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Detoxification and bioregulation are critical for long-term waterborne arsenic exposure risk assessment for tilapia

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Abstract Long-term metal exposure risk assessment for aquatic organism is a challenge because the chronic toxicity of chemical is not only determined by the amount of accumulated chemical but also affected by the ability of biological regulation or detoxification of biota. We quantified the arsenic (As) detoxification ability of tilapia and developed a biologically based growth toxicity modeling algorithm by integrating the process of detoxification and active regulations (i.e., the balance between accumulated dose, tissue damage and recovery, and the extent of induced toxic effect) for a life span ecological risk prediction. Results showed that detoxification rate (k_{dex}) increased with increasing of waterborne As when the accumulated metal exceeded the internal threshold level of 19.1 μg g^{-1}. The k_{dex} values were comparable to or even higher than the rates of physiological loss and growth dilution in higher exposure conditions. Model predictions obtained from the proposed growth toxicity model were consistent with the measured growth data. The growth toxicity model was also used to illustrate the health condition and growth trajectories of tilapia from birth to natural death under different exposure scenarios. Results showed that temporal trends of health rates and growth trajectories of exposed fish in different treatments decreased with increasing time and waterborne As, revealing concentration-specific patterns. We suggested that the detoxification rate is critical and should be involved in the risk assessments framework. Our proposed modeling algorithm well characterizes the internal regulation activities and biological response of tilapia under long-term metal stresses.

Keywords Arsenic · Bioavailability · Detoxification · Bioregulation · Tilapia · Risk assessment

Introduction

The exact dose exerts in target sites is difficult to be examined. The environmental concentration was traditionally adopted as the surrogate for the chemical dose to produce a given chemical effect to aquatic animals, e.g. the median lethal concentration (LC50) and the lowest observed effect concentration (LOEC). The concept of the body

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residue hypothesis states that the use of environmental chemical concentrations to gauge hazard could be misleading because the environmental concentration necessary to cause effect varies with the biouptake route, duration of exposure, type of exposure medium, and species used for testing (Niyogi et al. 2008; Sappal et al. 2009; Pan and Wang 2009). Therefore, the target organ/tissue or subcellular fraction imply an easier and more reliable way of referencing the dose with the continued assumption that the total accumulation reflects the concentration at the target site (De Schamphelaere and Janssen 2004; Escher and Hermens 2004; Seebaugh and Wallace 2009).

One of the major challenges for assessing the potential risk of heavy metal to aquatic organism in field ecosystems is to predict the time-dependent internal effective dose that causing the toxic effects. The responses of organism is not merely determined by the dose–response relationships but also effected by the ability of biological regulation or detoxification of biota during long-term exposures (Taylor et al. 2000; Schuler et al. 2004; Vijver et al. 2004). Once the metal and metalloids enter the organism, a certain number of them are subject to biotransformation or sequestered from the target sites by binding with metallothioneins or similar functioning molecules and stored as inactive forms in a slowly exchanging detoxified form variety of granules or structural tissue (Newman and Unger 2003; Sharma and Sohn 2009).

The sequestration of a large portion of metal in detoxification pool or stored as metabolites in aquatic organism as an accumulation and toxicity regulation strategy has been reported for many aquatic organisms (Rainbow 2002; Campbell et al. 2005; Voets et al. 2009). These processes reduce the amount of chemical available to cause toxic effects. Thus, another problem using body residues may be associated with the presence of metabolites. Metabolites are usually included in total chemical amounts in laboratory tests, thereby overestimating the critical body residue unless the metabolites contribute equally to the effect.

Long-term diet exposure of ingesting inorganic arsenic (As) in artesian well water has been demonstrated to induce blackfoot disease (BFD), a unique peripheral vascular disease that ends with dry gangrene and spontaneous amputation of affected extremities in southwestern coastal area of Taiwan (Chen et al. 2001). Nowadays, the local residents do not drink the well water. However, artesian well water is still used for aquaculture. The tilapia *Oreochromis mossambicus* is a traditional food fish for the people of Taiwan. It is also one of the most abundant species in local freshwater and estuary ecosystems in Taiwan. Tilapia have been the target species in several earlier studies inspecting the metal toxicokinetics, toxicodynamics, and physiological responses (Suhendrayatna et al. 2002; Liao et al. 2004; Wu et al. 2008). Farming tilapia is one of the promising practices in the southwestern coastal area of Taiwan because of its high market value. The As concentration in BFD area pond water showed wide spatial varieties ranging from 8.1 to 251.7 $\mu g\ L^{-1}$ (Singh 2001; Liao et al. 2003).

Arsenic contents in several farming ponds even exceed the water quality criteria for total As in the freshwater ecosystems (150 $\mu g\ L^{-1}$) documented by the Criterion Continuous Concentration (USEPA 2002). Our previous studies revealed that waterborne As significantly accumulated in specific organs (e.g., gill, liver, and intestine) and manipulated growth toxicity to tilapia (Liao et al. 2004; Tsai and Liao 2006). However, knowledge about the process that links biouptake, detoxification, bioregulation, and the adverse effect of As to tilapia is still limited.

