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Coupled dynamics of energy budget and population growth of tilapia in response to pulsed waterborne copper

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Abstract The impact of environmentally pulsed metal exposure on population dynamics of aquatic organisms remains poorly understood and highly unpredictable. The purpose of our study was to link a dynamic energy budget model to a toxicokinetic/toxicodynamic (TK/TD). We used the model to investigate tilapia population dynamics in response to pulsed waterborne copper (Cu) assessed with available empirical data. We mechanistically linked the acute and chronic bioassays of pulsed waterborne Cu at the scale of individuals to tilapia populations to capture the interaction between environment and population growth and reproduction. A three-stage matrix population model of larva-juvenile-adult was used to project offspring production through two generations. The estimated median population growth rate (λ) decreased from 1.0419 to 0.9991 under pulsed Cu activities ranging from 1.6 to 2.0 μg L^{-1}. Our results revealed that the influence on λ was predominately due to changes in the adult survival and larval survival and growth functions. We found that pulsed timing has potential impacts on physiological responses and population abundance. Our study indicated that increasing time intervals between first and second pulses decreased mortality and growth inhibition of tilapia populations, indicating that during long pulsed intervals tilapia may have enough time to recover. Our study concluded that the bioenergetics-based matrix population methodology could be employed in a life-cycle toxicity assessment framework to explore the effect of stage-specific mode-of-actions in population response to pulsed contaminants.

Keywords Tilapia · Copper · Population dynamics · Pulsed toxicity · Dynamic energy budget · Ecotoxicology

Introduction

Copper sulfate ($CuSO_4$) had been widely used to exterminate phytoplankton and control skin lesions in tilapia (*Oreochromis mossambicus*) cultured ponds in the Taiwan region (Carbonell and Tarazona 1993; Chen and Lin 2001; Chen et al. 2006). Tilapia is a commercially important farmed product and has a high market value to Taiwan's aquaculture (Fisheries Agency 2011) with wide farming distribution in the northwestern coastal areas of Taiwan.

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. 2002). 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 (Ng et al. 2010). Therefore, as waterborne metals are elevated, pollutants can inhibit growth, decrease reproductive ability and increase mortality potentially risking the health of tilapia. Decreases in tilapia health could result in severe economic losses nation-widely due to bans on harvesting of contaminated tilapia and the need for costly monitoring programmes.

It is recognized that acute tests of toxicity in the laboratory are insufficient for ecotoxicological risk assessment

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(Kimball and Levin 1985). On the other hand, tests of chronic exposure are needed to fully understand impacts of metal stressors in the aquatic environments (Luoma and Rainbow 2008). 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 (Chen et al. 2010). Moreover, the impact of environmentally pulsed metal exposure to aquatic organisms is still poorly understood.

Diamond et al. (2006) indicated that the effects of pulsed exposures of Cu and Zn on aquatic organisms rely on the frequency, magnitude, duration of pulses, and the recovery period between events, suggesting a need to incorporate pulsed frequency into regulatory decision-making. Ashauer et al. (2007) also implicated that the sequence where organisms are exposed to chemicals could matter just as much as the concentration and exposure duration.

To understand how Cu toxicity affects tilapia populations requires models at the population level that integrate energetic, growth, reproduction, and bioaccumulation. The amount of metal transfer depends on the energetic status and metal burden that depends on the environmental conditions experienced and the consequential energy acquisition and utilization (including reproduction). Thus energy budget and metal exposure dynamics are inherently linked.

The concept of dynamic energy budget (DEB) has been extensively employed to determine the growth of organisms and the productivity of ecosystems and further extended to explore the life-cycle toxicity assessment (Kooijman and Bedaux 1996; Beyers et al. 1999; Jager et al. 2004; Nichols et al. 2004; Klok et al. 2007; Ducrot et al. 2007; Klanjscek et al. 2007). Fish constantly consume energy to maintain life and offset effects of multiple stressors such as daily fluctuations of water temperature, availability of food, and pollutants in the environment (Wedemeyer et al. 1984). Barata and Baird (2000) suggested that the ecotoxicological modes of action of different chemicals can be determined bioenergetically by studying sublethal effects on food acquisition and hence on growth and reproduction rate.

The purpose of our study was to link a DEB model to a toxicokinetic/toxicodynamic (TK/TD) model to investigate the tilapia population dynamics in response to pulsed waterborne Cu. We mechanistically linked the acute and chronic bioassays of pulsed waterborne Cu at the scale of individual to tilapia populations to capture the interaction between environment and population growth and reproduction. Our analysis spanned a timescale ranging from several days to several months. We explored several pulsed exposure scenarios designed to reflect the transport of Cu to tilapia populations. These included single and multiple exposures to Cu pulses with a longer-term exposure.

Our study was based on the available empirical data that were drawn from controlled laboratory studies of acute pulsed Cu bioassays, Cu inhibition and recovery, growth and survival, and studies linking body mass to fecundity. Our study can also highlight important differences in the population-scale impacts of pulsed waterborne Cu that are attributable to differences in the rates at which juvenile tilapia recover growth function. This proposed DEB-based population-level model provided an understanding of the joint dynamics of body mass and population growth of tilapia in response to pulsed waterborne Cu.

Materials and methods

A flowchart illustrating the key steps in our study is shown in Fig. 1. The relationships used to link each steps are described in the subsequent sections.

Mechanistic models

To develop the stage-structured population growth model of tilapia exposed to pulsed waterborne Cu, a three-stage matrix model of larvae-juveniles-adults was used to project offspring production through two generations based on the body mass as suggested by Taiwan Fisheries Research Institute. The duration of each life stage of tilapia is estimated to be 1.5, 1, and 4 months, respectively, yielding a life span of 6.5 months.

