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Mode of Action and Growth Toxicity of Arsenic to Tilapia *Oreochromis mossambicus* Can Be Determined Bioenergetically

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Abstract. We present a bioenergetics-based approach to analyze the chronic effects and growth toxicity mode of action in tilapia *Oreochromis mossambicus* exposed to waterborne As and to predict fish growth under different exposure scenarios. 7-day exposure bioassays showed that tilapia accumulate As when exposed to waterborne As. We conducted growth bioassays to assess chronic As toxicity to tilapia. We incorporated a universal ontogenetic growth model with the DEB_{tox} theory to explore the mode of action of As toxicity. Our results show that the specific growth rates of exposed tilapia are inversely proportional to As concentrations and are calculated as 0.76% d^{-1} in 0 μg mL^{-1}, 0.57% d^{-1} in 1 μg mL^{-1}, 0.2 % d^{-1} in 2 μg mL^{-1}, and 0.04% d^{-1} in 4 μg mL^{-1} As, respectively. We showed that the internal threshold concentration did not change significantly with time, demonstrating that the critical body residue approach is applicable for As toxicity assessment. We distinguished between three modes of action of As, including direct effects on growth and indirect effects by way of maintenance and food consumption. Our results support that decreased feeding accounts for the growth decrease in the case of feeding *ad libitum*. The feeding decrease model also illustrates the growth trajectories of tilapia during the entire whole life span, suggesting that the maximum biomass of tilapia are 1038.75 g in uncontaminated water and 872.97 g in 1 μg mL^{-1}, 403.06 g in 2 μg mL^{-1}, and 336.65 g in 4 μg mL^{-1} As, respectively. We suggest that considering modes of action in ecotoxicology not only improves our understanding of the toxicities of chemicals, it is also useful in setting up models and avoiding pitfalls in species- and site-specific environmental risk assessment. This proposed framework for tilapia gives preliminary information relevant to aquacultural and ecologic management.

Long-term ingestion of the groundwater contaminated by inorganic As has been found to induce blackfoot disease (BFD) in residents of the southwestern coastal area of Taiwan (Chen *et al*. 2001). Currently, most of the people living in these areas do not drink water from artesian wells because tap water has been made available in this area. However, artesian well water is still used for aquaculture. Farming tilapia (*Orechromis mossambicus*) is one of the most promising aquatic endeavors in the BFD area because of its high market value. Liao *et al*. (2003) pointed out that the As concentration in BFD-area pond water ranged from 8.1 to 251.7 μg L^{-1}. Arsenic contents in several farming ponds exceed the water-quality criteria for total As in freshwater ecosystems (150 μg L^{-1}) as documented by the Criterion Continuous Concentration (United States Environmental Protection Agency [USEPA] 2002). If As levels in pond water increase, severe effects may occur to the health of farmed fish and may even pose a potential risk to the people who consume tilapia farmed in the BFD area.

The use of assimilated energy has been extensively employed by physiology and ecosystem scientists in recent years to determine the growth of organisms and the productivity of ecosystems (Kooijman and Bedaux 1996; Beyers *et al*. 1999; Sherwood *et al*. 2000). Fish constantly consume energy to maintain life and offset the effects of multiple stressors such as daily fluctuations in water temperature, availability of food, and pollutants in the environment (Wedemeyer *et al*. 1984). Therefore, assessing the impact of chronic exposure to chemicals by using energy metabolism as a performance response could be a rigorous physiologic and ecologic approach to toxicity assessment.

Organisms acquire energy by ingesting food from their environment. The assimilated energy is stored in reserve before biologic use. Pery *et al*. (2003) pointed out that exposure to a toxic chemical may decrease resource acquisition from the environment and cause a decrease in reproduction rate. Beyers *et al*. (1999) indicated that organisms must compensate for these chemical stresses with detoxicification mechanisms, which require energy, and their effect can be evaluated using a bioenergetic model. Because maintenance (including detoxicification) cost has priority over growth in fish bioenergetic theory, maintenance cost competes with growth investment for the allocation of energy that is used from the reserves, and a decrease in assimilation translates into a decrease in the amount of energy that is used from the reserves (Beyers *et al*.

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1999; Congdon *et al*. 2001). Therefore, an increase in the energy cost for life maintenance could leads to a decrease in growth rate (Congdon *et al*. 2001).