Trivalent (As(III)) and pentavalent (As(V)) arsenic entering animals may be rendered less toxic by methylation to monomethylarsonic and dimethylarsinic acids (Newman and Unger 2003; Sharma and Sohn 2009). Suhendrayatna et al. (2002) indicated that around 14–42% accumulated As were transformed to methylated As in tilapia under chronic exposure conditions. However, the rate of internal sequestration detoxification of metal is rarely quantified biokinetically (Croteau and Luoma 2009) and is often ignored during ecological risk assessment modeling, leading to the underestimation of the risk of metal toxicity.

To reveal how the aquatic organism regulating and acclimating the chemical stress, the damage assessment model (DAM) (Lee et al. 2002) had

been developed to depict quantitatively the time course of toxicity by integrating the mechanistic process between accumulated chemicals dose, the induced dynamics of tissue damage and recovery. Chemical elimination is not the only process for the recovery of organisms. Even after the chemical is eliminated, tissue damage will need to be repaired before the tissue will fully function (Schuler et al. 2004). This is particular true for sublethal and chronic exposure predictions. These mechanistic processes are particular true for long-term real field exposures risk assessments.

There is an urgent need that a comprehensive ecological risk assessment framework is supposed to link the toxicokinetics and toxicodynamics knowledge to elucidate a more reliable result for long-term exposure risk assessment. The purposes of this study are (1) to quantify the detoxification ability of tilapia and to predict the internal active dose responsible for As toxicity, (2) to develop a mechanistic-based algorithm that integrate the process of detoxification and biological regulations, and (3) to assess how the As affects on the tilapia growth in different exposure scenarios.

Materials and methods

Experiments

Male tilapia *Oreochromis mossambicus* with ages of 9 months (mean body length = 10.5 ± 1.3 cm (mean ± SD) and mean weight = 16.58 ± 1.52 g wet wt.) were hatched in laboratory and considered to be uncontaminated by As. Tilapia were allowed to acclimate in tap water at least 14 days before the initiation of exposure tests. Mortality was less than 5% of the population during the acclimatization, and no weight losses were observed. Experiments employed an aqueous exposure route. Chemical stock solutions were prepared by dissolving a calculated amount of reagent-grade sodium arsenite ($NaAsO_2$) in deionized water. Stock solutions were diluted to the nominal concentrations with local tap water. All experiments were carried out in 54-L indoor rectangular glass aquaria in that the dissolved oxygen in each tank was maintained at close to saturation by aeration (7.2 ± 0.1 μg mL^{-1}). The temperature in each aquarium was maintained at 26.7 ± 0.24°C using submerged heaters. The pH values maintained at 7.8 ± 0.02. The photoperiod was 16 h light/8 h dark with an intensity of 1,400 ± 100 lux.

Bioaccumulation assays were conducted to investigate the time course of biouptake of fish in As concentration of 1 μg mL^{-1} for 7 days based on the experimental setting and protocol of Suhendrayatna et al. (2002). The measured As concentration was 0.89 ± 0.06 μg mL^{-1}, and the experimental As concentrations were 20–50 times higher than that in the field environment to produce high As level in tilapia. The fish were fed with a commercial fish food once a day, 7 days a week at a low rate of 0.5% of fish biomass to avoid As contamination of feed remaining in the aquaria. Five fish were sequentially harvested from solutions after 0, 1, 2, 4, and 7 days of exposure. Fish were rinsed with deionized water and then were anesthetized in Benzocaine hydrochloride (Sigma Chemical Co., St. Louis, MO) solution. Fish samples were freeze-dried overnight and then ground to fine powder in a grinder (Tai-Hsiang S36–89, Taiwan). A 500-mg portion of the powder was digested in 10 mL concentrated HNO_3 (65% wt.) overnight at room temperature. The resulting solution was evaporated and the residues redissolved in 0.1 N HCl.

A 4-week bioassay was carried out for assessing the chronic toxic effects on tilapia growth. The nominal As concentrations were designed based on the result of preliminary text and were assigned as 0, 1, 2, and 4 μg mL^{-1}. The corresponding measured As concentrations are 0.93 ± 0.45, 1.85 ± 0.43, and 3.86 ± 0.7 μg mL^{-1}, respectively. All the chronic tests were repeated two times, and each concentration was assigned to two replicate tanks for 28 days. For each concentration of As, 10 tilapia were exposed. Fish were fed twice a day with commercial fish food of 4% of fish biomass. A 50% As solution was replaced every 1 to 2 days to avoid the regression of ambient water quality and As concentration. The whole As solution was replaced weekly in each tank. The specific daily growth rate (k_g, day^{-1}) of tilapia in different As concentrations was estimated as (Sherwood et al. 2000), $k_g = \ln(W_t/W_0)/dt \times 100\%$ where W_t and W_0 are the body weight of tilapia at time t and

the initial of experiment, respectively. Growth coefficient (mean body weight after 1, 2, 3, and 4 weeks/mean body weight at the start of the experiment) was calculated for each As concentration every week.