Based on the stage-specific survival and life stage transition rates and fecundity through juveniles and adults, a three-stage matrix population growth model can be constructed and expressed by a matrix population model (MPM) (Fig. 2) (Caswell 2001),

$$\begin{Bmatrix} N_1(t+1) \\ N_2(t+1) \\ N_3(t+1) \end{Bmatrix} = [A] \begin{Bmatrix} N_1(t) \\ N_2(t) \\ N_3(t) \end{Bmatrix} = \begin{bmatrix} P_1 & 0 & F_3 \\ G_1 & P_2 & 0 \\ 0 & G_2 & P_3 \end{bmatrix} \begin{Bmatrix} N_1(t) \\ N_2(t) \\ N_3(t) \end{Bmatrix}, \quad (1)$$

where $N_i(t)$ is the number of tilapia in stage i at time t, matrix $[A]$ is a population projection matrix, P_i is the probability of surviving and staying in stage i, G_i is the probability of surviving and growing from stage i to stage $i + 1$, and F_i is the per capita fertility of stage i within each projection interval. P_i, G_i, and F_i are also referred to as the life-cycle parameters or transition probabilities. To manipulate the simulation of bioenergetics-based stage-structured population growth model, a projection interval of 1 day was used.

Matrix $[A]$ can be used to estimate the asymptotic population growth rate (λ) by the dominant eigenvalue of

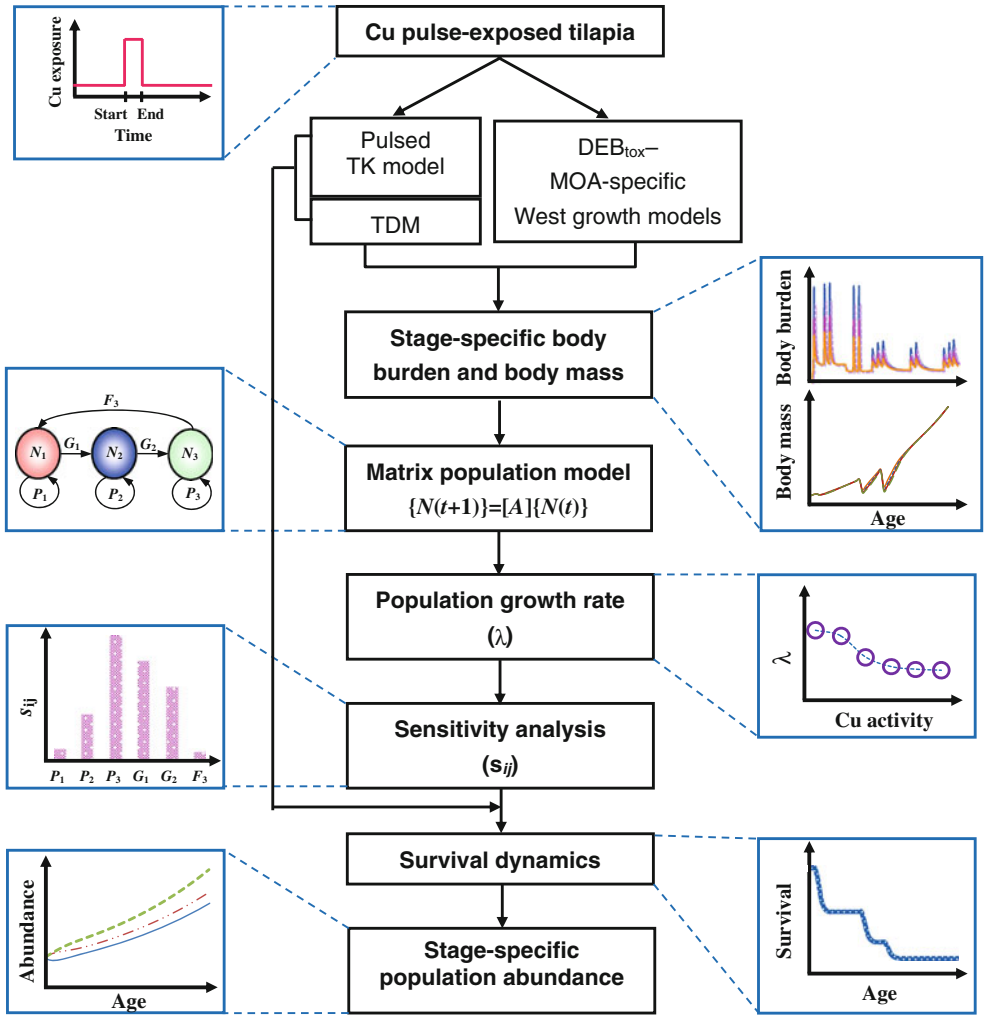

Fig. 1 A flowchart and mathematical models used in our study (see text for the symbol descriptions)

[*A*], reflecting the temporal trend in population abundance (Caswell 2001). When λ exceeds 1.00, the population is projected to increase over time, whereas the population is projected to decline when λ is <1.00. Table 1 summarizes the essential mathematical equations of the life-cycle parameters of P_i, G_i, and F_i.

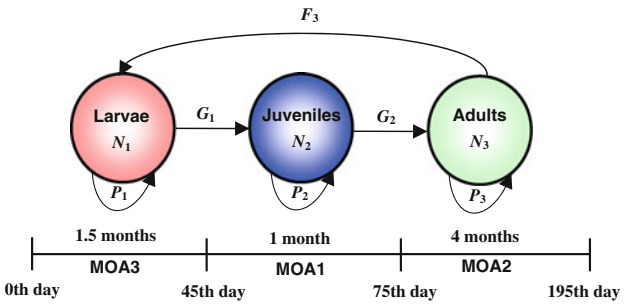

Fig. 2 A three-stage life-cycle graph of an individual tilapia *Oreochromis mossanbicus*. The symbol meanings are given in the text

Ashauer et al. (2007) modified the damage assessment model to develop a process-based threshold damage model (TDM) that laid the foundations for predicting survival of aquatic organisms after exposed to sequential pulsed and fluctuating patterns of contaminants. The primary focus of the out study was 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 Cu activity (the input), the internal damage (the bioaccumulation), and the hazard (the output). Table 2 lists the equations for pulsed TK and TD models.