A mode of action is defined as a common set of physiologic and behavioral signs that characterize a type of adverse biologic response (Landis and Yu 1999). Escher and Hermens (2002) indicated that elucidating the detailed chemical-specific modes of a metal's toxic action could enhance the prediction power of models by providing a mechanistic explanation for chemical risk assessment in ecotoxicology. Barata and Baird (2000) further suggested that the ecotoxicologic modes of action of different chemicals can be determined bioenergetically by studying sublethal effects on food acquisition and hence growth and reproduction rates.

In the present study, specific efforts were paid to quantitatively relate As concentrations in tilapia to extent of growth inhibition. We conducted bioassays to determine if growth decrease occurs in chronic-exposure conditions, including a 7-day bioaccumulation test to determine the toxicokinetic process of As and a chronic bioassay to observe the organism's growth trajectories in different exposure scenarios. We further developed bioenergetics-based mechanistic models to elucidate and predict growth effects of chronic As exposure. The objectives of this study were threefold: (1) to quantitatively determine the relations between As exposure and growth inhibition; (2) to identify the mode of action dominating As growth inhibition; and (3) to develop a residue-based mechanistic growth model to predict individual growth in different exposure scenarios. We believe a comprehensive understanding of the mode of action of As toxicity to tilapia will be of great benefit to aquaculture management.

Materials and Methods

Test Fish and Experimental Protocol

Male tilapia *Orechromis mossambicus*, age 8 to 9 months (mean body length 12.9 ± 1.54 cm (mean ± SD) and mean weight = 10.58 ± 1.52 g wet weight), were supplied by Taiwan Fisheries Research Institute (Tainan, Taiwan), where they are hatched in the laboratory and considered uncontaminated by As. Tilapia were visibly free of any deformities, lesions, or diseases. Fish were kept on ice during transport from Tainan to the Ecotoxicological Modeling Center, Department of Bioenvironmental Systems Engineering, National Taiwan University, Taipei, Taiwan. On arrival in our laboratory, the fish were allowed to acclimate in tap water at 27.7°C ± 0.24°C during a light-to-dark cycle of 12:12 for at least 14 days before the initiation of exposure tests. Fish were fed daily twice with artificial food, and water pH ranged from 7.6 to 7.8. Mortality was <5% of the population during acclimatization, and no weight losses were observed.

The experiments employed an aqueous exposure route, so all test media were prepared using deionized water. Chemical stock solutions were prepared by dissolving a calculated amount of reagent-grade sodium arsenite ($NaAsO_2$) in deionized water, and the new stock solutions were prepared as needed during the toxicity tests. All experiments were carried out in 54-L indoor rectangular fiberglass aquaria such that the dissolved oxygen in each tank was maintained at close to saturation by aeration (7.21 ± 0.1 μg mL^{-1}). The temperature in each aquarium was maintained at 26.7°C ± 0.24°C using submerged heaters. Water pH was maintained at 7.75 ± 0.02. The photoperiod was 16 hours of light to 8 hours of dark with a light intensity of 1400 ± 100 lux. All of the experiments were assigned to two replicate tanks. We replaced 40% to 60% As solution every 1 to 2 days to avoid the regression of ambient water quality. To keep the As concentration constant, the entire As solution was replaced weekly in each tank.

Three series of semistatic tests were conducted in this study. In the first series, we conducted a range-finding test to determine the As contamination level in the As bioaccumulation and chronic toxicity bioassays by exposing tilapia to differing As concentrations of 0.25, 0.5, 1, 2, 4, and 6 μg mL^{-1}. The results of the preliminary test revealed that the median lethal tolerance (LT_{50}) of tilapia at ≤ 4 μg mL^{-1} As was >28 days. In the second series, a bioaccumulation assay was conducted to investigate the time course of uptake and depuration of chemicals in tilapia. We conducted an uptake experiment in As concentration of 1 μg mL^{-1} for 7 days based on the suggestion by Suhendrayatna *et al*. (2002). The measured As concentration was 0.89 ± 0.06 μg mL^{-1} As in the bioassay. The As concentrations used in this experiment were 20 to 50 times higher than that in the field environment conditions needed to produce high As levels in tilapia.

