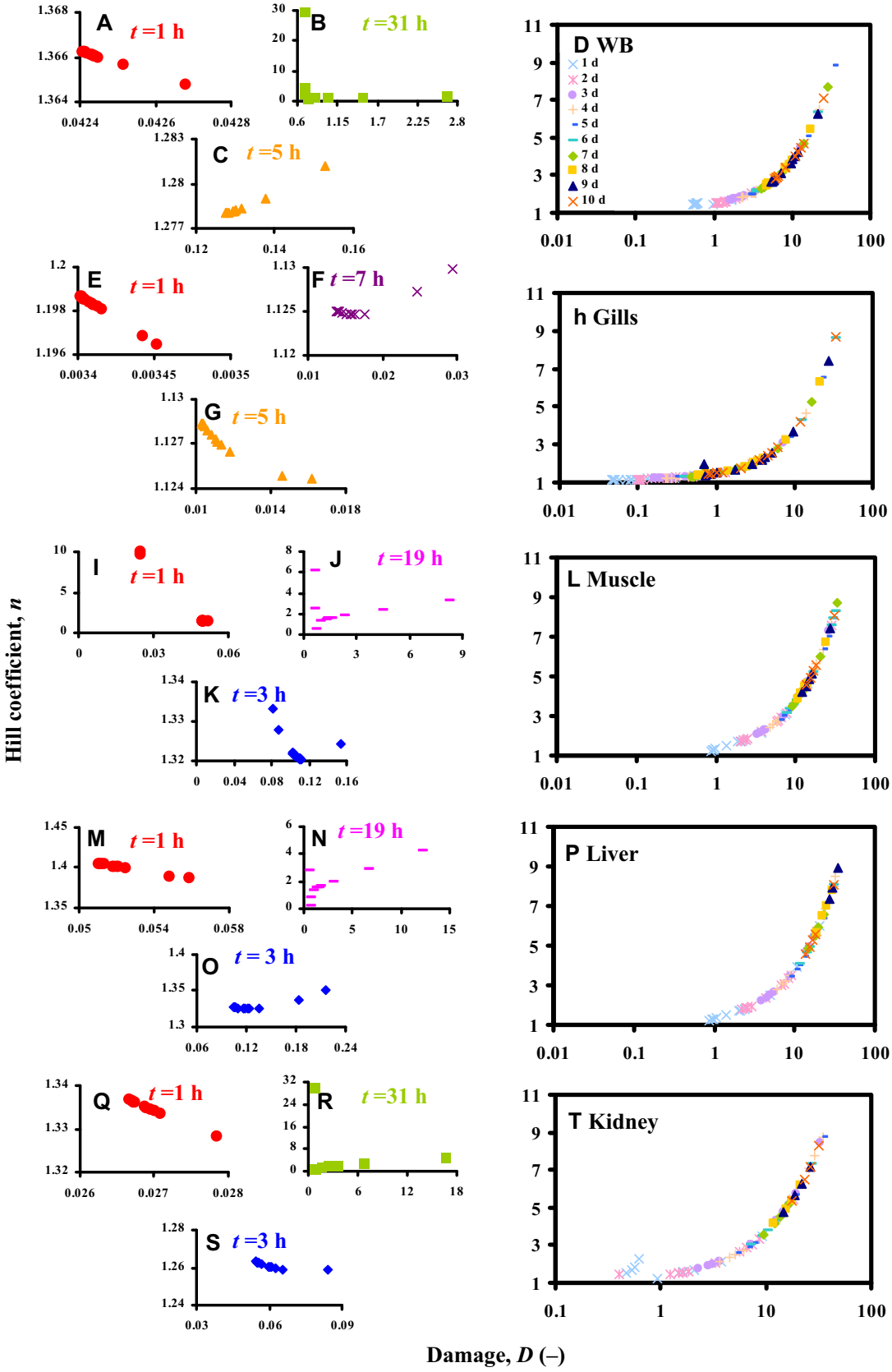

Fig. 5. Relationship among the exposure time (t), damage (D), and Hill coefficient (n) of (A)–(D) whole body, (E)–(H) gills, (I)–(L) muscle, (M)–(P) liver, and (Q)–(T) kidney.

