Assessing the effects of pulsed waterborne copper toxicity on life-stage tilapia populations

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1. Introduction

Tilapia Oreochromis mossambicus is a commercially important native species with wide farming distribution in the northwestern coastal areas of Taiwan. Tilapia has a high market value to Taiwan’s aquaculture (http://www.fa.gov.tw). Previous study (Liao et al., 2003; Chen et al., 2004) found significant accumulative correlations between farmed tilapia and waterborne metals of arsenic (As), cadmium (Cd), copper (Cu), manganese (Mn), ferrum (Fe), mercury (Hg) and zinc (Zn). Therefore, as waterborne metals are elevated, pollutant-induced changes in the mobility of growth inhibition, decreased reproductive ability, and mortality can occur. Thus the elevated metals have potentially risks on the health of tilapia, resulting in severe economic losses nation-wide due to bans on harvesting of contaminated tilapia and the need for costly monitoring program.

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. The relations between aquatic species exposed to fluctuating or pulsed contaminants and their responses are frequently studied (Meyer et al., 1995; Reinert et al., 2002; Diamond et al., 2006; Ashauer et al., 2007, 2010).

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 Cu and Zn 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 fleas survival exposed to Cu and Zn. Ashauer et al. (2007; 2010) have recently developed a process-based threshold damage model (TDM) based on damage assessment model (DAM) to simulate the survival of aquatic invertebrates after exposure to fluctuating and sequential pulses of pesticides. They suggested that TDM not only are capable of simulating the observed survival ($r^2 \approx 0.77–0.96$) 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 as important as the concentration and exposure duration.

TDM assumes that death occurs when the cumulative damage reaches a critical level (Lee et al., 2002; Ashauer et al., 2007). When initial damage overwhelms damage threshold, the damage is irreversible. Therefore, the recovery rate in TDM is species and metal specific. In a TDM scheme, 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. TDM thus provides a more comprehensive framework to...
investigate the time-dependent toxicity of chemical incorporating the co-influence of chemical accumulation and damage accumulation.

It has been recognized that biotic ligand model (BLM) can be used to predict metal bioavailability and toxicity in aquatic environments based on site-specific water chemistry parameters (Di Toro et al., 2001; Paquin et al., 2002a; Niyogi and Wood, 2004). Specifically, BLM quantifies the metal bioavailability and capacity of the gills (biotic ligand) of aquatic organisms to bind metals and relates this binding to acute toxicities (Niyogi and Wood, 2004; Slav chokeva and Wilkinson, 2005). Recently, the implications for risk assessment of BLM quantitatively on metal uptake by aquatic organisms.

In Taiwan, copper sulfate (CuSO4) had been widely used to exterminate phytoplankton for controlling skin lesion of fish in tilapia cultured ponds (Carbonell and Tarazona, 1993; Chen and Lin, 2001; Chen et al., 2006). Acute Cu toxicity is associated with inhibition of sites involved in active Na\(^+\) uptake at the gills, resulting in death from failure of NaCl homeostasis (Paquin et al., 2002b). Water chemistry and associated Cu speciation can greatly affect Cu toxicity. Naturally occurring cations (e.g., Na\(^+\)) can offer protection by competing with Cu\(^{2+}\) for binding sites on the gill, whereas naturally occurring anions can bind Cu\(^{2+}\), rendering it poorly available to gill sites (Di Toro et al., 2001; Paquin et al., 2002a; Niyogi and Wood, 2004).

Most toxicity tests are conducted in laboratories with constant exposure settings. Less is known about toxicological effects of metal stressors pulse moving through free-flowing runoff into cultured ponds, where farmed species and exposure conditions are more dynamic. Moreover, the impact of environmentally pulsed metal exposure to aquatic organisms is, however, still poorly understood experimentally. The purpose of this study was twofold: (1) to conduct 10-day exposure experiments to obtain toxicokinetic parameters for larva, juvenile, and adult tilapia exposed to pulsed waterborne Cu and (2) to link TDM and BLM to assess the survival probability for life-stage tilapia populations in response to pulsed Cu exposure. A mechanistic model based on cation-dependent toxicokinetics and stage-specific toxicodynamics was constructed to serve as a bridge between the experimentally measured pulsed responses and the predictions of survival probability.

