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ORIGINAL PAPER

Environmental stochasticity promotes copper bioaccumulation and bioenergetic response in tilapia

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Abstract Environmental change not only undergoes in mean environmental conditions but also in their degree of stochasticity. Changes in waterborne metal variability are often associated with altered disturbance regimes and temporal patterns of source availability. Here copper (Cu) was used as an example because Cu sulfate ($CuSO_4$) has been extensively used as a chemical tool to exterminate phytoplankton for controlling skin lesions and gill disease of fish in aquatic ecosystems. This study showed that increased variability of waterborne Cu concentrations strongly promotes a key process of biokinetics, bioaccumulation. In experimental tilapia populations, the mean growth cost coefficient in pulsed Cu exposures was 7 % lower than the control group. On the other hand, the double-pulse, constant low, and single-pulse scenarios had similar effect on biomass change (2.2–2.4 %). The greatest biomass change ($\sim$10 %) occurred where Cu concentrations were gradually increasing over time or at a constant high rate. Most importantly, this study demonstrated that chronic exposure of tilapia to a low Cu concentration rate that approximated a single large pulse of field-realistic levels damaged bioenergetic mechanisms and increased energy acquisition. This study also showed that interactions across multiple pulsed or fluctuating Cu exposures were involved in accumulation changes that could also be achieved by controlling pulse timing and duration. It can be concluded that increased metal variability can promote biokinetic and bioenergetic responses in fish; and that changes in environmental variability may interact with other global change processes and thereby substantially accelerate change in aquatic ecosystems.

Keywords Environmental variability · Copper · Tilapia · Bioaccumulation · Bioenergetics · Risk assessment

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1 Introduction

One of major challenges in ecotoxicology is to understand changes in environmental variability that disturb aquatic organisms in patterns similar to the temporal patterns of waterborne toxicants. The underlying complexity arises from the intertwined nonlinear and dynamic interactions among a variety of pulse conditions. For example, when the degree of stochasticity of environments increases or extreme events become more frequent, such as rainfall, accidental spillage of wastes, and periodic emission of anthropogenic contaminants, those events can generate pulsed patterns of toxicity. The diel metal cycles of

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biogeochemical processes are dynamics and cause short-term (daily and bihourly) variations in metal concentrations (Authman and Abbas 2007; Tercier-waeber et al. 2009). The occurrence of diel variations in heavy metal concentrations were found in the surface water (Gammons et al. 2007; Diamond et al. 2005; Parker et al. 2007; Pereira et al. 2009, 2010; Heier et al., 2010).

Copper (Cu) is widespread in the environment from anthropogenic and natural processes. Anthropogenic input can raise ambient Cu concentration to >100 mg L^{-1} and Cu can even reach levels as high as 200 mg L^{-1} in mining areas (USEPA 2007). In addition to mining activities, Cu in surface water also arises from fabricated metal producers, electric equipment, and the leather industry (Patterson et al. 1998). On the other hand, municipal waste waters, manure, fertilizers, and antifouling measures (paint and wood preservatives) further contribute Cu to the aquatic systems (Patterson et al. 1998). Cu is also the cheapest and most commonly used pesticide in the aquaculture industry and other aquatic systems.

Copper sulfate ($CuSO_4$) has been extensively used as a chemical tool to exterminate phytoplankton for controlling skin lesions and gill disease of fish in the cultured ponds (Boyd 2005; Mischke and Wise 2009; Miao et al. 2011). The treatment of culture ponds with >1 mg L^{-1} $CuSO_4$ is effective in killing algae and parasites (Banerjee et al. 1990). The algaecide suppresses the respiration and photosynthesis of algae, leading to a dissolved oxygen decrement in aquatic systems. Wise et al. (2006) indicated that $CuSO_4$ algaecide ranging from 2.5 to 5.0 mg L^{-1} (0.64–1.27 mg L^{-1} Cu) could effectively extirpate snails and induce adverse effects on fish health.

Numerous researchers reported that Cu in fish, e.g., *Megalops cyprinoids, Chanos chanos*, *Liza macrolepis*, *Mugil cephalus*, *Oreochromis* sp. ranged from 0.39 to 1 μg g^{-1} dry wt (Huang et al. 2001; Chen et al. 2004). However, Cu burdens in tilapia *Oreochromis mossambicus* are relatively higher ranging from 1.524 to 18 μg g^{-1} dry wt (Lin et al. 2005). Moreover, a growing body of evidence shows that Cu accumulated in the chloride cells is likely to inhibit brachial Na^+/K^+-ATPase activities and have the potential to decrease Na^+ transport in the gills of fish (Grosell and Wood 2002; Wu et al. 2003; De Boeck et al. 2007). Therefore, high Cu burdens could induce the disruption of branchial ion regulation, causing mortality in fish.