Uptake Experiment

The fish were fed a commercial fish food once a day, 7 days a week, at a low rate of 0.5% fish biomass to avoid As contamination of the feed remaining in the aquaria. Uneaten food and faces were siphoned from the bottom of the aquaria every day. To conduct analysis of As accumulation kinetics, five fish were sequentially harvested from solutions after 0, 1, 2, 4, and 7 days of exposure. The fish were rinsed with deionized water and then anesthetized in pH-neutralized tricaine methane sulfonate (MS-222) (Sigma Chemical, 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% weight) overnight at room temperature. The resulting solution was evaporated and the residues redissolved in 0.1 N HCl.

Chronic Toxicity Bioassays

We conducted a 4-week chronic toxicity bioassay to determine the toxic effects on the tilapia growth response when exposed to waterborne As concentrations. The nominal As concentrations for the chronic test were 1, 2, 4, and 0 μg mL^{-1}, and the corresponding measured As concentrations were 0.87 ± 0.35, 1.77 ± 0.34, and 3.56 ± 0.69 μg mL^{-1}, respectively. All of the chronic tests were repeated 2 times, and each concentration was assigned to 2 replicate tanks for 28 days. For each dose of As, 10 tilapia were exposed. Fish were fed 2 times/d with commercial fish food at a rate of 4% fish biomass. Uneaten food was siphoned from the aquaria 30 minutes after feeding. We replaced 50% to 60% As solution every 1 to 2 days to avoid the regression of ambient water quality and As concentration. The entire As solution was replaced weekly in each tank. Mortality was monitored at 0, 6, and 12 hours through the first day of exposure, then twice daily until the end of the test. Every week, the mean body weights of each exposed group were recorded, and we calculated the growth rates in different As concentrations.

Chemical Analysis

A Perkin-Elmer Model 5100PC atomic absorption spectrometer (Perkin-Elmer, Shelton, CT) equipped with an HGA-300 graphite furnace atomizer was used to analyze As. Analytic quality control was

achieved by digesting and analyzing identical amounts of rehydrated (90% H_2O) standard reference material (dogfish muscle, DORM-2; NRC-CNRC, Canada). Recovery rate was 94.6% ± 3.6%, and levels of detection were 0.62 μg As L^{-1} for water samples and 0.05 μg As g^{-1} for tissue samples. We detected As concentrations in each test media, and 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.

Data Analysis

The specific daily growth rate (k_g, % d^{-1}) of tilapia was estimated as (Sherwood *et al*. 2000),

$$k_g = \ln\left(\frac{W_t}{W_0}\right)/dt \times 100 \quad (1)$$

where W_t and W_0 is the body weight of tilapia at time t and the initial of experiment, respectively. Determination of toxicokinetic parameters was done by fitting concentration data to the integrated form of the kinetic equation for constant water exposure using iterative nonlinear regression (Reinfelder *et al*. 1998; Clason *et al*. 2003),

$$C_f(t) = C_f(0)e^{-(k_2+k_g)t} + \frac{k_1}{k_2+k_g}C_w\left(1-e^{-(k_2+k_g)t}\right), \quad (2)$$

where C_f is the time-dependent As concentration in tilapia (μg g^{-1}), k_1 is the tilapia uptake rate constant (mL g^{-1} d^{-1}), k_2 is the depuration rate (d^{-1}) constant, and t is the time in days. The bioconcentration factor (BCF) can be calculated as: BCF = $k_1/(k_2+k_g)$, representing the net accumulation ability that is the result of the competition between uptake and depuration associated with growth dilution, and C_w is the mean measure waterborne As concentration (μg mL^{-1}). Equation 2 provides a toxicokinetics-based approach to predict the accumulative As profile in constant-exposure scenarios.

We expressed the growth of tilapia as growth coefficient (mean body weight after 1, 2, 3, and 4 weeks/mean body weight at the start of the experiment) (Gomot 1997) for each As concentration every week with respect to initial body weight before exposure to As. The values of the growth coefficient for each concentration were plotted with arithmetic coordinates with corresponding regression equations. The curves obtained give an estimation of the external effect concentrations for 10% growth inhibition (EC_{10}). The USEPA (2000) recommended that the internal effect concentration causing 10% response (IEC_{10}) could be used as a surrogate threshold of regulatory end point in ecologic risk assessment and that the IEC_{10} can be estimated from the critical body residue (CBR) model as $IEC_{10} = EC_{50} \times BCF$ (McCarty and Mackay 1993).