2. Materials and methods

2.1. Pulsed exposure experiments

The sequential pulsed Cu exposure experiments were conducted to examine the accumulation abilities for life-stage tilapia O. mossambicus populations. Tilapia of 2-week old larva, 1-month old juvenile, and 8-month old mature adult with mean length 1.01 ± 0.24 cm (mean ± SD), 2.03 ± 0.38 cm, and 12 ± 1.44 cm and mean body biomass 8.24 ± 1.58 mg wet wt., 25.20 ± 15.10 mg wet wt., and 26.88 ± 9.29 g wet wt., respectively, were used as the study animals, respectively. Water source was tap water that has been dechlorinated before conducting the exposure experiments.

During the acclimation periods, fish were fed twice per day with commercial fish food. The exposure experiment was carried out with 42 adults under static conditions in three aquariums of 81 L volume filled with 70.2 L of exposure solution. Each aquarium was containing a stock density of 14 fish. For the exposure experiments of larva and juvenile stages, there were 42 larvae and 42 juveniles, respectively, hatched per glass tank (aquaria measuring 27 × 21 × 21 cm\(^2\)), containing 9 L of water in static conditions.

The copper sulfate (CuSO\(_4\)·5H\(_2\)O) stock solution was prepared with double-deionized water. The sequential pulsed Cu exposure assay was carried out with a 10-day exposure period exposed to Cu concentrations by increasing 1X to 3X mg L\(^{-1}\), i.e., from 30 to 90 mg L\(^{-1}\) for larvae and juveniles, and from 100 to 300 mg L\(^{-1}\) for adults. The sequential pulsed Cu exposure design was accomplished by siphoning the volume of Cu contaminated water in the test aquarium from X L to 1/3 X L, i.e., from 70.2 L to 23.4 L for adults, and filled water to X L in the test aquarium.

After water was siphoned from X L to 1/3 X L, Cu-amended water was filled to X L in the test aquarium to increase the pulsed concentration and vice versa. The pulsed Cu exposure timings were occurred twice during the exposure periods at days 1 and 6, respectively. The pulsed exposure duration was carried out 6 h in each event. The entire Cu solution was replaced and collected daily to avoid the regression of water quality. The feces were removed every 6 h. The forage debris was collected every 1 h after feeding in the aquarium. One adult fish was removed from each experimental tank on days 0, 1, 2, 3, 6, 12, 15, 21, and 30 of exposure. On the other hand, three larvae and three juveniles were respectively removed from experimental tanks. Tested fish were anesthetized with benzocaine hydrochloride solution during the sampling. The fish samples were cleaned with double-deionized water and freeze dried overnight, and then grounded to fine powder in a grinder (Tai-Hsiang Scientific, Taiwan). A 250 mg portion of the powder was digested with 2 ml 65% concentrated HNO\(_3\) and 1 ml 30% H\(_2\)O\(_2\) overnight at 95 °C. The 20 ml fish samples were stored at −4 °C in the dark until they were analyzed.