First, the first-order bioaccumulation model can be used to predict the body burden followed the exposure to sequential pulsed Cu activities (Eqs. (T2-1) and (T2-2)). Second, the time-dependent cumulative damage can be derived from the first-order damage accumulation model (Eq. (T2-3)). When a threshold for damage is exceeded, the

Table 1 Mathematical expression of transition probabilities in population projection matrix [*A*]

Probability of surviving and staying in stage i	
$P_i = \sigma_i(1-\gamma_i)$	(T1-1)[a]
Probability of surviving and growing from stage i to $i+1$	
$G_i = \sigma_i \times \gamma_i$	(T1-2)[a]
Fertility of stage i within each projection interval	
$F_3 = f_e \times E \times F_m$	(T1-3)[b]
where	
$\sigma_i = \frac{S_0(t+T)}{S_0(t)\times T} \times S_i(t)$	(T1-4)
$\gamma_i = \frac{\ln(W(t+\omega))-\ln(W(t))}{\omega}$	(T1-5)[c]
$f_{ei} = \frac{dF_{ei}(t)}{dt}$	(T1-6)
$F_{ei}(W(t)) = m[W(t)]^d$	(T1-7)[d]
$S_0(t) = \left(\frac{1-at}{1+bt}\right)^k$	(T1-8)[e]

Symbol meanings: σ_i and γ_i are the probabilities of stage-specific vital rates of survival and growth (d^{-1}), respectively, f_e is the number of eggs per mature female per unit time in stage i (d^{-1}), E is the egg eclosion rate (–), F_m is the proportion of mature female in stage i (–), $S_0(t)$ is the intrinsic survival probability of tilapia at time t (–), T is the projection interval (d), $S_i(t)$ is the stage-specific survival probability at time t (–), $W(t)$ is the body mass at time t (g), ω is the duration in stage i (d), $F_e(W(t))$ is the mass-dependent fecundity of tilapia at time t (egg female^{-1}), m and d are the fitted coefficients, a is the inverse proportion to the life-cycle duration (d^{-1}), b is the biological parameter (d^{-1}), and k is the fitted coefficient

[a] Adopted from Caswell (2001)

[b] Adopted from Simas et al. (2001)

[c] Adopted from Solomon and Boro (2010)

[d] Adopted from Blanchard et al. (2003)

[e] Adopted from Klok and deRoos (1996)

Table 2 Pulsed toxicokinetic and TDM

Pulsed toxicokinetic model	
$\frac{dC_{bi}}{dt} = k_{1i}\{C_w(t)\} - k_{2i}C_{bi}(t)$	(T2-1)
$\{C_w(t)\} = \{C_0\} + \{C_1\}\left[\sum_{n=1}^{N}(U(t-t_{2n-1}) - U(t-t_{2n}))\right]$	(T2-2)
Threshold damage model	
$\frac{dD_i}{dt} = k_{ki} \times C_{bi}(t) - k_{ri} \times D_i(t)$	(T2-3)
$\frac{dH_i(t)}{dt} = D_i(t) - D_{0i}$	(T2-4)
$S_i(t) = e^{-H_i(t)}$	(T2-5)

Symbol meanings: C_{bi} is the body burden in stage i (μg g^{-1}), k_{1i} is the uptake rate constant in stage i (L g^{-1} d^{-1}), k_{2i} is the elimination rate constant in stage i (d^{-1}), $\{C_w(t)\}$ is the Cu activity (μg L^{-1}), $\{C_0\}$ is the background Cu activity (μg L^{-1}), $\{C_1\}$ is the pulsed Cu activity (μg L^{-1}), U is the unit step function, t is the time (d), t_{2n-1} is the pulsed exposure start (d), t_{2n} is the pulsed exposure end (d), k_{ri} is the damage recovery rate constant in stage i (d^{-1}), k_{ki} is the killing rate constant in stage i (g μg^{-1} d^{-1}), $D_i(t)$ and $H_i(t)$ are the stage-specific damage and cumulative hazard at time t (–), respectively, D_{0i} is the stage-specific threshold of damage (–), and $S_i(t)$ is the stage-specific survival probability at time t (–)

time change of hazard (i.e., hazard rate) rises above zero, indicating the probability of the organisms suffering injury at a give time t (Eq. (T2-4)). Finally, the survival probability can be derived directly from TDM and is given by an exponential function of cumulative hazard (Eq. (T2-5)).

In our study, three pulsed exposure scenarios were modeled: (i) high pulse frequency exposure scenario: the pulse starts at days 2, 6, 13, 55, 62, 80, 87, 94, 131, 138, 182, and 188; (ii) median pulse frequency exposure scenario: the pulse starts at days 2, 16, 23, 55, 62, 80, 87, 94, 131, 138, 182, and 188; and (iii) low pulse frequency exposure scenario: the pulse starts at days 2, 30, 37, 55, 62, 80, 87, 94, 131, 138, 182, and 188. The pulsed duration for all pulse scenarios is 1 day.

The killing rate constant in Eq. (T2-3) is the proportionality factor describing the relation between the cumulative damage and hazard, whereas the recovery rate constant characterizes all processes leading to recovery such as repair

mechanisms on a cellular scale or adaptation of the physiology and other compensating processes (Ashauer et al. 2007). Thus, the recovery rate constant can be used to estimate the recovery time [i.e., recovery time = 1/(recovery rate constant)] of the organisms from internal damage. However, this study only considered that the estimates of killing and recovery rate constants are dependent on repair capacity for specific life stage.

West et al. (2001) developed a mechanistic model to describe ontogenetic growth trajectories of organisms (referred to as the West growth model) instead of the conventional growth model based on the biometric approach. The West growth model is a general quantitative model based on fundamental principles for the utilization of the consumed energy between maintenance of existing tissue and the reproduction of new biomass that has described the growth of many diverse species successfully (West and Brown 2004). This model characterizes the slowing of growth as the body size increases to limitations on the capacity to supply sufficient resources to support further increase in body mass. We adopted the West growth model as the tilapia growth model under nonexposed conditions (Table 3, Eqs. (T3-1) and (T3-2)).

On the basis of DEB_{tox} theory (Kooijman and Bedaux 1996; Beyers et al. 1999; Jager et al. 2004), this study assumed that only one mode of action on Cu growth inhibition in tilapia occurred at the same time, whereas the major mode action in each life-stage was different. Therefore, three modes of toxic action on the Cu growth inhibition of tilapia population model can be distinguished (Fig. 2): (i) decrease assimilation (feeding) (MOA1) on juvenile stage, (ii) increase the maintenance costs (MOA2) on adults stage, and (iii) increase the cost of growth (MOA3) on larval stage. Table 3 summarizes the bioenergetic models to describe MOA1, MOA2, and MOA3 for juvenile, adult, and larval stages, respectively (Eqs. (T3-4), (T3-5), (T3-6), (T3-7), (T3-8)).