Chemical and data analysis

A Perkin-Elmer Model 5100PC atomic absorption spectrometer (Perkins-Elmer, Shelton, CT, USA) equipped with an HGA-300 graphite furnace atomizer was used to analyze As. Analytical quality control was achieved by digesting and analyzing identical amounts of rehydrated (90% H_2O) standard reference material (Dog fish muscle, DORM-2, NRC-CNRC, Canada). Recovery rate was 96.4 ± 2.7% and the levels of detection were 0.62 μg As L^{-1} for water samples and 0.05 μg As g^{-1} for tissue samples. Arsenic concentrations were detected in each test medium; exposure water characteristics during the test were measured three times weekly in one selected replicated aquarium for analysis of As. The 10 mL water samples were acidified (pH < 1) with 5 mL 1 N HNO_3 and then stored at −4°C in the dark until they were analyzed.

The method to estimate biokinetic parameters was by fitting the integrated form of the biokinetic equation for constant water exposure to measured data, using iterative nonlinear regression (Newman and Unger 2003),

$$C_f(t) = C_f(0)e^{-(k_2+k_g+k_{dex})t} + \frac{k_1}{k_2+k_g+k_{dex}} \times C_w\left(1-e^{-(k_2+k_g+k_{dex})t}\right), \quad (1)$$

where C_f is the time-dependent As concentration in tilapia (μg g^{-1}), k_1 is the uptake rate constant (mL g^{-1} day^{-1}), k_2 is the depuration rate constant (day^{-1}), k_g is growth rate of fish (% day^{-1}), k_{dex} is the detoxicifcation rate (d^{-1}), C_w is the waterborne As concentration (μg mL^{-1}), and t is the exposure time in days. The bioconcentration factor (BCF) can be calculated as: $BCF = k_1/(k_2+k_g+k_{dex})$, representing the net accumulation ability that is the result of the competition between uptake and depuration associated with growth dilution and detoxification. The basic assumption of the bioaccumulation model is that the k_1 and k_2 are constant and are independent of the C_w.

The quantitative relation between the total accumulated As in tilapia and the corresponding exposure As concentrations was constructed based on the result of the present biouptake bioassay conducted in 1 μg mL^{-1} together with the published measurements in 5, 10, and 15 μg mL^{-1} (Suhendrayatna et al. 2002). Croteau and Luoma (2009) proposed a parsimonious method to quantify the metal detoxification rate constant by linking the biokinetic bioassays and biokinetic modelings. They assumed that the metal uptake influx is proportional to the waterborne metal concentration and the rate of metal loss is a function of the rate constant for physiological loss (k_2, day^{-1}), detoxification rate (k_{dex}, day^{-1}) and the internal accumulated metal concentration. The k_{dex} can be estimated when the rate of chemical influx equals or begins to exceed the combined rates of chemical loss and detoxification as,

$$k_{dex} = \left(\frac{k_1 \times C_w}{C_{IT}}\right) - k_2, \quad (2)$$

where C_{IT} is the critical chemical concentration in the organism at the influx threshold (μg g^{-1}). C_{IT} can be estimated by numerically solving the differential form of the fitted equation.

Mechanistic models

The bioregulation model, DAM, describes the mode of action (MOA) of compounds with rapid reversible binding to the target site as well as to those that act with irreversible binding. This model provides a more comprehensive framework to investigate the time course of toxicity by incorporating the co-influence of chemical accumulation and damage accumulation. The DAM-based median effect concentration [$EC_{50}(t)$] is derived from the first-order damage accumulation model and given as (Lee et al. 2002),

$$EC_{50}(t) = \frac{D_{E,50}/k_a}{\left(\frac{e^{-k_r t}-e^{-(k_2+k_g+k_{dex})t}}{k_r-(k_2+k_g+k_{dex})} + \frac{1-e^{-k_r t}}{k_r}\right)} BCF^{-1}, \quad (3)$$

where k_a is the damage accumulation rate (g μg^{-1} day^{-1}), k_r is the damage recovery rate constant (day^{-1}), $D_{E,50}/k_a$ is a coefficient reflects the compound equivalent toxic damage level required for 50% toxic effect (μg day g^{-1}). Here $D_{E,50}/k_a$ and k_r are referred to as the bioregulation parameters. With sufficient $EC_{50}(t)$ data of a given duration range, the best-fit values of the $D_{E,50}/k_a$ and k_r in Eq. 3 could be estimated by using a nonlinear regression technique.

Our previous study revealed that the growth toxicity of As to tilapia was exerted by reducing the food assimilation efficiency and could be predicted by a bioenergetical-based ontogenetic growth model (West et al. 2001; Tsai and Liao 2006; Tsai et al. 2009) as,

$$W(t) = W_{\max}\left\{1 - \left[1 - \left(\frac{0.05}{W_{\max}}\right)^{1/4}\right] e^{-a_0 t/4W_{\max}^{1/4}}\right\}^4, \tag{4}$$