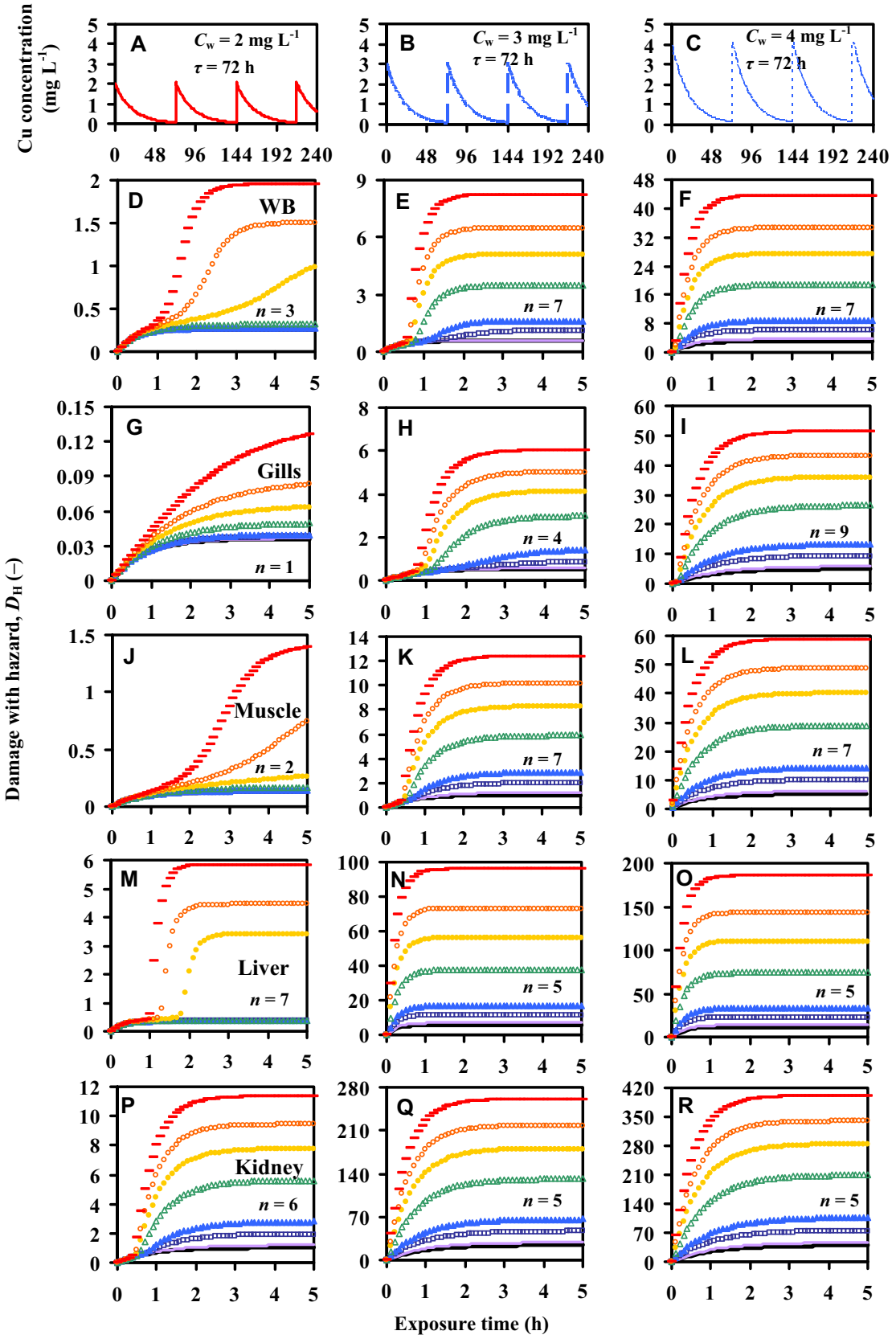

Fig. 6. (A)–(C) Sequential pulse Cu exposure scenarios. Dynamic damage with hazard behaviors varied with strength of feedback (k_f) in tilapia (D)–(F) whole body, (G)–(I) gills, (J)–(L) muscle, (M)–(O) liver, and (P)–(R) kidney. (Symbols for k_f values: —— 0; —— 0.01; □ 0.05; ▲ 0.1; △ 0.3; ● 0.5; ○ 0.7; — 1).