Model parameterization

All parameters including point values and maximum likelihood estimates used to calculate the vital rates of individual tilapia were summarized in Table 4. The experiments carried out by Liao et al. (2006) and Chen et al. (2012) were adopted to represent the actual culture operations and management of tilapia farms. Therefore, all parameters listed in Table 4 are obtained under comparable experimental conditions. This study chose the survival rate of age 23rd, 60th, and 135th days old as the σ estimates of larva, juvenile, and adult stages. Then, we considered growth periods of 1, 1.5, and 4 months of each life stage to estimate γ. This study used the daily fecundity at age 135th days old to determine the F_3 estimate. Caswell (2001) pointed out that the initial condition has no influence on the stable age distributions as well as population growth rate. Therefore, the initial number of tilapia of each stage (N_1, N_2, N_3) was arbitrarily assumed to be 10, 10 and 10, respectively, yielding an initial population density of 30 individuals per unit area.

Sensitivity and uncertainty analyses

The influence of each population matrix element, a_{ij}, on λ was assessed by calculating the sensitivity values for the transition matrix [A]. The sensitivity of matrix element a_{ij} equals to the rate of change in λ with respect to a_{ij}, defined by $\delta\lambda/\delta a_{ij}$ as (Caswell 2001),

$$[S] = \frac{\partial \lambda}{\partial a_{ij}} = \frac{v_i \times w_j^T}{v \times w} = \begin{bmatrix} s_{11} & s_{12} & s_{13} \\ s_{21} & s_{22} & s_{23} \\ s_{31} & s_{32} & s_{33} \end{bmatrix}, \quad (2)$$

where [s] is the sensitivity matrix that can be used to estimate the changed amount of population growth rate when life-cycle parameter (P_i, G_i, and F_3) were changed, w is the right eigenvector, v is the left eigenvector, w_j^T is the transpose of w_j, and s_{ij} is the sensitivity coefficient with respective to a_{ij}.

Uncertainty arises from estimation of both exposure and adverse effects. To quantify this uncertainty and its impact on the estimation of population growth rate, a Monte Carlo simulation was performed including input distributions for the parameters of the vital rates of tilapia population. Largely because of limitations in the data used to derive model parameters, inputs were assumed to be independent. The result shows that 10,000 iterations are sufficient to ensure the stability of results. The 95 % confidence interval (CI) is defined as the 2.5 and 97.5th percentiles obtained from the Monte Carlo simulation. Model simulations and the determinations of asymptotic population growth rate under different scenarios were performed using the MATLAB® software (The Mathworks Inc., MA, USA). The Monte Carlo simulation and sensitivity analysis were implemented using Crystal Ball® software (Version 2000.2, Decisioneering, Inc., Colorado, USA).

Results

Pulsed Cu impacts on body burden and mass

Individual-based outcomes of body burden and body mass for constant and all pulsed Cu exposure scenarios were shown in Fig. 3. The TK model incorporated with the West growth model at stage-specific MOA and stress function (Tables 2, 3) can be used to calculate stage-specific body burden and body mass.

Table 3 West growth and bioenergetics models

West growth model[a]

$$W(t) = W_{\max 0}\left\{1 - \left[1 - \left(\frac{W_{b0}}{W_{\max 0}}\right)^{1/4}\right] \times e^{-A_0 t/4W_{\max 0}^{1/4}}\right\}^4 \quad \text{(T3-1)}^{b}$$

$$A_0 \equiv B_0 m_c E_{c0}^{-1} \quad \text{(T3-2)}^{b}$$

Stress function[b]

$$s_i(t) = [C_{bi}(t) - \text{IEC1}_i]/\text{IEC10}_i \quad \text{(T3-3)}^{c}$$

MOA1 for juvenile stage[c]

$$W(t) = W_{\max}\left\{1 - \left[1 - \left(\frac{W_{b0}}{W_{\max}}\right)^{1/4}\right] \times e^{-A_0 t/4W_{\max}^{1/4}}\right\}^4 \quad \text{(T3-4)}^{d}$$

$$W_{\max} = W_{\max 0}[1 - s_i(t)] \quad \text{(T3-5)}^{d}$$

MOA2 for adult stage[c]

$$W(t) = W_{\max 0}\left\{1 - \left[1 - \left(\frac{W_{b0}}{W_{\max 0}}\right)^{1/4}\right] \times e^{-A_0 t/4W_{\max 0}^{1/4}}\right\}^4 \times [1 + s_i(t)]^{-1} \quad \text{(T3-6)}^{d}$$

MOA3 for larval stage[c]

$$W(t) = W_{\max 0}\left\{1 - \left[1 - \left(\frac{W_{b0}}{W_{\max 0}}\right)^{1/4}\right] \times e^{-At/4W_{\max 0}^{1/4}}\right\}^4 \quad \text{(T3-7)}^{d}$$

$$A = B_0 m_c\{E_{co}[1 + s_i(t)]\}^{-1} = A_0[1 + s_i(t)]^{-1} \quad \text{(T3-8)}^{d}$$

Symbol meanings: $W(t)$ is the body mass at time t (g), W_{b0} and W_{max0} are the body mass at birth and the maximum body mass in control groups (g), respectively, A_0 is the species-specific growth coefficient in control groups ($g^{1/4}$ d^{-1}), B_0 is the taxon-specific constant (J $g^{-3/4}$ d^{-1}), m_c is the mass of a cell (g), E_{c0} is the metabolic energy required to create a cell (J), C_{bi} is the body burden in stage i (μg g^{-1}), IEC1_i is the body burden for 1 % growth inhibition as chronic growth inhibition threshold (μg g^{-1}), IEC10_i is the body burden for 10 % growth inhibition as level of toxicity once C_{bi} exceeds IEC1_i (μg g^{-1}), and MOA is the mode of toxic action