where a_0 is a species-specific the growth coefficient (g$^{1/4}$ day^{-1}). It can be estimated by optimal fits of the original form of West growth model (West et al. 2001) to the measured growth profile of control fish. $W(t)$ is the time-dependent body weight, W_{max} is the ultimate body weight (g) of contaminated tilapia related to chemical stress as $W_{max} = W_{max\,0} \times S(t)$ where W_{max0} is the maximum body weight (g) of tilapia in uncontaminated water, and was recorded as 1,130 g (www.fishbase.org/home.htm). We replaced the original semi-empirical linear chemical stress function with a DAM-based safety function, $S(t)$, to relate the health rate of organism to body residues and cumulative damage as $S(t) = e^{-H(t)}$ where $H(t)$ is the cumulative hazard (dimensionless). $H(t)$ can be linked to tissue damage as $H(t) = k_3 \times D(t)$ where k_3 is a proportionality constant (dimensionless), $D(t)$ is the time-dependent cumulative damage (dimensionless) and can be estimated from the solution of first-order damage accumulation model, $dD(t)/dt = k_a C_f(t) - k_r D(t)$ where $C_f(t)$ is the accumulated chemical in organisms which could be predicted from Eq. 1. This leads to the following expression for $S(t)$ as (Lee et al. 2002)

$$S(t) = e^{-\left[k_3 k_a \times \mathrm{BCF} \times C_w\left(\frac{e^{-k_r t} - e^{-(k_2+k_g+k_{dex})t}}{k_r-(k_2+k_g+k_{dex})} + \frac{1-e^{-k_r t}}{k_r}\right)\right]}. \tag{5}$$

Kooijman and Bedaux (1996) introduced a constant, called killing rate ($k_\dagger$), to represent a measure for the toxicity of a compound and has the dimension [(tissue concentration × time)$^{-1}$] in that $k_\dagger = k_3 k_a$. The killing rate is the proportionality factor that describes the relation between the accumulated hazard and the cumulative damage. In the case of 50% effect, $k_\dagger$ values are calculated as $\ln 2/\left(D_{E,50}/k_a\right)$, followed the scheme of Lee et al. (2002).

Model validation and statistic analysis

The growth toxicity model was validated if the model predictions fall within the error limits of the measured growth data. We validated the DAM-based safety function and estimated the biokinetic (k_1 and k_2) and DAM-based bioregulation parameters ($D_{E,50}/k_a$ and k_r) by using the non linear regression option of the Statistica® software (StatSoft, Tulsa, OK, USA). Statistica® software was also used to calculate the coefficient of determination (R^2) of nonlinear curve fittings and for statistical analyses, $p < 0.05$ was considered significant.

Results

Bioaccumulation and detoxification of As

Biokinetic parameters, i.e., the biouptake rate constant (k_1) and biodepuration rate constant (k_2), were estimated to be 0.49 mL^{-1} g^{-1} day^{-1} and 0.17 day^{-1}, respectively (Fig. 1b), by optimal fitting the Eq. 1 to the time series of measured accumulated As burden ($p < 0.05$). Figure 1a shows that the As burden in the end of 7-day exposures was proportional to their waterborne concentrations when $C_w < 5$ μg mL^{-1}. However, at the concentrations of 10 and 15 μg mL^{-1}, the

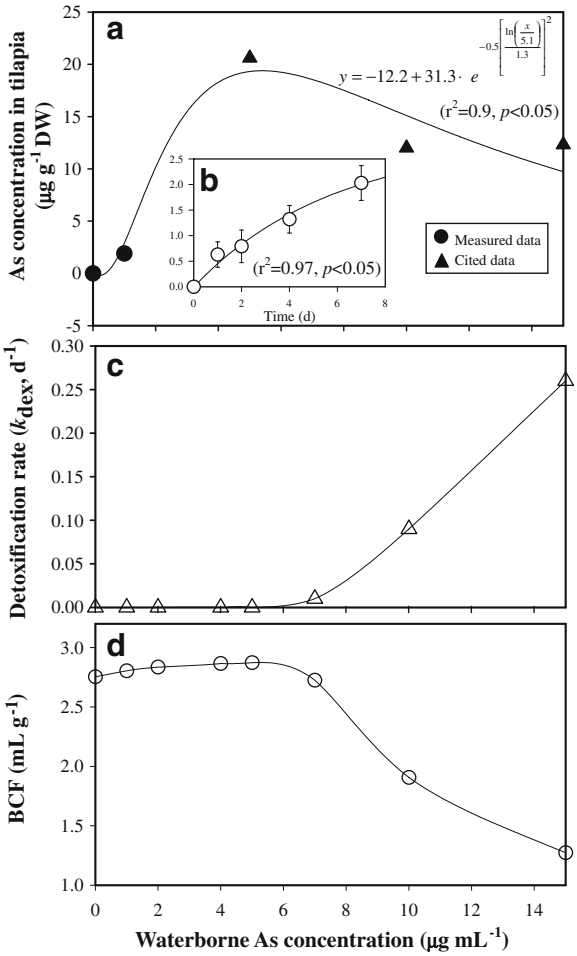

Fig. 1 Quantitative relations between **a** accumulated As by tilapia in the end of 7-day biouaptake bioassay and waterborne As concentration (C_w). The *solid line* is the best-fit regression curve. **b** Bioassays of tilapia exposed to 1 μg mL^{-1} waterborne As during a 7-day uptake. *Symbols* represent mean ± 1 SE ($n = 5$). The *solid line* is the best-fit regression curve from the bioaccumulation model. **c** Detoxification rate of tilapia and C_w, and **d** BCF and C_w

accumulated As was less because the higher concentration of As stressed to the fish, thus reducing accumulation of As by tilapia (Suhendrayatna et al. 2002). The critical chemical concentration in the organism at the influx threshold (C_{IT}) was estimated to be 19.1 μg mL^{-1}.