Standard laboratory bioassays designed to investigate the toxicity of Cu on aquatic organisms inaccurately estimate the chemical toxicity and risk of Cu (Reinert et al. 2002; Bearr et al. 2006; Ashauer et al. 2007). Some studies indicated that pulsed/fluctuating exposures could induce latent effects on aquatic organisms that were different from these seen with constant concentration exposure (Diamond et al. 2006; Nimick et al. 2007; Ashauer et al., 2010). Nimick et al. (2007) have conducted field bioassays to monitor the diel waterborne metal cycle and measure the survival probability for cutthroat trout (*Oncorhynchus Clarki Lewisi*). This important endpoint revealed that the latent mortality and the mortality related the prior fluctuating events were observed in cutthroat trout bioassays. The results suggested that exposure to diel-fluctuating concentrations was less toxic than exposure to a constant concentration for cutthroat trout when the average concentration for the fluctuating and constant exposures was the same. However, there is limited information related to growth inhibition of aquaculture species that suffered from a non-constant metal concentration exposure.

Gammons et al. (2007) and Heier et al. (2010) indicated that pulsed and fluctuating Cu concentrations had the inverse relations with pH and temperature, whereas a positive correlation was found with water flow and dissolved organic carbon. The Cu toxicity depends on the external (environmental stochasticity) and internal (biological) factors. The external factors such as temperature, pH, hardness, and specific ions levels may affect the mechanisms of the bioavailability of Cu to aquatic organisms (Niyogi and Wood 2004).

Recently, the majority of studies have focused on carrying out the acute toxicity and exposure bioassays to determine effective Cu concentrations in tilapia in a constant exposure scenario (Abdel-Tawwab and Mousa 2005; van Aardt and Hough 2006; Chen et al. 2012a). However, there is little understanding of how chronic Cu accumulation rates would change and potentially affect the regulation mechanisms in aquaculture species under a pulsed/fluctuating condition that more closely resembles field exposure. In this study, "pulse" was used to denote a broad spectrum of repetitive phenomena that range from irregular and stochastic to more uniform and periodic dynamics. Pulse characteristics include frequency, duration and amplitude (i.e., concentration).

Farm raising of tilapia is a promising aquatic practice because of the high market values of tilapia. Anything that causes weight loss of tilapia may pose an economic loss of aquaculture products. Therefore, the tolerances of aquaculture species to fluctuating Cu toxicity need to be estimated. To date, the ecotoxicological consequences of changes in variability; and their potential interactions with other drivers of change are not well understood. Particularly, there is a lack of experimental studies that truly tested for the consequences of changes in variability by explicitly manipulating environmental variability. The purposes of this study were to provide information on bioaccumulation and toxicity during chronic exposure to pulsed Cu associated with Cu toxicity on growth in farmed tilapia *O. mossambicus*. This present study carried out a 28-days pulse

exposure bioassay to obtain toxicokinetic parameters. A 28-d growth toxicity bioassay of chronic exposure to pulsed Cu was also conducted to examine the relationships between growth inhibition and pulsed Cu concentration.

2 Materials and methods

2.1 Chronic bioaccumulation bioassay

An experiment for chronic exposure to sequential pulsed Cu was conducted to investigate the ability of 8-month old mature subadult tilapia *O. mossambicus* with mean length 10.09 ± 1.69 cm (mean ± SD) and mean body biomass 17.02 ± 9.80 g wet wt to accumulate Cu. Fish were cultured at the *Department of Biological Science and Technology*, China Medical University, Taichung, Taiwan. The tilapia acclimation conditions for 14 days are listed in Table 1. During the acclimation period (August 16th–September 13th, 2010), the fish were fed twice per day with commercial fish food.

An exposure experiment was carried out with 48 subadult fish under static conditions in three aquaria of 81 L ($60 \times 30 \times 45$ cm^3) volume filled with 70.2 L of exposure solution. Each aquarium contained a stock density of 16 fish. The copper sulfate stock solution ($CuSO_4 \cdot 5H_2O$) was prepared using deionized water. The sequential pulsed Cu exposure bioassay was carried out with a 28-days exposure period starting with initial concentrations of 100 and 300 μg Cu L^{-1} of pulsed concentrations. The sequential pulsed Cu exposure design was accomplished by siphoning the volume of Cu contaminated water in the test aquarium from *X* L (70.2 L) to 1/3 *X* L (23.4 L). Later, after water was siphoned from *X* L to 1/3 *X* L, and Cu-amended water was added and filled to *X* L in the test aquarium in order to increase the pulsed concentration and vice verse. The exposure bioassay included a control group and two replicated experiments.

Table 1 Summary of tilapia acclimation conditions and water chemistry characteristics

Variable	Unit	Value
Temperature	°C	28 ± 0.76[a, b]
pH	–	7.8 ± 0.13[b]
Light cycle	h	12
Dissolved oxygen (DO)	mg L^{-1}	7.5 ± 0.48[b]
Alkalinity	mg $CaCO_3$ L^{-1}	91.1
Calcium (Ca)	mg L^{-1}	59.6
Magnesium (Mg)	mg L^{-1}	13.17
Sodium (Na)	mg L^{-1}	9.40
Potassium (K)	mg L^{-1}	2.73
Hydroxide (OH^-)	mg L^{-1}	0.013
Carbonate (CO_3^{2-})	mg L^{-1}	0.012

[a] Mean ± SD

[b] Sample size = 4

The pulsed Cu exposure timings occurred twice during the exposure periods at days 0.5 and 25, respectively. The pulsed exposure duration was 1 day in each event. The entire Cu solution was replaced and collected daily to avoid the regression of water quality and feces were removed every 6 h and forage debris was collected 1 h after feeding. Three or four fish were removed from experimental tanks on days 0, 0.5, 1.5, 4, 7, 11, 14, 18, 21, 25, 26, and 28 of exposure. The tilapia were anesthetized with benzocaine hydrochloride solution during the sampling. The dissected tissue samples were cleaned with double-deionized water (ddH_2O) and freeze dried overnight. We analyzed Cu concentration in gill, liver, kidney, muscle, gonad, carcass, bone, intestine, and blood per individual to estimate the Cu accumulation in the whole body.