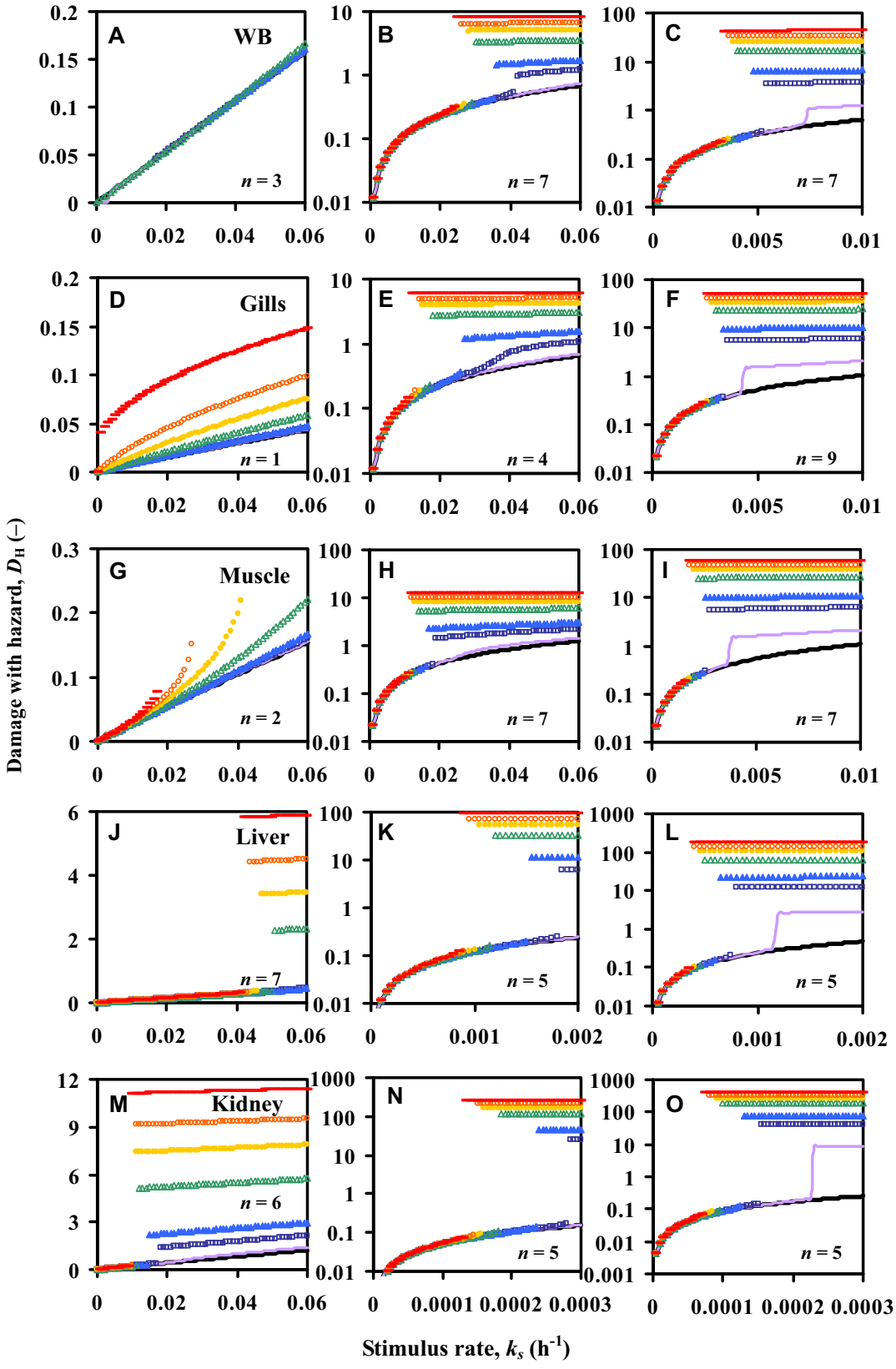

Fig. 7. Relationships between environmental Cu stimulus rate (k_s) and the steady-state response of damage with hazard varied with strength of feedback (k_f) for tilapia exposed to fluctuating Cu concentrations for (A)–(C) whole body, (D)–(F) gills, (G)–(I) muscle, (J)–(L) liver, and (M)–(O) kidney. (Symbols for k_f values: —— 0; —— 0.01; □ 0.05; ▲ 0.1; △ 0.3; ● 0.5; ○ 0.7; — 1).