By incorporating k_1, k_2 and C_{IT} into Eq. 2, the waterborne concentration-dependent detoxification rate (k_{dex}) can be estimated. Result showed that values of k_{dex} were zero, i.e., no detoxification occurs, when the fish were exposed to waterborne As <5 μg mL^{-1}. In contrary, k_{dex} showed positive values, i.e., detoxification activating when C_w raised higher than the C_{IT}. Values of k_{dex} increased with increasing waterborne As concentration and were estimated to be 0.01, 0.09 and 0.26 day^{-1} in 7, 10, and 15 μg mL^{-1} of As, respectively (Fig. 1c). Values of k_{dex} was comparable to or even higher than the k_2 when the fish were exposed to waterborne As >10 μg mL^{-1}. k_{dex} was estimated to be 45, 1,800, and 65,000 times higher than the growth dilution rate when the tilapia exposed to 7, 10, and 15 μg mL^{-1} As solution, respectively. Result suggested that the rate of detoxification should not be ignored in higher exposure conditions.

Growth toxicity and bioaccumulation factor

Results of chronic toxicity bioassays showed that the growth of tilapia were significantly inhibited by assigned treatments comparing to control group ($p < 0.05$) and showed concentration-dependent growth trajectories (Fig. 2a). Figure 2a shows the concentration-specific growth coefficient of tilapia in 4-week chronic bioassays. The growth coefficients were negatively proportional to the external As concentration. They were all above 1 besides those of the tilapia groups in 4 μg mL^{-1} during the 2nd, 3rd weeks, indicating that not only inhibitions of growth were observed but also the thinning of the body weight occurred in this study. The concentration-specific growth coefficients were derived to establish the regression equations to calculate $EC_{50}(t)$ values on a weekly basis. $EC_{50}(t)$ values decrease from 3.3 1.99 μg mL^{-1} in selected weeks (Table 1).

In the control groups, the specific growth rate (k_g) was calculated to be 0.8% per day. k_g of control group fell within the reported values ranging from 0.4% to 0.18% per day (Uchida et al. 2003; Tsai and Liao 2006). k_g of exposure tilapia were negatively correlated to C_w and were calculated as 0.5% per day in 1 μg mL^{-1}, 0.28% per day in 2 μg mL^{-1}, and 0.1% per day in 4 μg mL^{-1}. The tilapia almost stopped growing in 4 μg mL^{-1}, the k_g value approximately 41-fold lower than that in control condition.

The quantitative relationship between k_g and C_w were well described by fitted statistical model

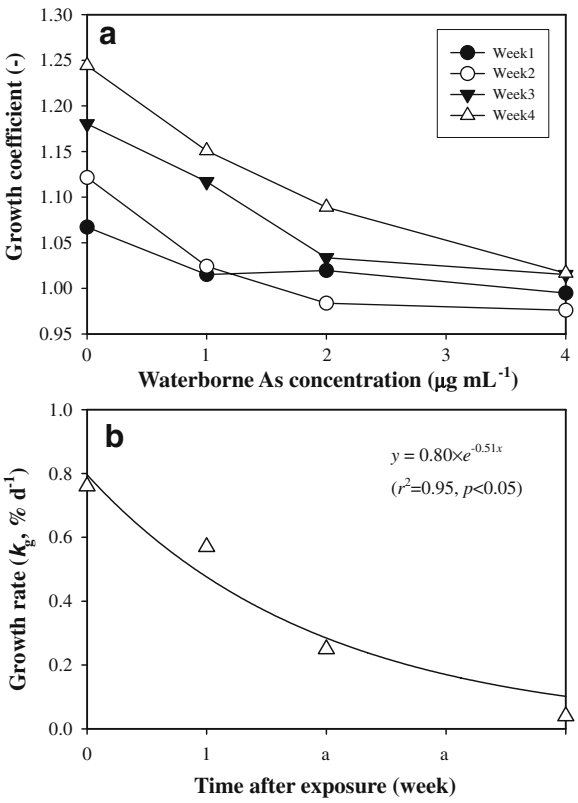

Fig. 2 **a** Growth coefficient of tilapia versus waterborne As concentrations during different exposure periods. **b** The growth rate of fish in different treatments. The *solid line* is the best-fit regression curve

of $y = 0.80 \times e^{-0.51x}$ ($r^2 = 0.95$; Fig. 2b) and the k_g in higher exposure concentrations (5–15 μg mL^{-1}) could be predicted by that fitted model. BCF in different concentrations could be calculated as $BCF = k_1/(k_2 + k_g + k_{dex})$ which increased from 2.75 to 2.87 mL g^{-1} when the fish were exposed to C_w lower than 5 μg mL^{-1}. In the contrary, BCF decreased from 2.73 to 1.27 mL g^{-1} when C_w was raised above the C_{IT} because the activating of detoxification (Fig. 1d).