2.2 Growth toxicity bioassays

Tests of growth of subadult fish were performed with 10 fish in control and pulsed Cu exposures, respectively, using fish 8–9 months of age with a mean body length 9.39 ± 0.39 cm and a mean body weight 13.99 ± 1.29 g wet wt. Tilapia were acclimatized for 14 days before the Cu exposure, and water condition characteristics were the same as for the chronic bioaccumulation bioassay. Two replicated tanks were used in the control and exposure treatments for 28 days. The pulsed Cu exposure protocol was also the same as in the chronic bioaccumulation exposure bioassay.

During the exposure period, the tilapia in both treatments were fed twice per day with fish food at a rate of 4 % tilapia body biomass, and the water was renewed twice per day after feeding. The Cu concentrations were monitored every day before feeding to assess the water quality. In each exposure tank, the mean body biomass and body length of fish were measured at days 0, 0.5, 1.5, 4, 7, 11, 14, 18, 21, 25, 26, and 28.

2.3 Chemical analysis

A flame atomic absorption spectrometer (Perkin Elmer AA-200, USA) was used to analyze Cu in fish tissues. Analytical quality control was achieved by digesting and analyzing identical amounts of rehydrated standard reference material (Merck, Darmstadt, Germany). Fish tissues were digested with 2 mL 65 % HNO_3 and 1 mL 30 % H_2O_2 overnight at 95 °C. The 20 mL fish samples were stored at −4 °C in the dark until they are analyzed. Analytical quality control was achieved by digesting and

analyzing identical amounts of rehydrated (90 % H_2O) standard reference materials (dogfish muscle, DORM-2; NRC-CNRC, Canada). The recovery rate was 95.6 ± 3.4 %. The 15 mL water samples with 65 % HNO_3 were digested for 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).

2.4 Data analysis

In the experimental groups, the body length and weight of tilapia were measured at sample timing without dissection. The daily growth rate (k_g, % d^{-1}) of tilapia was calculated as (Allen et al. 2006),

$$k_g = \ln\left(\frac{W_t}{W_i}\right)/t \times 100, \tag{1}$$

where W_i (g wet wt) are the body length or weight of tilapia at the beginning and W_t (g wet wt) are the body length or weight of tilapia at time t (d), respectively.

Here we used the condition factor (K) as an indicator to reflect the fish well-being. The condition factor can be calculated as (Fulton 1904),

$$K = 100 \times \left(\frac{W}{L^3}\right), \tag{2}$$

where W is the whole body weight (g wet wt) and L is the body length (cm).

A first-order bioaccumulation model was used to fit the Cu accumulation data of tilapia following the exposure to sequential pulsed Cu concentrations (Luoma and Rainbow 2005),

$$\frac{dC_b(t)}{dt} = k_u C_w(t) - k_e C_b(t), \tag{3}$$

where k_u is the uptake rate constant (mL g^{-1} d^{-1}), k_e is the elimination rate constant (d^{-1}), $C_b(t)$ is the body burden of Cu at time t (μg g^{-1} dry wt), $C_w(t)$ is the sequential pulsed Cu concentration in water (μg mL^{-1}) and can be expressed as

$$C_w(t) = C_0 + C_1[U(t-T_1) - U(t-T_2) + U(t-T_3) - U(t-T_4)], \tag{4}$$

where $U(t - T_i)$ is the unit step function, C_0 and C_1 represent initial and pulsed concentrations (μg mL^{-1}), respectively, and T_i is pulsed exposure timing (day).

Specifically, when water concentration is a constant, the uptake and elimination rate constants can be determined by fitting the following integrated form of the kinetic rate equation to bioaccumulation data (Luoma and Rainbow 2005),

$$C_b(t) = C_b(t=0)e^{-k_e t} + \frac{k_u}{k_e} C_w(1 - e^{-k_e t}), \tag{5}$$

where $C_b(t = 0)$ is the initial dependent concentration of Cu in fish tissue (μg g^{-1} dry wt). The bioconcentration factor (BCF) can then be determined from the equation as: $BCF = k_u/k_e$.

Organisms could fuel ontogenetic growth by allocating absorbed energy to synthesize new biomass and to maintain existing biomass. The ontogenetic growth model (West et al. 2001) can be used to describe the organism ontogenetic growth (biomass) trajectory from birth to maturity based on energy allocation without toxicity. On the other hand, DEBtox can be used to describe the mode of action (MOA) of metal toxicity that alters the energy allocation including metabolism, growth, and reproduction (Alunno-Bruscia et al. 2009). Hence, the integration of the ontogenetic growth model and DEBtox theory can effectively predict allometric relationships between the growth rate and the life history events under metal stresses.