[a] Adopted from West et al. (2001)

[b] Adopted from Kooijman and Bedaux (1996)

[c] Adopted from Liao et al. (2006)

In the larval stage, Cu body burden increased rapidly then reached a steady-state, whereas body mass increased monotonically at the constant exposure (Fig. 3a, b). Due in part to a higher elimination rate constant in juvenile stage, Cu body burden decreased nearly 80 % of the larvae over 195 days to adult stage (Fig. 3a). Notably, the constant exposed tilapia had the same growth trajectory for entire life span (Fig. 3c). The time course of the body burden and body mass for Cu pulse-exposed tilapia is depicted in Fig. 3g–l subjected to three different pulsed scenarios (Fig. 3d–f). Generally, different time courses of body burden reflect different recovery periods during the pulse exposure (Fig. 3g, i, k). Of the different pulsed exposure scenarios modeled, the low pulsed frequency exposure scenario produced the largest fluctuation in body mass in larvae stage (Fig. 3h, j, l).

Pulsed Cu impacts on tilapia population

The survival and fertility rates computed for each scenario by the individual-based model were used to parameterize the population model. The model was then run and outcomes were used to assess potential changes in productivity due to changes in population growth rate (λ). Generally, population growth rates decreased with increasing Cu activities for constant and all pulsed exposure scenarios (Fig. 4a, b). The estimated λ for the unexposed population was 1.0865 (95 % CI: 1.0742–1.0987), reflecting potential population growth.

Our results indicated that median λ estimates decreased from 1.0487 to 0.9998 under constant Cu exposures from 1.6 to 2.0 μg L^{-1} (Fig. 4a). On the other hand, for Cu pulse-exposed tilapia population, the estimated median λs decreased from 1.0085 to 0.9991, 1.0174 to 0.9991, and 1.0419 to 0.9998, respectively, for high, median, and low pulsed frequency exposure scenarios. By comparison, the growth rate of the tilapia population exposed to low pulsed frequency exposure scenario was higher. However, exposure to low pulsed frequency exposure scenario caused the largest reduction in population growth, followed by high and median pulsed frequency exposure scenarios (Fig. 4b).

A sensitivity analysis of the matrix for the unexposed population of tilapia revealed that population growth rate (λ) was most strongly influenced by changes in surviving and growing from juvenile to adult stages ($s_{32} = 0.92$), yet with increasing Cu activities, the influences were shifted to surviving and growing from larval to juvenile stages ($s_{21} = 0.41$–1.19), followed by surviving and staying in adult stage ($s_{33} = 0.45$–0.98) (Fig. 4c, d). For high and median pulsed frequency exposure scenarios, however, λ was most strongly influenced by changes in surviving and

Table 4 Parameter values used to calculate the vital rates of tilapia populations

Parameter	Larval stage	Juvenile stage	Adult stage	Unit
Survival probability				
k_1[b]	3.576 ± 2.448[a]	6.240 ± 3.480	0.432 ± 0.216	L g^{-1} d^{-1}
k_2[b]	0.576 ± 0.456	1.632 ± 1.008	0.360 ± 0.240	d^{-1}
k_k[b]	20.40	1.44	1.20	g μg^{-1} d^{-1}
k_r[b]	262.32 ± 0.744	1152 ± 51.36	422.64 ± 0.336	d^{-1}
D_0[b]	0.842 ± 0.248	0.597 ± 0.075	0.996 ± 0.489	–
a[c]	0.0051			d^{-1}
b[d]	0.076 ± 0.037			d^{-1}
k[d]	0.049 ± 0.011			–
Growth rate				
ET	3[e]	3[f]	7 [g]	d
R_{max}	0.134[e]	1[f]	1	–
n	1.84 ± 0.73[e]	16.36 ± 1.63[f]	4.96 ± 1.83[g]	–
EC50	125 ± 24[e]	166 ± 1[f]	250 ± 19[g]	μg L^{-1}
IEC50	4.54[e]	4.89[f]	10.15[g]	μg g^{-1}
IEC10	1.37[e]	4.28[f]	6.52[g]	μg g^{-1}
IEC1	0.37[e]	3.70[f]	4.02[g]	μg g^{-1}
W_{b0}[h]	0.00054			g wet wt
W_{max0}[i]	2000			g wet wt
A_0[g]	0.03547 ± 0.00017			$g^{1/4}$ d^{-1}
Fertility rate				
m[j]	–		49.20 ± 2.13	–
d[j]	–		0.49 ± 0.01	–
E[c]	–		0.9	–
F_m[c]	–		0.5	–

Symbol meanings: k_1 is the uptake rate constant, k_2 is the elimination rate constant, k_r is the damage recovery rate constant, k_k is the killing rate constant, and D_0 is the threshold of damage, a is the inverse proportion to the life-cycle duration, b is the biological parameter, k is the fitted coefficient, *ET* is the exposure time, R_{max} is the maximum response of growth inhibition, n is the Hill coefficient, EC50 is the copper concentration for 50 % growth inhibition, IECx is the body burden for x % growth inhibition, W_{b0} and W_{max0} are the body mass at birth and the maximum body mass in control groups (g), respectively, A_0 is the species-specific growth coefficient in control groups, m and d are the fitted coefficients, E is the egg eclosion rate, and F_{mi} is the proportion of mature female in stage i

[a] Mean ± SE

[b] Adopted from Chen et al. (2012)

[c] Adopted from Liao et al. (2006)

[d] Estimated from Pathiratne (1999) based on Eq. (T1-8)

[e] Estimated from Wu et al. (2003) based on Hill model

[f] Using the IEC10 and IEC1 in adult stage to estimate the growth rate parameters in juvenile stage by Eqs. (T3-3) and (T3-4)

[g] Estimated from Huang (2010) based on Eqs. (T3-1) and (T3-2)

[h] Estimated from Houde (1992) and Lin et al. (2001)

[i] Adopted from Chotivarnwong (1971)

[j] Estimated from Riedel (1965) based on Eq. (T1-7)

staying in adult stage (mean $s_{33} = 0.95$ and 0.91, respectively), followed by surviving and growing from larval to juvenile stages (mean $s_{21} = 0.47$ and 0.55, respectively) (Fig. 4e, f). A notable finding was that for Cu pulse-exposed tilapia population under low pulsed frequency exposure scenario, survival change in adults (mean $s_{33} = 0.81$) and survival and growth changes in entire life span (mean $s_{ij} = 0.19$–0.68) had relatively strongly influence on population growth rate (Fig. 4g). Taken together, our results revealed that the influence on population growth rate was predominately due to changes in the adult survival and larva survival and growth function.