Biodynamic parameters and model validation

We assessed the DAM (Eq. 3) and estimated the essential bioregulation parameters ($D_{E,50}/k_a$ and k_r) by optimal fits Eq. 3 to the $EC_{50}(t)$ data (Fig. 3). The input parameters include k_2 and BCF derived from the bioaccumulation experiment. $D_{E,50}/k_a$ and k_r are estimated to be 4.1 ± 3.8 μg day g^{-1} and 1.3 ± 1.2 day^{-1}, respectively ($r^2 = 0.97$, $p < 0.05$). Figure 4a–d shows the results of the model prediction comparisons to the measured growth data. The predicted values all fell within the error limits of the observations in C_w of 0–2 μg mL^{-1}. Although the quality of the prediction was relative low in C_w of 4 μg mL^{-1}, most of the predicted value was still within error limits, characterizing the trend of the measured data.

Table 1 Estimated chronic toxic effects of regressive equations of As on growth for tilapia *O. mossambicus* after one to four weeks

Time after exposure (week)	Regression equation[a]	r^2	EC_{50} (μg mL^{-1})[b]
1	$y = -0.017x + 1.05$	0.85	3.302
2	$y = -0.033x + 1.10$	0.89	2.201
3	$y = -0.030x + 1.18$	0.93	2.012
4	$y = -0.045x + 1.24$	0.95	1.998

[a] x is waterborne arsenic concentration and y is the concentration-specific growth coefficient
[b] EC_{50} is the estimated external effect concentration for 50% growth inhibition

Health rate and life-cycle risk assessment

Figure 5a shows the temporal trends of health rate ($S(t)$) under chronic and sublethal exposure conditions ($LC50(\infty)$ = 5.9 μg mL^{-1},

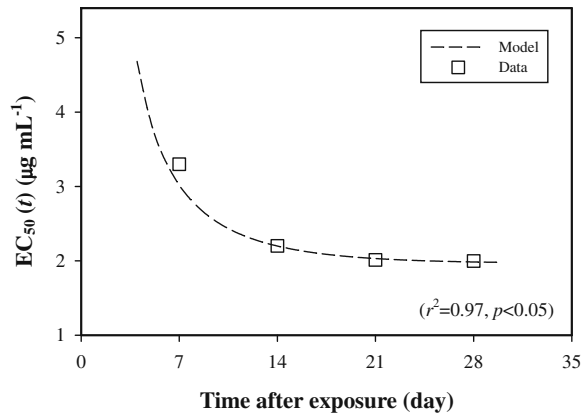

Fig. 3 Temporal trends of $EC_{50}(t)$ value in selected time and optimal fits of the DAM to data

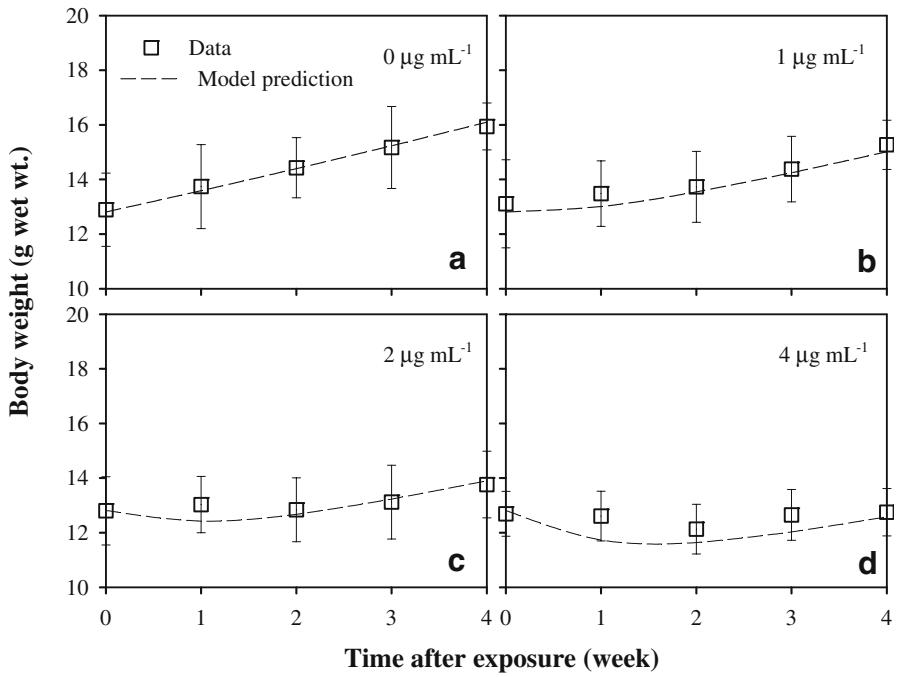

Fig. 4 Comparisons between the model prediction and the corresponding measured data (mean ± SD) obtained from 28-day growth toxicity experiments in different waterborne As concentrations

Tsai et al. 2006 predicted by Eq. 5 with the input of chronic bioregulation parameters ($D_{E,50}/k_a$ and k_r) and biokinetic parameters (k_2, k_g, k_{dex} and BCF). The safety function characterized the time trends of the concentration-specific heath rates. Result showed that the control group kept 100% health throughout the simulation. Rates of exposed fish decreased with C_w and shows a dramatic decreasing from 100% initially and then slowly approached to a steady-state values of 0.70, 0.48, 0.23, and 0.16 in concentrations of 1, 2, 4, and 5 μg mL^{-1}, respectively. Simulations revealed the potential biologically regulation of organisms when exposed to chemicals, especially in the first few days of exposures. When the fish were exposed to waterborne ≧7 μg mL^{-1}, S(t) values quickly dropped to <0.5 in the 2nd day of exposure and remained in the rate ≦0.1 after 14th day of the exposure.