Tsai and Liao (2006) revealed that MOA of reducing food assimilation efficiency can be well predicted by the ontogenetic growth model. The ontogenetic growth model was adapted to assess the growth coefficient of tilapia under control and pulse exposure conditions

$$W(t) = \left[W_{\max,0} \cdot (1 - s(t))\right] \times \left\{1 - \left[1 - \left(\frac{W_0}{W_{\max 0} \cdot (1 - s(t))}\right)^{1/4}\right] \times \exp\left(-\frac{A_0 t}{4(W_{\max 0} \cdot (1 - s(t)))^{1/4}}\right)\right\}^4, \tag{6}$$

where $W(t)$ is the time-dependent body biomass (g), W_0 is the body biomass at birth of tilapia that approximately 0.05 g in uncontaminated environment (www.fishbase.org/home.htm), $W_{\max 0} \cdot (1 - s(t))$ is the ultimate body biomass of tilapia under the contaminated environment in which $W_{\max,0}$ is the maximum body biomass that approximately 1,130 g in uncontaminated environment, $s(t)$ is the stress function, and A_0 is the growth coefficient ($g^{1/4}$ d^{-1}). To assess the performance of model predictability, this study used the mean absolute percentage error (MAPE) as $\text{MAPE} = \frac{1}{N}\sum_{n=1}^{N} \frac{|C_{o,n} - C_{m,n}|}{C_{o,n}} \times 100\,\%$ to compare the growth biomass between prediction and observed data where N denotes the number of observation, $C_{o,n}$ is the experimental data, and $C_{m,n}$—is the modeled result corresponding to data point n. The MAPE < 50 % presents reasonable prediction, 10 % ≤ MAPE < 20 % is the good predictions, and MAPE < 10 % is an excellent prediction.

McCarty and Mackay (1993), Kooijman and Bedaux (1996), and Pery et al. (2003) suggested that the extent of adverse effects could be proportional to the difference

between accumulated chemical concentration [C_b(t)] and IEC10 of the 10 % effect threshold for chronic growth inhibition. Kooijman and Bedaux (1996) and Pery et al. (2003) further introduced a stress function [s(t)] to describe the extent of adverse effect as: $s(t) = b(C_b(t) - \text{IEC10})$ where b accounts for the level of toxicity (g g^{-1}) once C_b exceeds IEC10. Here $s(t) = (C_b(t) - \text{IEC}_{10})/\text{IEC}_{10}$ is used to describe the extent of adverse effect that body burden exceeds IEC_{10} where IEC_{10} value is 6.52 μg g^{-1} adopted from (Chen et al. 2012b).

This study employed the TableCurve 2D (Version 5, AISN Software, Mapleton, OR) to optimal fit the published and experimental data to obtain optimal statistical models. A Monte Carlo (MC) technique was performed to obtain 2.5th- and 97.5th- percentiles as the 95 % confidence interval (CI) for all fitted models. The MC simulation was performed for 10,000 iterations to ensure the results. The Crystal Ball® software (Version 2000.2, Decisionerring, Inc., Denver, Colorado, USA) was employed to implement MC simulation. Mathamatica® (Version 5.1, Wolfram Research Inc., Champaign, IL, USA) was used to perform all simulations of the bioaccumulation to time-varying Cu exposures.

3 Results

3.1 Toxicokinetic parameters

The results showed that a rapid accumulation along with a gradual decreasing of tissue concentration over time was found (Fig. 1a) for the tilapia subjected to two Cu pulses in the course of 28 days (Fig. 1b). The toxicokinetic rate equation in Eq. (3) was fitted to the exposure data to obtain the accumulation parameters, resulting in an uptake rate constant (k_u) of 25.18 ± 4.61 mL g^{-1} d^{-1} (mean ± se) and an elimination rate constant (k_e) of 1.09 ± 0.29 d^{-1} ($r^2 = 0.61$, $p < 0.05$) (Fig. 1a). Therefore, the steady-state BCF is 23.10 mL g^{-1}. Our result also revealed that the MAPE between model prediction and double pulsed accumulation experiment was 17.05 %, indicating a good prediction.

3.2 Daily growth and condition factors

The tilapia grew substantially in body length and weight both in the control and pulsed Cu exposure groups. The body length and weight of tilapia in each treatment at sampling timing were monitored to calculate the daily growth rate (Fig. 2a, b). However, tilapia exposed to pulsed Cu experienced relative lower daily growth rates (k_g) than those in control groups (Fig. 2). Notably, the growth of body length was inhibited in chronic exposure to pulsed Cu

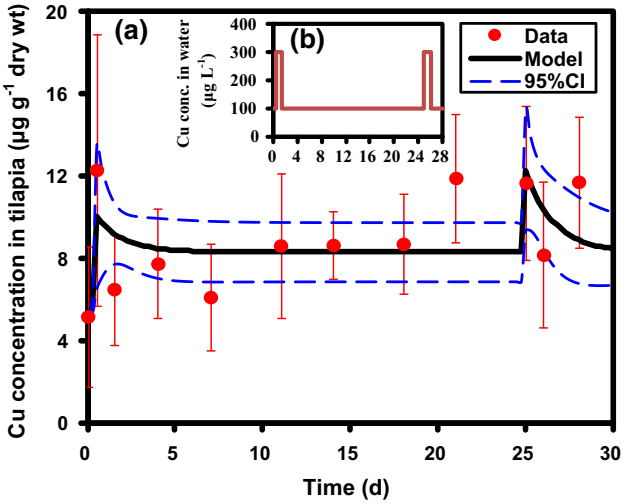

Fig. 1 Pulsed Cu exposure bioassay in **a** Cu accumulation of tilapia exposed subjected to **b** initial Cu concentration of 100 μg L^{-1} and pulsed concentration of 300 μg L^{-1} during a 28-d exposure. *Error bars* are SD from the mean

at days 0.5 and 1.5, whereas the growth of body weight was inhibited at days 1.5 and 4.