The flame atomic absorption spectrometer (Perkin Elmer AA-200, USA) were used to analyze fish Cu body burdens and water quality. Analytical quality control of tissue sample was achieved by digesting and analyzing identical amounts of rehydrated (90% H\(_2\)O\(_2\)) standard reference material (dogfish muscle, DORM-2; NRC-CNRC, Canada). The waterborne ions concentrations, such as total Cu, Ca\(^{2+}\), Mg\(^{2+}\), Na\(^+\), and K\(^+\), were analyzed followed the standard methods based on APHA (1998). Standard solutions of ions were used to establish standard curves (Merck, Darmstadt, Germany). The 15 ml water sample with 200 μL HNO\(_3\) were digested 2–3 h at 95 °C, then the water characterizations were determined by Inductively Coupled Plasma Mass Spectrometer (Perkin Elmer ELAN DRC ROMAN II, USA). All samples were analyzed in three times. The recovery rate was 94.6 ± 3.6% and the levels of detection were 20 μg Cu L\(^{-1}\) for water sample and 20 μg Cu g\(^{-1}\) for tissue sample. The experimental materials used in the exposure experiment included glassware and plastic implements. All glassware and plastic materials were cleaned by immersion in 10% Decon for 1 day, then in 25% H\(_2\)O\(_2\) for 2 days and finally rinsed with ultrapure water and dried. All experimental processes were performed by ultraclean technique during the sample manipulations.

2.2. Mechanistic models

The process-based TDM can be described by four dynamic equations as (Lee et al., 2002; Ashauer et al., 2007),

\[
\frac{dC_{B(t)}}{dt} = k_1 C_{w(t)} - k_2 C_{B(t)},
\]
Given the fitted DL50/k2 estimate, the killing rate constant (k2) can be calculated as k2 = ln2/(DL50/k3) (Lee et al., 2002).

2.3. Data analyses

Toxicokinetic parameters of uptake and elimination rate constants (k1 and k2) can be determined by fitting one-compartment toxicokinetic model (Eq. (1)) to exposure data under pulsed waterborne Cu exposures, Cw(t), as

$$C_w(t) = C_0 + C_1[U(t-t_1)-U(t-t_2)+U(t-t_3)-U(t-t_4)]$$

where C0 is the initial Cu concentration (μg L⁻¹), C1 is the pulsed Cu concentration (μg L⁻¹), and U(t-ti) is the unit step function in which ti to t4 are the pulsed timings at the 24th-, 30th-, 144th-, and 150th-h.

To obtain the stage-specific LC50(t) values, the published mortality data were adopted, respectively, from Wu et al. (2003) for larvae, Nussey et al. (1996) for juveniles, and de Vera and Pocsidio (1998) for adults. The Cu forms used to derived 96-h Cu LC50(t) values were total Cu for juveniles and dissolved Cu for adults. However, Wu et al. (2003) did not provide the form of Cu data for larvae. Here a Hill-based standard 3-parameter sigmoidal dose–response equation was used to fit the published data of stage-specific mortality-Cu concentration to estimate LC50(t) and/or 96-h LC50 values,

$$M(t,C_w) = \frac{M_{\text{MAX}}}{1 + \left(\frac{\text{LC50}(t)}{C_w}\right)^{n}}$$

where M(t, Cw) is the mortality rate depending on time t and Cw (%), MMAX is the maximum mortality (%), and n is the Hill coefficient.

A simple critical body residue (CBR) model was used to estimate LC50(t) values when only 96-h LC50 is available (Tsai et al., 2006),

$$\text{LC50}(t) = \frac{\text{CL50}}{\text{BCF} \left(1 - e^{-k_2 t}\right)}$$

where CL50 = 96-h LC50 × BCF(1−e−k2 t×96h) is the 50% effect of lethal body burden (μg g⁻¹).

TableCurve 2D (Version 5, AISEN Software Inc., Mapleton, OR, USA) was performed to fit the published data to obtain the optimal fitted statistical models. A p-value <0.05 was taken as significant. WHAM (Windermere humic aqueous model) software (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. 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 solve TDM under pulsed Cu exposures.