We employed the growth toxicity model to illustrate the growth trajectories of tilapia from birth to natural death in different exposure scenarios (Fig. 5b). The reported life-span of tilapia *O. mossambicus* is recorded as 11 years (approximately 4,000 days; www.fishbase.org/home.htm). Tilapia in different treatments were all assumed to be able to live to the end of their whole lifespan. The biomass of fish in different treatments were predicted to increase exponentially with time, decreased with C_w, and the growth trajectories showed concentration-specific patterns when the exposed to $C_w \leqq 5$ μg mL^{-1}. The maximum body weight (W_{max}) of the control tilapia was predicted to be 1,038.9 g. For the groups exposed to 1, 2, 4, and 5 μg mL^{-1} of As, the predicted W_{max} values were 744.6, 526.6, 258.4, and 180.2 g, respectively (Fig. 5b). When the fish was exposed to $C_w > 7$ μg mL^{-1}, the growth curves were almost consistent with the treatments. Biomass stopped increasing after the 1500th day to the end of life. The temporal pattern of the predicted growth inhibition was governed by the temporal of corresponding health rate in different concentrations.

Discussion

Our study showed that the detoxification rate (k_{dex}) was initiated and increased with the waterborne metal concentration once the accumulated

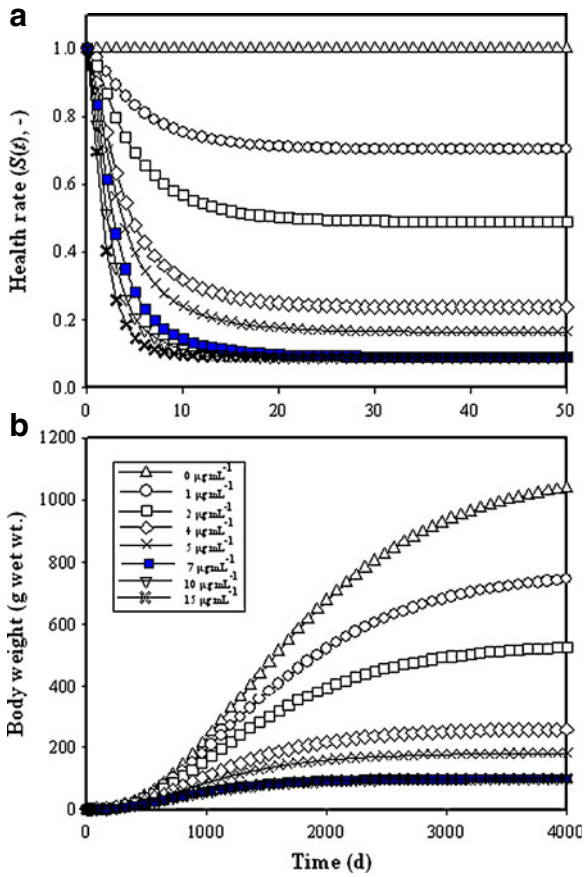

Fig. 5 **a** Temporal trends of predicted health rate profiles varied with different waterborne As concentrations applied by the DAM. **b** Predictions of the time course of the biomass of tilapia *O. mossambicus* during their entire life span in different waterborne concentrations by using the proposed growth toxicity model

metal concentration exceeded the metal influx threshold (C_{IT}) during short-term exposure conditions. However, when the metal burden is lower than the C_{IT}, the detoxification rate is nearly zero, indicating physiological loss process (k_2) is the major role for regulating the metal content. As to our knowledge, no studies have reported the biokinetic and bioregulation processes of arsenic in freshwater fish, although the active regulation of fish for other metals has been studied. Dang et al. (2009) found that k_1 and k_2 does not change significantly between treatments and durations during chronic exposures. They also found that the detoxification processes, e.g., metallothionlike proteins fraction account for the newly accumulated Cu in tissues increase with time and internal metal concentrations. Croteau and Luoma (2009) revealed that freshwater snail *L. stagnalis* detoxified the accumulated metal, rather than reducing uptake or intensifying excretion when exposed to different treatments. The detoxification rate for Cu, Cd and Ni in snail was observed to be two orders of magnitude higher than the k_2. Voets et al. (2009) indicated that the storage of accumulated metal in detoxified subcellular component increase with metal concentration in the organisms, especially in more polluted individuals. These studies support our findings about the active role of detoxification during the biokinetic process.