This study also found that the both daily growth rates of body length and weight in pulsed exposure group were lower than those in control group during the Cu plused concentrations. The k_g estimates of body length in control and pulsed exposure groups ranged from 0.14 to 5.97 and from −1.06 to 0.42 % d^{-1}, respectively (Fig. 2a). On the other hand, the k_g estimates of body weight were from 0.34 to 13.25 and from −0.66 to 1.09 % d^{-1}, respectively, in control and pulsed exposure groups (Fig. 2b). Thus, chronic exposure to pulsed Cu concentration affected the growth mechanism in farmed tilapia.

A different growth pattern in the condition factors (Ks) was also found (Fig. 3). The results indicated that K in the pulsed exposure group was higher than that in the control group at pulsed timings of days 0.5, 1.5, and 26 (Fig. 3). After a pulsed Cu period, the Ks were significantly decreased, revealing that chronic pulsed Cu exposure induced lagged-growth inhibition.

3.3 Growth coefficient

The growth coefficients (A_0) for tilapia were estimated by fitting the ontogenetic growth model [Eq. (6)] to time-dependent body weight data in Fig. 4a and b, resulting in 0.0274 ± 0.0022 $g^{1/4}$ d^{-1} in the control ($r^2 = 0.82$, $p < 0.05$) and 0.0256 ± 0.0015 $g^{1/4}$ d^{-1} in the pulse group ($r^2 = 0.91$, $p < 0.05$). The results indicated that chronic exposure to pulsed Cu substantially influenced growth coefficients of tilapia, revealing a decreasing of growth

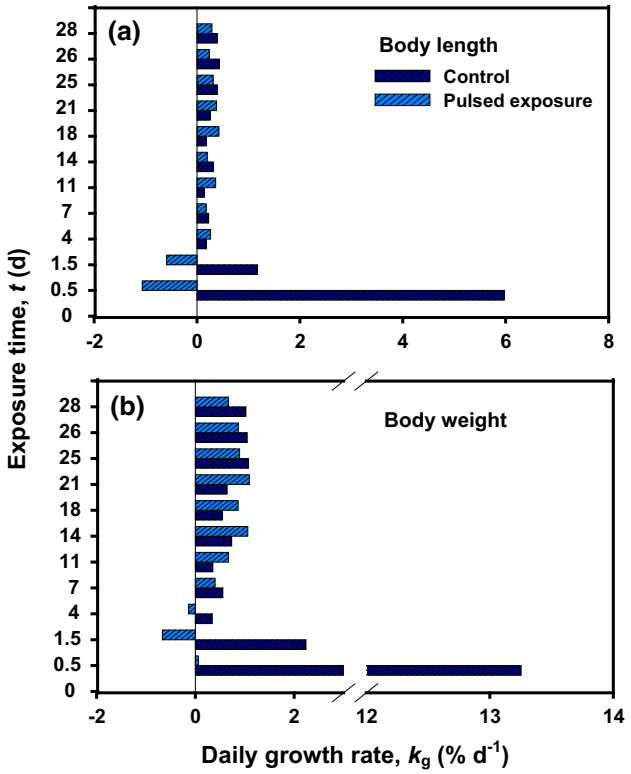

Fig. 2 **a** Calculated daily growth rate of body length and **b** body weight of tilapia with and without pulsed Cu exposure during 28 days bioassays

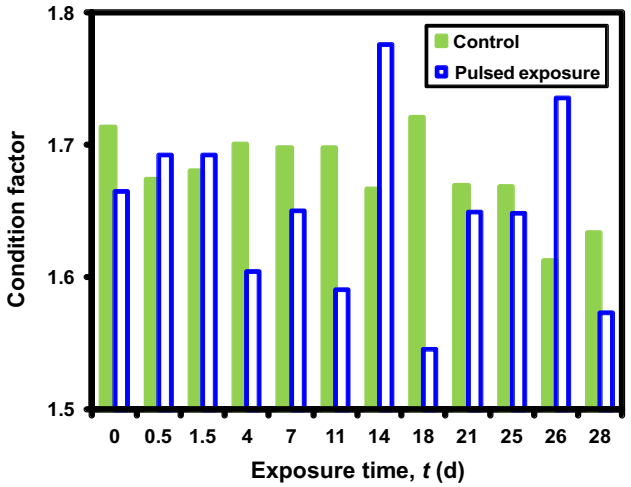

Fig. 3 Condition factor in tilapia during the 28 days of exposure in control and pulsed Cu

energy acquisition. Overall, the results indicated that the ontogenetic growth simulations with validation were reasonably agreed with experimentally determined value of the growth biomass under without Cu and double pulsed Cu exposures. The growth biomass of without Cu and double pulsed exposures revealed the excellent predictions with MAPEs of 3.83 and 2.27 %, respectively (Fig. 4c). This study also compared the growth biomass between without Cu and double Cu pulse exposures, revealing that the biomass change was 2.4 %.