3. Results

3.1. Stage-specific toxicokinetic parameters and LC50(t) estimates

A toxicokinetic rate equation (Eq. (1)) with a pulsed waterborne Cu concentration function (Eq. (7)) was best-fitted to pulsed exposure data (Fig. 1A, C, E) under pulsed Cu exposure patterns (Fig. 1B, D, F) to obtain stage-specific k1 and k2 (Table 1). The results showed that k1 and k2 estimates were 0.149 L g⁻¹ h⁻¹ and 0.024 h⁻¹ (r² = 0.47, p < 0.05), 0.260 L g⁻¹ h⁻¹ and 0.088 h⁻¹ (r² = 0.51, p < 0.05), and 0.018 L g⁻¹ h⁻¹ and 0.015 h⁻¹ (r² = 0.62, p < 0.005) for larvae, juveniles, and adults, respectively (Table 1). Meanwhile, a highest BCF of 6200 mL g⁻¹ was found in larvae. The BCFS of juveniles and adults...
were estimated to be 3803 and 1208 mL g⁻¹ wet wt, respectively. Thus a relative high Cu accumulation capacity was found in tilapia populations. The elimination half-life in larvae (27.73 h) was higher than in juvenile tilapia (10.14 h), indicating that larvae take a longer time to eliminate Cu in the same pulse exposure situation (Table 1). On the other hand, the elimination half-life of adult was estimated to be 46.18 h.

To obtain LC50(t) values for larvae, we first fitted Hill equation (Eq. (8)) to available 96-h acute toxicity data. The resulted 96-h LC50 estimate was 0.24 ± 0.01 mg L⁻¹ (mean ± se) (see Fig. S1A in Supplementary data). Then, a CBR model (Eq. (9)) was used to calculate LC50(t) values at 12-, 24-, 32-, 48, and 72-h (Table 1). Given the available acute toxicity data covering from 24-h to 96-h, a nonlinear least-squares analysis was performed by fitting Hill model to each data set to obtain LC50(t) estimates for juveniles, resulting a 96 h-LC50 of 2.55 ± 0.03 mg L⁻¹ (Table 1 and Fig. S1A in Supplementary data). The method for larvae was also performed to adults with only 96 h acute toxicity data available (Table 1 and Fig. S1C in Supplementary data). The resulted 96-h LC50 estimate was 0.24 ± 0.01 mg L⁻¹ with permissions, respectively.

### Table 1

<table>
<thead>
<tr>
<th>Toxicokinetic parameter</th>
<th>Larva</th>
<th>Juvenile</th>
<th>Adult</th>
</tr>
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<tbody>
<tr>
<td>k₁ (g⁻¹ h⁻¹)</td>
<td>0.149 ± 0.102</td>
<td>0.260 ± 0.145</td>
<td>0.018 ± 0.009</td>
</tr>
<tr>
<td>k₂ (h⁻¹)</td>
<td>0.024 ± 0.019</td>
<td>0.068 ± 0.042</td>
<td>0.015 ± 0.010</td>
</tr>
<tr>
<td>BCF (mL g⁻¹)</td>
<td>6200</td>
<td>3803</td>
<td>1208</td>
</tr>
<tr>
<td>t₁/₂ (h)</td>
<td>28.88</td>
<td>10.14</td>
<td>46.18</td>
</tr>
</tbody>
</table>

LC50(t) estimate (mg L⁻¹)

| 12-h | 4.86  | NA   | 25.85  |
| 24-h | 4.92  | 3.60 ± 0.53 | 15.57  |
| 32-h | 4.92  | 3.52 ± 0.01 | 12.35  |
| 48-h | 3.32  | 2.96 ± 0.03 | 9.17   |
| 72-h | 2.06  | 2.77 ± 0.04 | 7.13   |
| 96-h | 0.24 ± 0.01 | 2.55 ± 0.03 | 6.23 ± 0.40 |

Supplementary data

**Fig. 1.** Pulsed exposure experiments in the Cu accumulation of (A) larva, (C) juvenile, and (E) adult tilapia exposed to (B, D) 30–90 μg L⁻¹ and (F) 100–300 μg L⁻¹. Error bars are standard deviation from mean (n = 3). Photo credits: Larva, juvenile, and adult tilapia were adopted from LarvalBase, Taiwan e-learning and digital archives program (TEDAP), and Kent Andersson, Sweden, 2003 with permissions, respectively.