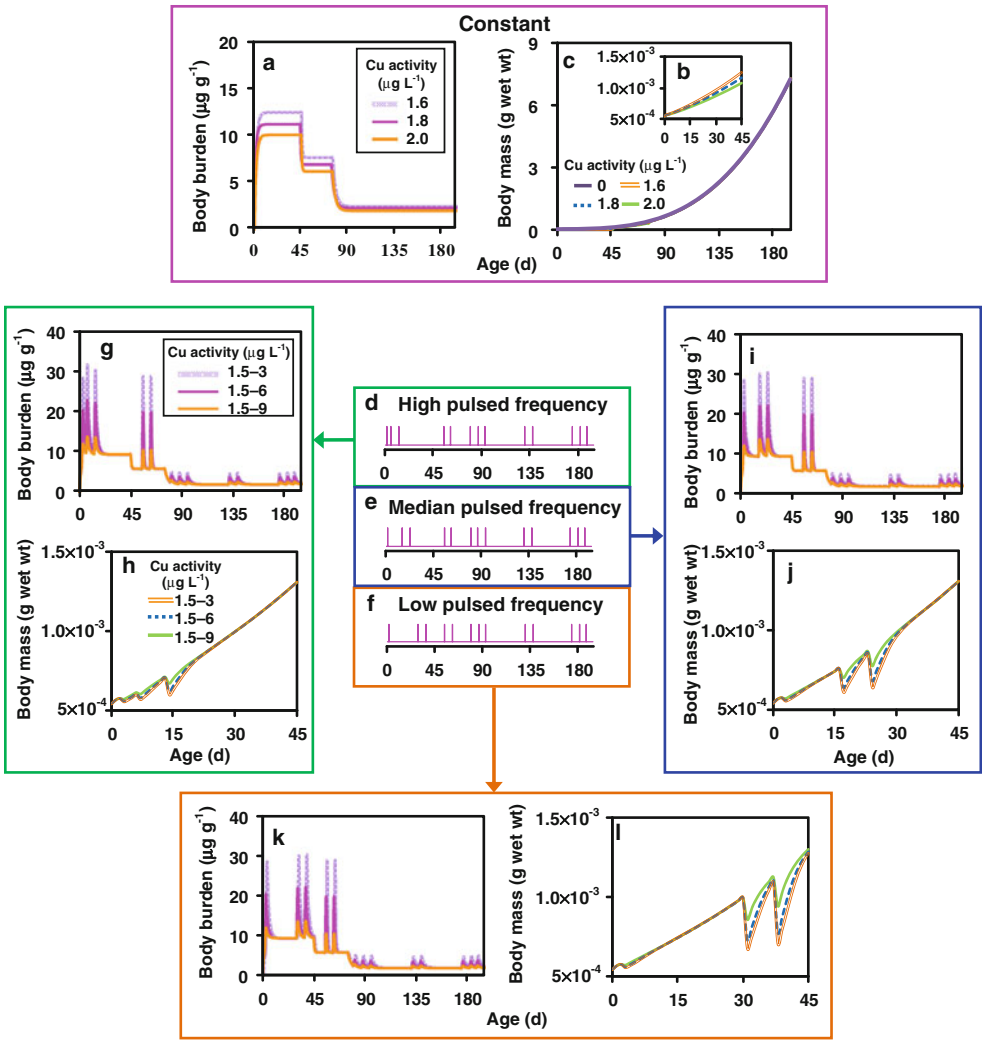

Fig. 3 **a** Simulations of body burdens of tilapia expose to constant Cu activities ranged from 1.6 to 2 μg L^{-1}. Simulations of growth trajectory of tilapia b larval stage and c whole life stage expose to different constant Cu activities. **d–f** The graphs of sequential pulsed. **g**, **i**, **k** Simulations of body burdens of tilapia expose to sequential pulsed Cu activities ranged from 1.5 to 3, 1.5 to 6, and 1.5 to 9 μg L^{-1} under different pulsed scenarios. **h**, **j**, **l** Simulations of growth trajectory of tilapia larval stage expose to different sequential pulsed Cu activities under different pulsed scenarios

Survivorship and population abundance

To understand the contribution of larva survival and growth to population growth, we estimated survival probability over time for larval stage based on Eq. (T2-5) of TDM (Table 2). Our results indicated that significant changes occurred in larva survival dynamics for constant and all pulsed scenarios (Fig. 5). Relative to the constant exposures (mean survival probabilities decreasing from 1 to 0.3), all of the Cu-pulse exposure scenarios showed lower survival probabilities in that the high pulsed frequency exposure scenario produced the largest reduction in survival in larval stage (mean survival probabilities decreasing from 0.70 to 0.09) (Fig. 5). Conversely, exposure to low pulsed frequency exposure scenario produced the smallest reduction (mean survival probabilities decreasing from 0.90 to 0.14). Population abundance of different Cu activities exposure was shown in supplementary materials (Fig. S1).