3.4 Model applications

By examining the individual-based outcome of body burden and biomass change, this study carried out model applications in which tilapia were exposed under five different scenarios: (i) constant low or (ii) constant high, (iii) gradual increase, (iv) a single large pulse or (v) multiple pulses of different magnitudes along with the present pulse experiment (Fig. 5a–e). Compared with (i) and (ii), (iii)–(v) thus constituted scenarios with increased environmental exposure variability. A first-order bioaccumulation model incorporated with ontogenetic growth model at MOA of reducing food assimilation efficiency and stress function were used to calculate body burden and weight (Fig. 5f–i). The results showed that accumulation of Cu in tilapia increased significantly between the timings of first and second pulses, particularly in the large pulse scenarios. The results also showed that the time-evolving body burdens of tilapia reflected the different recovery periods during the pulsed exposures.

This study found that the present pulsed experiment, constant low, and single pulse scenarios had similar effects on the total biomass change (2.2–2.4 %) (Figs. 4d, 5k). If exposure was unpredictable through multiple pulses of varying magnitudes rather than exposed at a constant low rate or a single pulse, the biomass change more than doubled (4.4 %) (Fig. 5k). The greatest biomass change (~10 %) occurred when Cu concentrations were gradually increasing over time or at a constant high rate (Fig. 5k).

4 Discussion

4.1 Environmental stochasticity on Cu bioaccumulation

The analytical approach described in this present study provided an opportunity to examine and to quantify metal accumulation dynamics for fish in response to environmental variability-induced non-uniform metal exposures. The present measurements and analyses of metal accumulation dynamics are a fundamental step in understanding the effects of metal toxicity on coping mechanisms of aquatic organisms. The important finding from this study is that increased environmental exposure variability can strongly influence Cu accumulation capacity of tilapia and

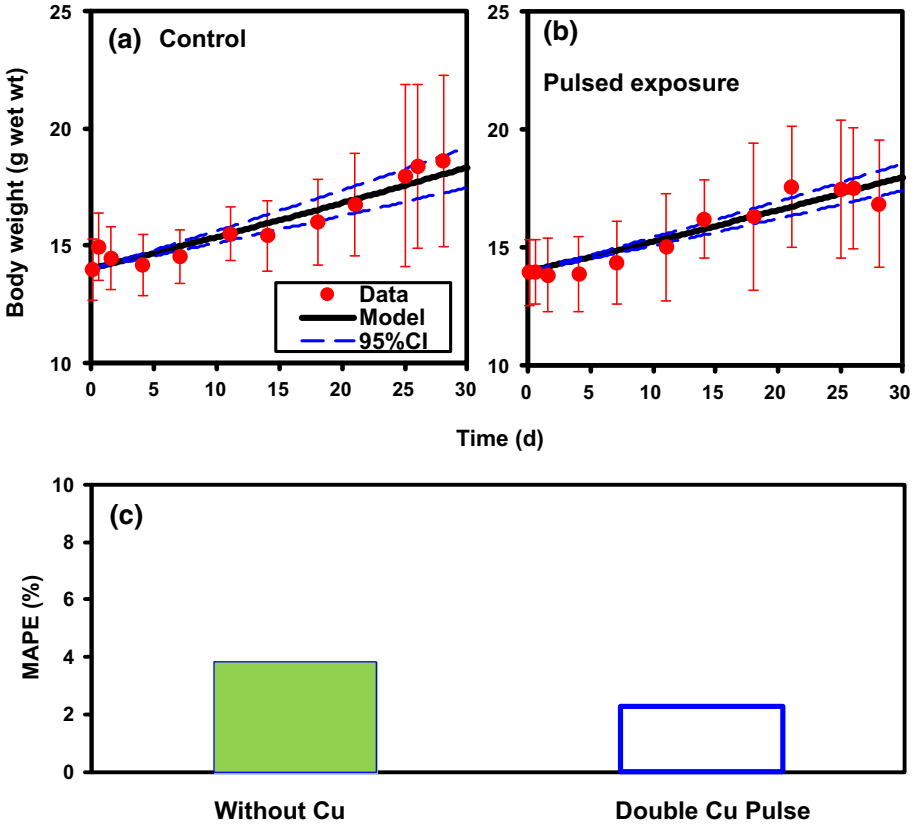

Fig. 4 Best-fitting regression models of body weight of tilapia in **a** control and **b** pulsed Cu exposure groups during 28-days Cu growth toxicity bioassays. **c** Mean absolute percentage error (MAPE) measured and simulated biomass of without Cu and double pulsed exposures