### 3.2. BLM-TDM parameters

To obtain time course fraction of the total number of Cu binding sites occupied by Cu at 50% effect (F₄₅₀(t)) for tilapia populations, BLM (Eq. (5)) was used to fit stage-specific LC50(t) estimates in Table 1 (Fig. 2A, C, E). BLM-data sets (Table 2), i.e., water chemistry characteristics and stability constants, that used to calculate binding constants [a] and [b] in Eq. (5) can be obtained and estimated from the literature (see Table S2 in Supplementary data). To account for the uncertainty of logKf in different versions, an optimal fitted lognormal distribution was found (see Table S2 in Supplementary data). The binding constants [a] and [b] were calculated to be 9.01, 4.43, and 5.12 and 1.22 × 10⁹, 1.25 × 10⁸, and 1.36 × 10⁸ M⁻¹ for larvae, juveniles, and adults, respectively. Table 3 lists the best-fitted equations describing stage-specific F₄₅₀(t) with r² = 0.98–0.99.

Given the estimated F₄₅₀(t) values, BLM-based LC50(t) (i.e., LC50-BLM(t)) can be determined followed by Eq. (5). The optimal fits of TDM (Eq. (6)) to stage-specific LC50-BLM(t) data were shown in Fig. 2B, D, F. The stage-specific TDM parameters of 50% mortality damage level (DL50/k₂) and k₁ can then be estimated by incorporating k₂ and BCF values in Table 1 (Table 3). A good quality of fit for TDM was found (Table 2). Table 3 indicates that the estimated k₁s were 10.93, 48, and 17.61 h⁻¹ and the k₂s were calculated to be 0.85, 0.06, 0.05 g⁻¹ h⁻¹ for larvae, juveniles, and adults, respectively.

### 3.3. Process dynamics of TDM

To understand the process dynamics and physiological consequences of these change for tilapia populations exposed to pulsed Cu, simulations of TDM (Eqs. (1)–(4)) were performed (Fig. 3). First, stage-specific damage threshold (D₀₅) has to be determined.
Practically, $D_0$ can be determined by the present fitted TDM-based dose–response model describing survival probability of tilapia versus waterborne Cu concentration based on 96-h dose–response data (see Fig. S1 and Table S1 in Supplementary data). We used $k_1$, $k_2$, $k_3$, and $k_4$ parameters and given exposure waterborne Cu to estimate $D_0$ via TDM by $D_0 = D(t) + d\ln S/dt$. This resulted in $D_0 = 0.842, 0.597 \pm 0.075, and 0.996 \pm 0.489$ for larvae, juveniles, and adults, respectively.

Given the stage-specific pulsed Cu patterns (Fig. 3A, F, K), the dynamics of body burden (Fig. 3B, G, L), damage (Fig. 3, H, M), cumulative hazard (Fig. 3D, I, N), and survival probability (Fig. 3E, J, O) can be obtained by using TDM. Results showed that tilapia survival in response to pulsed Cu exposure with survival time causing 50% effect (ST50) was estimated to be 149.8, 151.2, and 155.9 h, respectively, for larvae, juveniles, and adults (Fig. 3E, J, O).

To understand the relative contributions of pulsed and constant exposures to physiological responses, we estimated three mean survival rates at exposure times of 32nd-, 96th-, and 152nd-hr for each life stage of tilapia (Fig. 4). An apparent difference of survival probability time course between pulsed and constant Cu exposures was found for each life stage in that constant-exposed survival probability can be estimated from Fig. S1 (see Supplementary data) followed $S(t) = 1 - M(t)$ at 96-h (Fig. 4A, B, C). Fig. 4D shows the survival rate changes attributed to pulsed and constant Cu exposures for each life stage.

The juvenile survival rates at constant exposure were higher than those at pulsed exposure over the exposure time course. For larvae and adults, survival rates in the 32nd- and 96-th-h did not change substantially at the pulsed exposure, yet a rapid decreased survival rate was found in the exposure time post the second pulse. Overall, the change in the exposure patterns did change substantially the survival rates for each life stage of tilapia populations.