Discussion

Energy budget and population dynamics in ecotoxicology

Our study provides a novel assessment framework to analyze the mode of action of pulsed metal toxicity to aquatic organisms by incorporating the West growth model and the DEB_{tox} theory into a population stage-structure model. The single stage-specific MOA proposed in our model may not be reliable in higher concentration due in part to the inherent assumption of the DEB_{tox} theory that

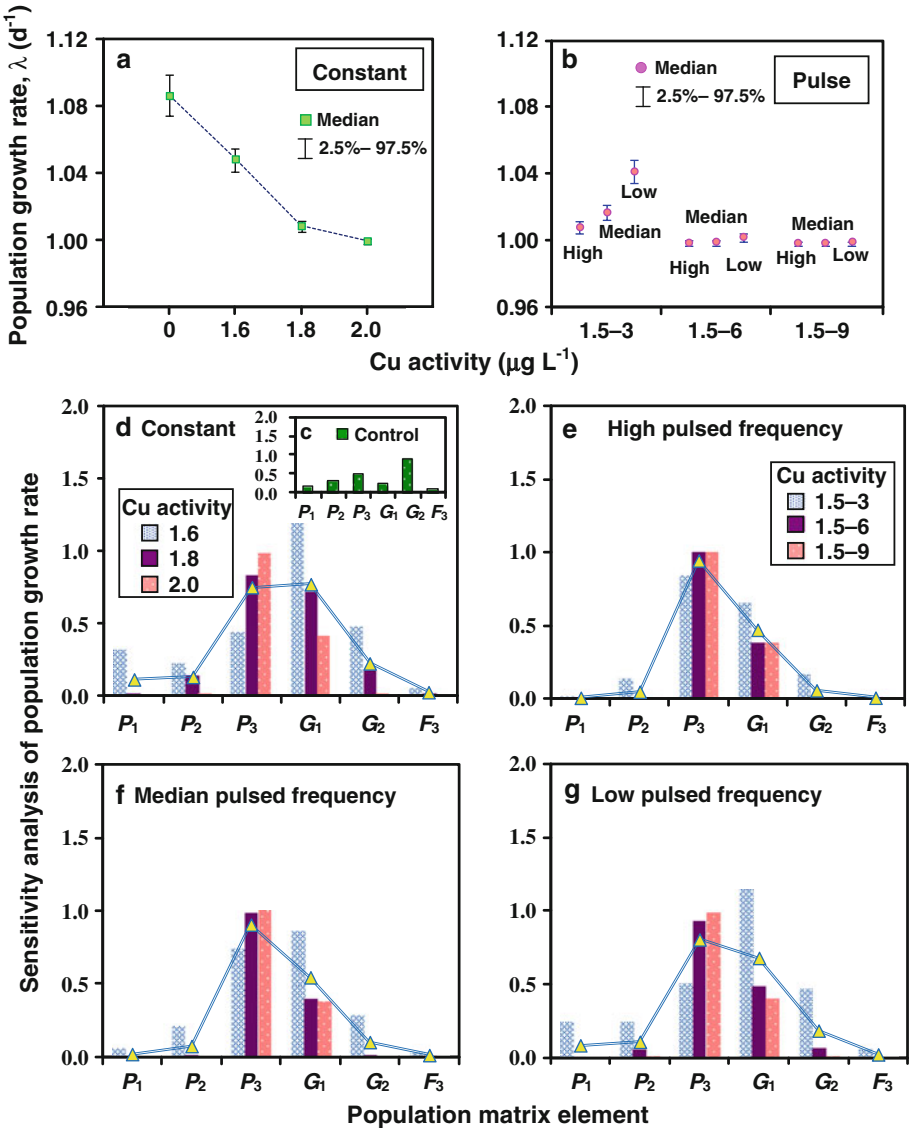

Fig. 4 Effects of increasing waterborne Cu activities on the asymptotic population growth rate of tilapia under a constant and b sequential pulsed exposure scenarios. The sensitivity of the asymptotic population growth rate to the population projection matrix elements for unexposed populations c and varied with Cu activities (μg L^{-1}) of d constant and **e**, **f**, **g** sequential pulsed exposures. (*open triangle* represents the mean sensitivity)

only one of growth effects occurs at the same time in the lower effect range of the chemical (Kooijman and Bedaux 1996). However, by linking the West growth model into the DEB_{tox} theory, population growth rate changing with different Cu activities in pulsed exposure scenarios could be evaluated successfully. Furthermore, we also showed that the population growth rate was predominately affected by the changes of survival condition in the adults and the survival and growth function in larval stages.

The West growth model describes the universal properties of individual growth based on the first principles on the basic of conservation of metabolic energy, the allometric scaling of metabolic rate, and the energetic cost of producing and maintaining biomass. The capability of this model has been validated for quantitatively predicting growth curves from birth to the mature body sizes for all multicellular organisms. This universal growth model provides a basis for understanding the general and fundamental features governing the organism growth. Although some criticisms indicated that the conceptual foundation of this model is not applicable to the growth of birds and their life-history properties (Ricklefs 2003). This model does not intend to account for all of the observed variation in growth rate and life histories but it indeed provide a baseline for developing more detailed treatments of ontogenetic growth (West and Brown 2004).

The species-specific growth coefficient (A_0) relates the rate of energy allocation of producing a cell to the rate of the whole-organism metabolic rate that fuels this biosynthesis in terms of normalization (West and Brown 2004). Our study shows that the values of A_0 changed significantly in different pulsed Cu exposures, indicating pulsed exposures disturbed the energy translations between life-sustenance activities and new biomass production. The growth