4. Discussion

4.1. Hill coefficient-based stochastic switch-like signal

In order to account for the reversible–irreversible interactions in aquatic organisms exposed to metal stressors, the deterministic DAM typically takes the damage-recovery dynamics into account, even in the recently developed unified threshold model (Jager et al., 2011). The existing published experimental evidence also supports the capability of DAM on simulating the survival of aquatic organisms exposed to contaminants (Lee et al., 2002; Reinert et al., 2002; Diamond et al., 2006; Ashauer et al., 2007, 2010). 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).

A positive feedback mechanism is widespread in a variety of natural systems, ranging from population to cellular scales (Goutelle et al., 2008; Scheffer et al., 2001; Kim and Gelenbe, 2012; Deris et al., 2013). A growing body of evidence indicates that positive feedback is often associated with uncontrolled processes, leading to alternative stable states (Scheffer et al., 2001; Staver et al., 2011; Feng et al., 2014).

Biologically, ultrasensitivity is used to describe a system that is more sensitive to environmental stimulus and can be characterized by a Hill function (Koshland et al., 1982; Hinze et al., 2007; Goutelle et al., 2008; Kim and Gelenbe, 2012; Feng et al., 2014). A form of ultrasensitivity can also be used to explain the stochastic switch-like behavior (Sprinzak et al., 2010; Kim and Gelenbe, 2012).

Generally, not all terms or parameters in an ecotoxicology model are known or observable directly, and the abundances of all the key state variables cannot be measured simultaneously and continuously. It is often difficult, if not impossible, to perform all of the necessary experiments to measure missing parameters directly. Frequently, these measurements are themselves subject to considerable uncertainty, or are only possible to make under very different conditions. Thus, models are tools to reveal mechanisms that cannot be directly observed. On the other hand, the mathematical and computational modeling of biological systems allows the exploration of hypotheses, if it used and interpreted appropriately with attention paid to inherent uncertainties. The model parameter of damage feedback k_f is assumed to reflect the chemical exposure blocked enzyme induced cytotoxic in aquatic organisms (Chen et al., 2012a,b). The abnormal cell-cycle regulation promotes damage dynamics. However, the damage feedback of pulsed copper exposure in aquatic organisms is still unknown. However, the ranged values of k_f are assumed in the model implementation. The different toxicants have typical toxic effect in cell scale with different exposure timing, dose, and period. The uncertainly of damage-recovery dynamics could also cause misestimates by k_f that should be take into account in evaluating the parameter based on pulsed waterborne Cu concentration and exposure timing in the field aquatic environments.

In this study, we used the deterministic nonlinear models for describing the damage-recovery dynamics in fish and produced response surfaces that depict the interactions between killing/recovery rate and Cu pulse interval. We showed that the stochastic switching arose from competition among killing-recovery–Cu-pulse frequency. This competition resulted in an ultrasensitivity appeared in whole body, gills, muscle, liver, and kidney with Hill coefficients $\geq$7, 4, 7, 5, and 5, respectively, at Cu 3 mg L^{-1}, dilution rate 0.05 h^{-1}, and pulse interval 72 h, indicating that a stochastic switch-like response was generated. We suggest that the role of gill-associated Hill coefficient as an early warning signal of the stochastic switch-like response in the fish damage-recovery dynamics in response to pulse metal stressor can serve as a sensitive indicator for risk detection in fluctuating environments (Acar et al., 2008; Drake, 2013).

4.2. Implications to ecotoxicology and information in fluctuating environments

Kussell and Leibler (2005) indicated that organisms in fluctuating environments must constantly adapt their behavior to survive. Acar et al. (2008) argued that stochastic switching behavior in organisms could be seen as a survival strategy in fluctuating environment. Here we show that the stochastic switch-like behavior described by a Hill function can be favored as a sensing probe of the damage-recovery dynamics in freshwater fish when the environment changes infrequently. The optimal switching rates then mimic the statistics of environmental changes (Drake, 2013). In this study, we derive a relation between the killing/recovery rates of the organisms and the information available about its fluctuating environment.