### Discussion

#### 4.1. Life-stage factor in susceptibility of tilapia to Cu toxicity

In this study, we incorporated BLM into process-based TDM with laboratory-derived toxicokinetic parameters to assess the impact of pulsed Cu toxicity on survival of life-stage tilapia populations. We conducted 10-day pulsed Cu exposure experiments to obtain life-stage-specific toxicokinetic parameters. We have recalculated the
BCF based on Cu concentration instead of Cu activity (Table 1) from one-compartment toxicokinetic model to compare with other studies. We found that the BCFs were 341, 209, and 174 mL g$^{-1}$ for larvae, juveniles, and adults, respectively, indicating that our BCF estimates were higher than those of other studies (Pelgrum et al., 1994; McGeer et al., 2003). Pelgrum et al. (1994) showed that BCF values ranged from 64 to 108 mL g$^{-1}$ with Cu concentrations of 100–400 μg L$^{-1}$ at 96 h exposure for juvenile tilapia.

We showed that the change in the exposure patterns did change substantially the survival rates for each life stage of tilapia. However, the results of simulated situation could not fully describe the inherently different toxic effects in life-stage tilapia populations. This is due in part to the different exposure water chemistry conditions and toxicity responses studied. Differences in life-stage survival rates for each life stage of tilapia populations.

<table>
<thead>
<tr>
<th>Estimated BLM parameter $f_{\text{BCF}}(t)$ and TDM parameters for larva, juvenile, and adult tilapia.</th>
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<tbody>
<tr>
<td><strong>BLM parameter: $f_{\text{BCF}}(t) = a + b \exp(-t/c)$</strong></td>
</tr>
<tr>
<td>Larva</td>
</tr>
<tr>
<td>$a$</td>
</tr>
<tr>
<td>0.74±1.25×10$^{-3}$</td>
</tr>
<tr>
<td>$r^2$</td>
</tr>
<tr>
<td><strong>TDM parameter</strong></td>
</tr>
<tr>
<td>$k_1$ (h$^{-1}$)</td>
</tr>
<tr>
<td>$k_{12}$ (g μg$^{-1}$ h$^{-1}$)</td>
</tr>
<tr>
<td>$k_{21}$ (g μg$^{-1}$ h$^{-1}$)</td>
</tr>
<tr>
<td>$r^2$</td>
</tr>
<tr>
<td>p-value</td>
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</table>

$a$ Mean±se.

$b$ $k_i = \ln(2)/(DL50/k_i)$.

by existing ecotoxicological modeling methods and assays. The present approach provided a window into ecotoxicological scheme and opens the way for understanding how aquaculture species show different responses to pulsed Cu exposures. In the present study, a BLM-based TDM, 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. Thus, the BLM-based TDM containing nonlinearities can predict survival dynamics of aquaculture species under sequential pulsed Cu exposures.

Although the present used models were 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 allometric scaling of energy acquisition and expenditure. More importantly, the relevant biological features captured by the model enabled us to look beyond the behaviors for their internal coping mechanisms (Buchwalter et al., 2008) and led to predictions that can be tested by future experiments. Using these confrontations between theory and experiment, the important linkages between eco-physiology and life-history traits can be found that explain dynamics of organism responses in fluctuation-driven environments.

Owing to direct contact with ambient water, gills are proposed to be the first and most important targets of tilapia exposed to waterborne metals (Kamaruzzaman et al., 2010). Gill regulation is a time-dependent acclimation characterizing by both how quickly the acclimation is activated to prevent further effects and how long the acclimation stays in place during nonstressed conditions to put in action when the next pulse comes (Diamond et al., 2006). In practice, the BLM has been successfully applied to predict both acute and chronic toxicity of metals on aquatic organisms (Schwartz and Vigneault, 2007; Hatano and Shoji, 2010; Ng et al., 2010; Veltman et al., 2010). Given that both physiological parameters of aquatic organisms and geochemistry parameters of ambient water are considered, this approach was of potential utility to develop and refine the ambient water quality criteria (Veltman et al., 2010). Hence, the integration between BLM and toxicokinetics/toxicodynamics 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 exposures (Ashauer et al., 2010).