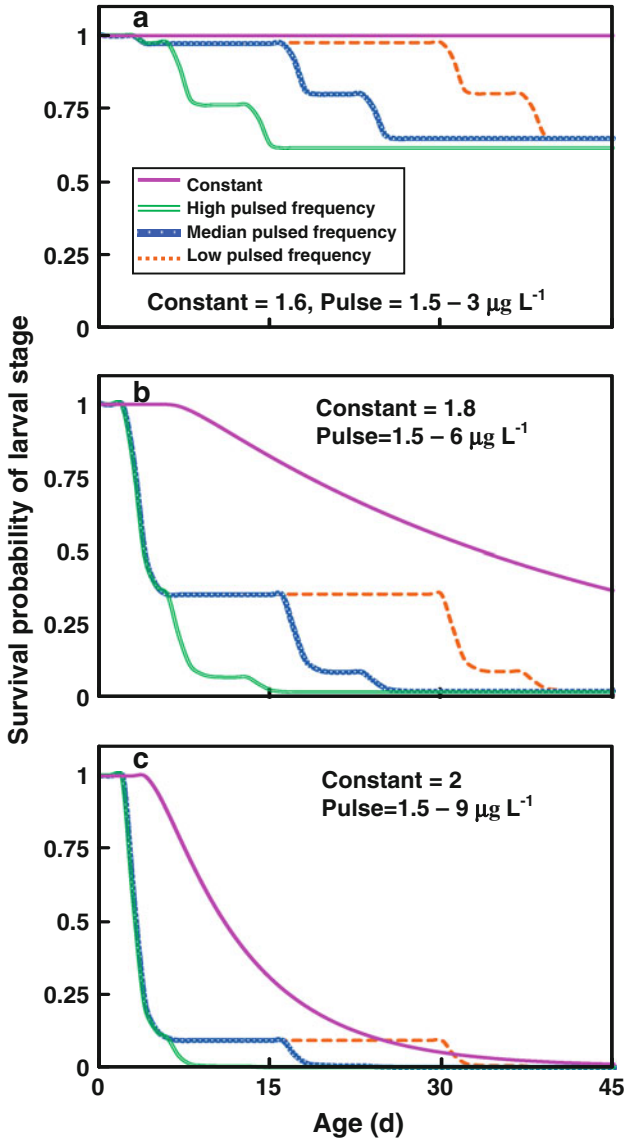

Fig. 5 The survival probabilities of larval stage with exposure to waterborne Cu activities ranged from **a** 1.6 and 1.5 to 3 μg L^{-1}, **b** 1.8 and 1.5–6 μg L^{-1}, and **c** 2 and 1.5–9 μg L^{-1}, respectively

inhibition by pulsed Cu exposures is not induced by increasing the energy cost to propagate new body tissues. The concentration–effect tilapia growth trajectories could be well described by MOA1 in juvenile stage and MOA3 in larval stage for decreasing the values of maximum biomass (W_{max}) depicted in the West growth model.

Other assumption not incorporated in the MPM included density-dependent parameters and its influence on survival, fecundity, and carrying capacity. Therefore, the effect of the self-limitation is negligible. Dealing with density-dependence in MPM in Eq. (1) results in most of the analysis for MPM inapplicable (Caswell 2001). Consequently, populations no longer grow exponentially, and solutions can no longer be resolved in terms of eigenvalues and eigenvectors (Caswell 2001; Klanjscek et al. 2006). Therefore, estimates of outcomes in different pulsed scenarios should be interpreted with caution.

Limitations and uncertainty

We estimated uncertainties based on a combination of quantitative methods. There were critical data gaps that affected both the results presented here and our ability to report and verify changes in pulsed Cu toxicity to tilapia populations. Data were substantially lacking for mortality and growth inhibition of tilapia populations in response to pulsed Cu toxicity. There was also a lack of measurement data of chronic pulsed Cu toxicity bioassay that is needed to parameterize TDM and to validate MPM. Moreover, temperature variation may influence the population metabolic, growth, and survival probabilities and feeding efficiency in uncontaminated conditions (Price et al. 1985; Pandit and Nakamura 2010; Dillon et al. 2010). In our study, the constant-temperature assumption was used in all model simulations.

It is recognized that quality of the outcomes from any modeling study depends on the reliable input data. In our study, the limited database used to calculate vital rates of tilapia populations in response to pulsed Cu toxicity may result in the source of uncertainty. Thus, the prediction of Cu toxicity for pulsed activities should be improved with larger sets of fluctuating ecotoxicity data.

Implications for risk assessment

It has been clear that typical assessment methods do not incorporate pulse timing and sequence, which are insufficient for ecological risk assessment (Reinert et al. 2002). We showed that pulse timing have potential impact on physiological responses and population abundances. Our study indicated that increasing time interval between first and second pulses decreased mortality and growth inhibition of tilapia populations, implicating that during the long interval, tilapia may have enough time to recover the damage (Bearr et al. 2006; Hoang et al. 2007; Hoang and Klaine 2008). Therefore, in light of the aquaculture management, this present study may provide a useful tool to schedule the potential algaecide application time.

Diamond et al. (2006) indicated that pulse timing matters in metal exposures, suggesting that there is a need to incorporate frequency (e.g., number of pulses and recovery time between pulses) into regulatory decision-making and risk assessment protocol. The recovery time of different exposure scenarios can be estimated from recovery rate constant estimates that obtained by using the TDM (Eqs. (T2-3), (T2-4), (T2-5)) to fit the survival probabilities of different exposure scenarios. Ashauer et al. (2010) further

pointed out that pulsed toxicity tests together with TDM-based toxicokinetic parameters could be used in risk assessment to improve biomonitoring systems in the real-world exposure patterns.

Generally, adult tilapia can be used as bioindicator of metal stressors in environmental systems (Liao et al. 2003; Liao and Ling 2003; Ling et al. 2005). However, our study indicated that adults were much less susceptible to Cu stress than larvae and juveniles with different life-stage variables. Therefore, if Cu toxicity is being evaluated with a traditional toxicological approach, such as constant exposure-derived toxicokinetic parameters, theses effective concentrations may be devastating to populations of a species that have much lower Cu susceptibility. Thus, the use of Cu effective 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.

The use of traditional constant exposure-derived toxicokinetic parameters will severely underestimate the true risks to populations subjected to pulsed metal exposure. Our results suggested that such margins might vary substantially among species in different life stages 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 (Ducrot et al. 2007; Klanjscek et al. 2007; Klok et al. 2007). 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 in pulse responses can incorporate into the studies of species interactions under different disturbance regimes and can expand the fundamental basis of ecotoxicology beyond reliance on short-term acute toxicity to include delayed, chronic, and indirect effects operating over longer periods (Vindenes et al. 2011).

In conclusion, the current study presented how a mechanistic perspective based on the chemical effects on the fish energy budget can promote life-cycle pulsed toxicity assessment. Our study showed that the bioenergetics-based matrix population methodology could be employed in a life-cycle toxicity assessment framework to explore the effect of stage-specific MOAs in population response to pulse contaminants (Linkov and Seager 2011). Our bioenergetics-based MPM yields population endpoint along with MOA allowing the comparison between different environmental pulse stressor scenarios. An important implication of our study was that we used mathematical models to examine the key population-level endpoints (the asymptotic population growth rate and stage-specific mode of toxic action) of the population dynamics and evaluate the effect of bioenergetics-based MOAs in field tilapia population response to pulsed waterborne Cu.

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