The concept depicted in this study further implies that the conditions that maximally utilize the information capacity of a sensing probe (e.g., a sensory system) representing by the Hill coefficient for the damage-recovery dynamics in freshwater fish should reflect fluctuating environments. This conditions can be computed from controlled laboratory observations, enabling a form of "inverse modeling analysis in ecotoxicology" that is sometimes the only feasible way to gain insight into an organism's fluctuating surroundings (Dowd and Meyer, 2003; Grami et al., 2011). The practical implication of damage-recovery signal model should be collected the exposure pattern of fluctuating Cu concentration in the real systems. By substituting background and pulsed Cu concentrations and pulsed timing into Eq. (3), we could estimate average killing and recovery rates. Finally, the damage dynamics can be estimated, and be substituted into Hill coefficient-based switch-like behavior model. The estimation seems complicated. In the future, the Hill coefficient-based switch-like signal model can be programmable to overcome this circumstance to apply to a biomonitoring system in aquatic environments.

To determine the frequency with which a metal stressor occurs in an organism's fluctuating environment, the population of organisms can be exposed to the metal stressor in an experimental setting. Individual organism responses can be measured at a range of doses, providing essential information that can be used to reveal information about an organism's fluctuating environment. In the real ecological systems, environmental factors such as temperature, salinity, light ranges along with mixture of contaminants of different sources, types, exposure timing, frequency, and concentration affect the responsive toxic level. Actually, this study recommended that future research can focus on the interactions of fluctuating Cu with water characteristics in aquatic organisms, and incorporates into the Hill coefficient-based switch-like signal model. However, this study provides the potential effectiveness of contamination exposure dynamic behavior of ecological systems to the rapid detection of sensitive indicator that can be derived for comprehensive assessment and management in aquatic environments.

To link information theory and inverse ecotoxicology, a conditional probability distribution of $P(response|signal)$ should be provided first from the experimental measurements. It is assumed that an organism is optimized to obtain the most information about the frequency of doses it encounters in its fluctuating environment and can be expressed as a probability density function of $P(signal)$. In the information theory framework (Cover and Thomas, 2006), we may use the Hill-coefficient based stochastic switch-like signal (n) as a proxy input source of information about fluctuating environment and the damage with hazard as an output response (R) to capture the damage-recovery dynamics in organisms in response to

fluctuating stressors. This reflects the dose frequency in the organism's fluctuating environment (Drake, 2013).

Therefore, a communication channel can be used to link an input source of information (i.e., n) to an output R. However, the signal processing may cause noise that includes noisy and incomplete experimental data from input signals ns, leading to an overlap of possible output response R. Thus, the uncertainty for an organism to acquire an accurate information content of fluctuating environment can be induced from the overlapping response to n.

An information process between signal (n) and response (R) can be expressed mathematically as the mutual information (MI) of MI(R; n) (Cover and Thomas, 2006) as,

$$MI(R;n) = \sum_{R,n} P(R,n)\log\frac{P(R,n)}{P(R)P(n)} = -\sum_{R} P(R)\log P(R) - \left[-\sum_{R,n} P(R,n)\log P(R|n)\right] = H(R) - H(R|n), \quad (11)$$

where $P(R,\ n)$ is a joint probability function determining the marginal probability functions $P(R)$ and $P(n)$ and can be expressed as $P(R,\ n) = P(n) \times P(R|n)$ in that $P(R|n)$ is a conditional response distribution, $H(R)$ is the Shannon entropy of a random variable R with a probability mass function $P(R)$, and $H(R|n)$ is the conditional entropy for a conditional response probability $P(R|n)$.

The Shannon entropy $H(R)$ can be used to measure information content of the fluctuating environment, measuring how unpredictable is the different environmental transitions in the time sequence considered (Kussell and Leibler, 2005). In particular, $H(R)$ can also be used to measure inherent uncertainties of fluctuating environments (Cover and Thomas, 2006). The plausible connection between the fields of ecotoxicology and information theory in handling environmental stochasticity is worthwhile studying in the future.

5. Conclusions

We present an analytical model appraised with the experimental data to show that the integrated ecotoxicology approach can be used to reveal a stochastic switch-like response in the fish damage-recovery dynamics in response to fluctuating metal stressors. We also showed implicitly that Hill coefficient-based switch-like signal and its damage with hazard response can be linked in an information theoretic framework to handle environmental stochasticity in the fields of ecotoxicology. Furthermore, our model provides detailed insight into the ecophysiological dynamics of Hill coefficient-based stochastic switch-like signal-activated regulation (Acar et al., 2008; Locke et al., 2011). Because the presented experimental and computational tools are applicable to any chemical stressor response, this integrated approach can lead to insights into stochastic pulse regulation for other aquatic organisms.

Conflict of interest

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2016.03.038.

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