4.3. Implications for risk assessment

Typical assessment methods do not incorporate pulse timing and sequence, which are critical in assessing realistic situations (Reinert et al., 2002). Our results showed that pulse timing 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.
Ashauer et al. (2007) further pointed out that pulsed toxicity tests together with TDM-based toxicokinetic parameters could be used in risk assessment to improve biomonitoring systems that estimate quantitatively the effects based on real-world exposure patterns.

Temporal variability adds complexity to exposures and adds uncertainties to characterizations of risks from contamination (Luoma and Rainbow, 2008). Efficient and accurate risk assessment is critical in determining the extent to which chemicals pose a risk to the environment and ecological communities. Generally, adult tilapia can be used as bioindicator of metal stressors in environmental systems (Liao et al., 2003; Liao and Ling, 2003; Chen and Liao, 2004; Ling et al., 2005). However, results of our study indicated that adults were much less susceptible to Cu stress than larvae and juveniles with different life-stage variables. Our result was in consistent with the previous studies, indicating that a serious

Fig. 3. Simulation of sequential pulsed Cu exposure of larva, juvenile, and adult tilapia, respectively. (A, F, K) Sequential pulsed Cu ranged from 1.8 to 5.3 µg L⁻¹, 91.8 to 275.4 µg L⁻¹, and 291.4 to 874.28 µg L⁻¹ activities, respectively, to larva, juvenile, adult tilapia. (B, G, K) Body burdens simulations by Eq. (1). (C, H, M) Time course of damage simulations by Eq. (2). (D, I, N) Cumulative hazard simulations by Eq. (3). (E, J, O) Survival probability simulations by Eq. (4).
consequence for living population was found on larva and juvenile stages influenced by chemicals (Kammenga et al., 1996; Ramskov and Forbes, 2008). Especially, the developing larvae have higher susceptibility to Cu than the older stages of tilapia. Therefore, if Cu toxicity is being evaluated with a traditional toxicological approach, such as constant exposure-derived toxicokinetic parameters, these concentrations may be devastating to populations of a species that have much lower Cu susceptibility. Thus, the use of Cu concentrations that are safe for adults or juveniles as aquaculture management criteria for risk assessment may have disastrous consequences for the protection of farmed species.

Our results suggested that such margins might vary substantially among species in different life-stage with widely different life growth traits. One way to improve risk assessment is to compare life-stage-specific variables for species that are most likely to be exposed to a toxicant under temporal variability of exposure. Unfortunately, most data exist only for constant-derived parameters and life-stage-specific parameters were scarcely. Once more data are collected, we can then move from individual response to population responses to toxic disturbances.

Furthermore, susceptibility to toxicants cannot be attributed solely to the toxic properties of a compound and/or the physiology of the exposed organisms and will vary as a function of population growth rates. Therefore, life-stage factor needs to be incorporated into studies of species interactions under different disturbance regimes. The availability of tilapia-specific toxicokinetic data of pulsed Cu toxicity exposure is important because they provide opportunities to design observations to better understand metal exposures and effects (Ashauer et al., 2010). Moreover, by incorporating species sensitivity distribution model describing the variation in sensitivity of species to a particular stressor (Posthuma et al., 2002) into BLM-based TDM should improve the risk assessment process for aquatic organisms exposed to sequential pulses of contaminants with fluctuating concentrations.

**Fig. 4.** Comparisons between pulsed and constant Cu exposure of survival probabilities for (A) larva, (B) juvenile, and (C) adult tilapia. (D) Time-specific 32nd-, 96th, 152nd-hr survival probabilities between pulsed and constant Cu exposure for larva, juvenile, and adult tilapia. The used constant Cu activities were 1.981, 103.8, and 329.5 μg L⁻¹ for larvae, juveniles, and adults.
Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.scitotenv.2011.12.043.

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