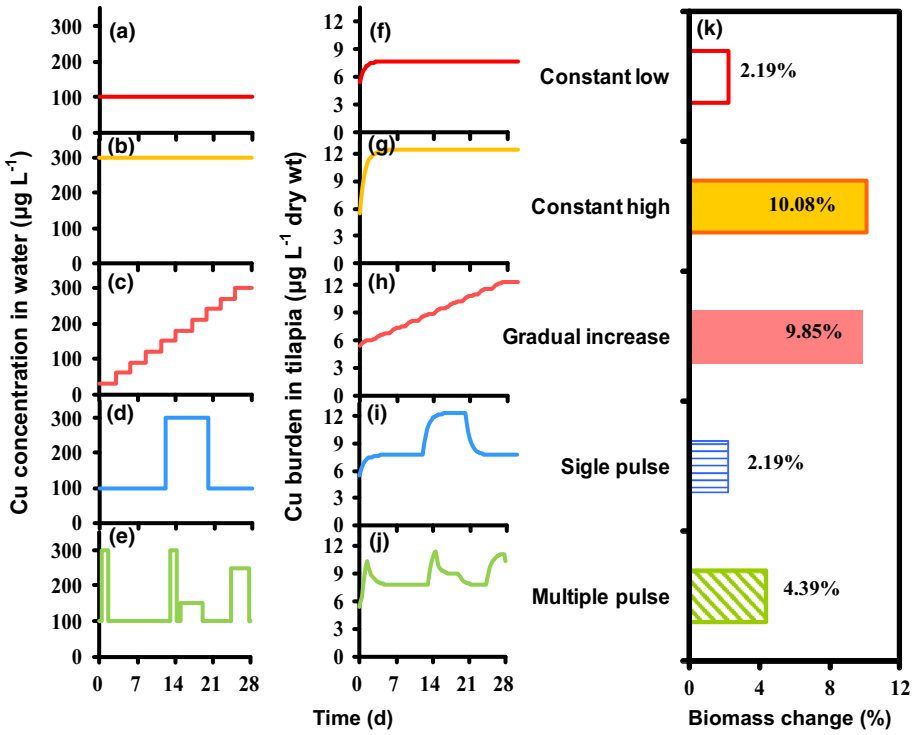

Fig. 5 **a–f** Temporal patterns of the present constant low, constant high, gradual increase, single large pulse, and multiple pulses of different magnitudes scenarios. **g–l** Simulations of Cu body burden of tilapia exposed under five different exposure scenarios. **m** Percent biomass change under five different exposure scenarios

their biomass development. The major environmental exposure variability included pulse concentration, duration, and interval.

Diamond et al. (2006) indicated that the different accumulation levels between the short- and long-term chemical exposures can in part be explained by the

acclimation mechanisms in aquatic organisms. Gill regulation is a time-dependent acclimation characterized by both how quickly the acclimation is activated to prevent further effects and how long the acclimation stays in place (Diamond et al. 2006). Hoang et al. (2011) also indicated that the toxicity of a metal second pulsed exposure was substantially influenced by the first pulsed exposure, depending on the pulse concentration, duration, and the interval between the pulsed exposures.

This study indicates that predominant exposure frequencies of Cu pulses may switch the safety growth probability for tilapia. Diamond et al. (2006) indicated that frequency and recovery time between chemical pulses have significant effects on the responses of aquatic organisms. The present results indicated that during the pulsed Cu exposure, the recovery time may play a crucial role in affecting tilapia safe growth. In view of the tilapia internal responses, long duration between pulses had higher safe growth probability than that of short duration.

Previous studies indicated that some level of elimination or detoxification to chemical during the chemical-free period may decrease the toxic effect, depending on the duration between pulses (Reinert et al. 2002; Zhao and Newman 2006). Moreover, the recovery time is a critical factor in influencing the safe and survival capacities in aquatic organisms during the pulsed chemical exposures. Diamond et al. (2006) demonstrated that the longer recovery times were associated with diminished hazardous effect of sequential chemical pulses, whereas the more significant hazard effects were observed at the more closely spaced pulses of exposure.

4.2 Environmental stochasticity on bioenergetics

The ontogenetic growth model considers that the maximum body biomass is affected by the chemical stress. Hence, the safe growth probability could provide the relationships between chemical stress and growth body biomass. This study demonstrated that the growth biomass dynamics of tilapia at different life-stage can be determined by various pulsed Cu exposure patters. The economics of tilapia aquaculture mostly depend on the abundance of the subadult tilapia population. Hence, bioenergetics response in growth biomass is an important factor in determining and promoting the economic benefits. Therefore, the proposed 28 days-growth bioassay data for chronic exposure of subadult tilapia to sequential pulsed Cu is extremely valuable in determining the growth inhibition in whole life span of tilapia.

The ontogenetic growth model can also be used to elucidate the growth inhibition over the entire life cycle based on the limited growth information at subadult tilapia stage exposed to pulsed Cu concentrations. However, larval and juveniles stages have higher growth abilities than that of subadult tilapia. The specific-stage growth coefficient should be fitted in the appropriate tilapia population stages to assess the growth biomass dynamics. Thus the appropriate growth dynamic features in each life-stage tilapia populations can be realized under a chronic exposure to pulsed Cu stressor. The growth weight dynamics can be influenced by the metal susceptibility that is specific to different life-stages of tilapia. It should further incorporate the available specific toxic effect models that can be used to describe the dynamics of growth inhibition for whole life-stage of tilapia population, in particular; for the developing larvae that have higher susceptibility to Cu than the older stages of tilapia (Ramskov and Forbes 2008).