Acute-to-chronic rations are widely employed to derive quality standards for prolonged exposure. However, changes in toxicity upon long-term exposure might be attributed to a change in the mode of action and the induction of physiological acclimation or genetic adaption to local contaminant regimes (Forrester et al. 2003; Muyssen and Janssen 2005). This would cause the limitations to assess the long-term chemical effects by acute toxicity data. Because it seems plausible that organisms might somehow become weakened after enduring long-term chemical loading, and nonspecific, initially sublethal effects might worsen with time. Our study showed that BCF decreased with waterborne metal concentration (C_w) in higher exposure conditions ($C_w > 5.1$ μg mL^{-1}). However, the corresponding predicted health rate (Fig. 5a) and growth performance of fish (Fig. 5b) still decreased with C_w. This indicated that the metal toxicity could not be predicted accurately merely depended on the amount of accumulated metal. The processes of toxicodynamics or bioregulation are critical to be involved in the scheme of the exposure risk assessment. We developed a mechanistic-based scheme by linking the process of detoxification, bioregulation and mode of toxic action to predict the chronic toxicity of As on organisms in their entire life span. This approach improved the using of short-term lab data for extrapolating the long-term prediction, even though there are still some limitations needed to be overcome.

The proposed models were validated and were performed by assuming that the essential bioki-

netic (BK) parameters (k_1, k_2, and BCF) are time- and concentration-independent. The modes of toxic effect (MOA) are also assumed to be identical throughout the whole life span. Because the model parameters were analyzed in view of the biological assumptions of the models, and the fit of model was strongly affected by these input parameters. Therefore, the uncertainties in the input values of k_1, k_2 and BCF affect the validation and the performance of the models. However, these might be controversial and would cause uncertainty in predictions. Because identical BK parameters and mode of action imply that there will be similar mechanisms between the exposure concentration and time. If the acclimation/adaptation occurs, this results in the changes of BK and BD processes or the MOA. These changes should be considered in longer term risk assessment.

Several studies showed an inverse relationship exists between BCF and exposure concentration. The relatively higher value of BCF obtained from lower chemical exposures may result from the active regulation or acclimation of organism to chemicals. Liao et al. (2003) revealed that the field tilapia featured with higher As accumulation ability (BCF = 143–421) than those adopted in their 7-day lab bioaccumulation assays (BCF = 1.04–4.19), in which the lab group exposed to the waterborne As concentration about 30 times higher than the field group. Kraemer et al. (2008) revealed that fish can alter their ability to decrease uptake and increase elimination cadmium in longer time or higher concentration in the field situation. Thus, the first-order bioaccumulation model for metals might only applicable for residue predictions in lower range of exposures, in which the uptake process is not limiting the rate of uptake (McGeer et al. 2003). Thus, the BK parameters in higher concentration or longer exposure were suggested to be re-evaluated in the future studies.

Our study assessed the life-cycle risk assessment of metal to aquatic organisms by linking the West growth model and the DEB_{tox} theory in a bioenergetics-based aspect. The DEB_{tox} theory distinguishes three types of MOA on growth toxicity, including direct effects and indirect effects via maintenance and assimilation. The inherent assumption of the theory is that only one of these MOAs exerts at the same time in the lower effect range of the chemical (Kooijman and Bedaux 1996) because that multi-MOA effects might concur to induce the toxicity in higher concentrations. For example, Sherwood et al. (2000) indicated that the growth inhibition of yellow perch in heavy metal- (Cd, Cu, and Zn) polluted lake was attributed to a reduced food conversion efficiency of the fish and not just simply to a reduced food intake. Tsai et al. (2006) revealed that the incipient external median lethal concentration value of As to tilapia is 5.9 $\mu g\ mL^{-1}$, and the quality of our toxicity model prediction decreased in $C_w = 4\ \mu g\ mL^{-1}$, implying that the performance of the single MOA-based models may be restricted in chronic and sublethal exposure ranges.

A life-cycle toxicity test provides vital knowledge for chemical risk assessment in population and community levels. Although providing a wealth of information, these tests are usually extremely hard to analyze, because testing life-cycle consequences is too complicated and too expensive for routine applications. Consequently, short-term testing with selected life cycle is used as a surrogate (Jager et al. 2004). Here, we adopted a 28-day growth bioassay data of sub-adult tilapia to predict growth trajectories of tilapia in whole life span. The merit of West growth model can elucidate the growth trajectories of organism over the entire life cycle solely based upon the growth information in a selected time interval. However, the difference in chemical sensitivity between life stages should be further considered if the life-cycle-specific toxic response is observed. Alternatively, some studies assumed that chemicals affect organisms by impairment of those life-cycle variables that are most sensitive to these toxicants. For example, to assess the impact of contaminants on organisms, a general toxicological approach is to quantify the response of juvenile, because this is often known to be the most sensitive life cycle variable with respect to chemical stress (DeLonay et al. 1993; Kammenga et al. 1996). To explicitly assess the chemical effects for a longer-term aspect, multi-life stage toxicity bioassays should be involved in the process of toxic texts to explicitly assess the metal toxicity to the entire life cycle of organisms.

Conclusions

In conclusion, the proposed detoxification- and bioregulation-based growth toxicity model facilitates us to make a comprehensive survey of growth effects in entire life cycle of an organism stressed by metals. It is important to address the fact of the obvious portion of inactive metal metabolite stored in the tissue and the physiologically regulation of organism when suffering long-term metal exposures. The ignorance of metabolite, however, might lead to underestimation of the metal toxicity. Moreover, the change in modes of toxic action can have similar effects at the individual level yet very different consequences when the data are integrated at the population, community, or ecosystem levels (Barata and Baird 2000). We believed that the proposed mechanistic-based study improves any attempt to set up predictive models for metal ecotoxicological assessment.

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