4.3 Impact of pulsed exposure on species response

The traditional Cu formulation used as an algaecide is Cu sulfate containing 26 % elemental Cu by weight (Boyd 2005). Generally, the guidance of algaecide concentrations and application rates in aquaculture ponds and other aquatic systems are recommended on the basis of ecotoxicological tests using a single algaecide set at a level for field use, e.g., LD50 (a lethal dose to 50 % aquatic animals or plants) (Boyd 2005; Mischke and Wise 2009; Ashauer et al. 2007). However, the risk of chronic exposure to sequentially pulsed Cu is currently not considered when assessing the safety of algaecide for farmed fish.

Given the potential impacts on body biomass growth in tilapia, it is concerning that at present there are no guidelines for testing chronic effects of Cu sulfate algaecide with pulsed exposure patterns on aquaculture species. Therefore, the current guidance of a maximum exposure of 96-h appears to be insufficient (Mischke and Wise 2009). The present results indicate the importance of the need for longer term ecotoxicity testing on both subadult tilapia and larvae in a chronic pulsed exposure means to detect cumulative toxicity effects and to employ risk assessment for different life-stage populations. More general, this study indicates that increased metal stress variability, e.g., in multiple pulses of different magnitudes or in a gradually increasing over time, can significantly influence safe growth of farmed species; and that changes in environmental variability may interact with other change processes and thereby substantially accelerate ecophysiological change in farmed species.

4.4 Implications and limitations

One important conclusion from this study is that chronic exposure of subadult tilapia to commonly encountered doses of waterborne Cu with pulse can substantially affect growth of body length and weight, with potential

contributions to bioenergetics risk. Most importantly, this study demonstrated that chronic exposure of tilapia to a low Cu concentration rate that approximate a single large pulse or multiple pulses of same magnitude of field-realistic level damages bioenergetics mechanisms and increases energy acquisition. On the other hand, the multiple pulses of varying magnitudes could lead to significant reductions in biomass development. Furthermore, the extent to which exposures affect growth appears dependent on pulse concentration, duration, interval, and frequency, and consequently the constant concentration level could not analogize the multiple pulses of same magnitude of field-realistic level.

There were two potential sources of certainty in our analysis. The first was the bioaccumulation rates that derived from the exposure experiment. Therefore, there is a need to conduct a more extensive characterization of the distribution of pulse exposures within given population groups. It would be useful to characterize better the distribution of exposures by life-stage of aquatic organisms exposed. The second source of potential uncertainty was the ontogenetic growth model we adapted to assess the growth coefficient of tilapia. We acknowledge that the ontogenetic growth model as a source of uncertainty even still, but note that a full analysis of this source of certainty was beyond the scope of this study. In our analysis, we limited our consideration to the most common, linear model of stress function representing the relationship between the extent of adverse effect that body burden exceeds IEC_{10}, despite the limited plausibility of such simple relationship, and limited our description of environmental variability to changes in bioenergetics in tilapia, as these have the strongest support in the literature. As our understanding of the complex relationships between environmental stochasticity, metal ecotoxicology, and bioenergetics in aquatic organisms, these decisions bear revisiting in future analysis.

A major difficulty in studying ecotoxicological modeling from a systems perspective has been the lack of information regarding timing and sequence in which organisms are exposed to chemicals. One way to address this issue is to develop a mathematical framework that estimates the potential advantage of a conditioned response in a given fluctuating environment. Intuitively, the physiological response depends on environmental and biological parameters. These parameters are in turn dependent on the typical time constants of the environment, for example, the time interval between the appearances of the two stimuli. Notably, quantitative predictions can be drawn by incorporating into the model biologically meaningful and realistic parameters, such as recovery time and bioenergetics cost. A theoretical understanding will improve the ability to interpret such data, uncover biological principles and design appropriate experiments. With limited information on site-specific parameters, numerical simulations can be undertaken for randomly selected parameter values in an attempt to discern typical behaviors.

This idea can be tested through computational studies together with basic bioassay data, beginning with an ordinary differential equation model of bioaccumulation model describing the ecotoxicological processes. These computational studies help understand the recovery mechanism, toxicokinetics and parameter values that reflect the MOA in physiological response processes. Models of the type described in this paper are largely explored through simulation in terms of their predictive power. More experiments are needed to validate the model predictions, especially related to physiological acclimation of copper exposed fish. The proposed analysis will be useful for us in designing the experimental protocol.

In conclusion, this present approach helps reveal how previously known toxicokinetics under pulsed conditions are combined to effect ecophysiological responses to environmental stochasticity. The methodology described here could discern the key MOA that regulates the pulsed Cu exposure events. This study indicates that the overall control design for aquatic organisms in response to pulse toxicant exposure depends on quantitative system characteristics that can be achieved by controlling pulse frequency and duration. The data presented here may be used for further computational analyses such as multivariate statistics and large-scale structural or toxicokinetic models. Moreover, the pulsed Cu bioassays based mechanistic study may assist in understanding the interactions among toxicokinetic, toxicodynamic, recovery, and bioenergetics mechanisms that reflect the MOA in ecophysiological responses for chronic exposure of aquaculture species to a sequential pulsed metal stressor. The information obtained from the pulsed Cu exposure experiments could be used to test the predictions of bioenergetics responses under the field-level exposure patterns and related probabilistic risk assessment implications (Sullivan 2011; Gao et al. 2013; Jang et al. 2013) based on the proposed mechanistic model.

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