Models

We attempted to construct a bioenergetics-based model that reflects the mode of action to simulate the growth of tilapia under different exposure scenarios. The DEB_{tox} theory (Kooijman *et al*. 1996) describes the modes of action of chemical toxicity based on the emphasis of resource allocation, a bioenergetics-based viewpoint that differs from the general aspect describing changes in physiology or behavior inhibition. The basic assumption of the DEB_{tox} theory is that an organism must take up chemicals before they can exert an effect. Second, once the chemical is inside the target tissues, it increases the probability of an adverse response and affects a parameter of the general ontogenetic growth model (*e.g*., the assimilation rate). DEB_{tox} indicates that chemical effects act by way of three types of mode of action including direct effects on growth and indirect effects on maintenance and food assimilation and that only one of these effects occurs at a time in the lower effect range of the chemical (Kooijman *et al*. 1996).

West *et al*. (2001) developed a mechanistic model, referred to as the West growth model, to describe ontogenetic growth trajectories of organisms instead of using the conventional growth model based on a statistical approach. The West growth model is a general quantitative model based on fundamental principles for the use of the consumed energy between maintenance of existing tissue versus the reproduction of new biomass, and it has described the growth of many diverse species successfully (West and Brown 2004). This model characterizes the slowing of growth as body size increases as being related to limitations on the capacity to supply sufficient resources to support further increase in body mass. We adapted the West growth model as the growth model without toxicity (West *et al*. 2001):

$$W(t) = W_{\max 0}\left\{1-\left[1-\left(\frac{W_0}{W_{\max 0}}\right)^{1/4}\right]e^{-A_0 t/4W_{\max 0}^{1/4}}\right\}^4, \quad (3)$$

where W_{max0} and W_0 are maximum body weight (g) in uncontaminated water and mass at birth (g), respectively. A_0 is a species-specific growth coefficient ($g^{1/4}$ d^{-1}) in that $A_0 \equiv B_0 m_c E_{c0}^{-1}$, where B_0 is a taxon-specific constant (W), m_c is the mass of a cell (g), and E_{c0} is the metabolic energy required to create a new cell (J). A_0 can be estimated by optimal fitting Equation 3 to the body-growth profile in control exposure conditions.

We distinguished three modes of toxic action on As growth inhibition in tilapia: (1) increased cost of growth, (2) increased cost of maintenance, and (3) decrease feeding. McCarty and Mackay (1993), Kooijman and Bedaux (1996), and Pery *et al*. (2003) suggested that we may relate the extent of adverse effects proportional to the difference between accumulated chemical concentration ($C_f(t)$) and IEC_{10} in that IEC_{10} is adapted as the effect threshold for chronic growth inhibition. We introduced a stress function ($S(t)$) to describe the extent of adverse effect as (Kooijman and Bedaux 1996; Pery *et al*. 2003):

$$S(t) = b[C_f(t) - IEC_{10}(t)], \quad (4)$$

where b accounts for the level of toxicity (g g^{-1}) once C_f exceeds IEC_{10}. We predicted C_f in various exposure scenarios by incorporating experimental-derived toxicokinetic parameters into Equation 2.

In the case of increase growth energy cost, *i.e*., C_f exceeds IEC_{10}, we assume that the metabolic energy (E_c) required to create a new cell are multiplied by [1+$S(t)$] and expressed as $E_c = E_{c0}[1+S(t)]$, where E_{c0} is the growth energy cost in control condition. We have $S(t) = 0$ and $E_c = E_{c0}$ when $C_f \le IEC_{10}$. We substituted the effect function into the West growth model, obtaining the mode of action on growth cost:

$$W(t) = 1130\left\{1-\left[1-\left(\frac{0.05}{1130}\right)^{1/4}\right]e^{-At/4\times 1130^{1/4}}\right\}^4, \quad (5)$$

where $A = B_0 m_c (E_{c0}[1+S(t)])^{-1} = A_0[1+S(t)]^{-1}$ and the two constants of 1130 and 0.05 represent the maximum biomass and the mass at

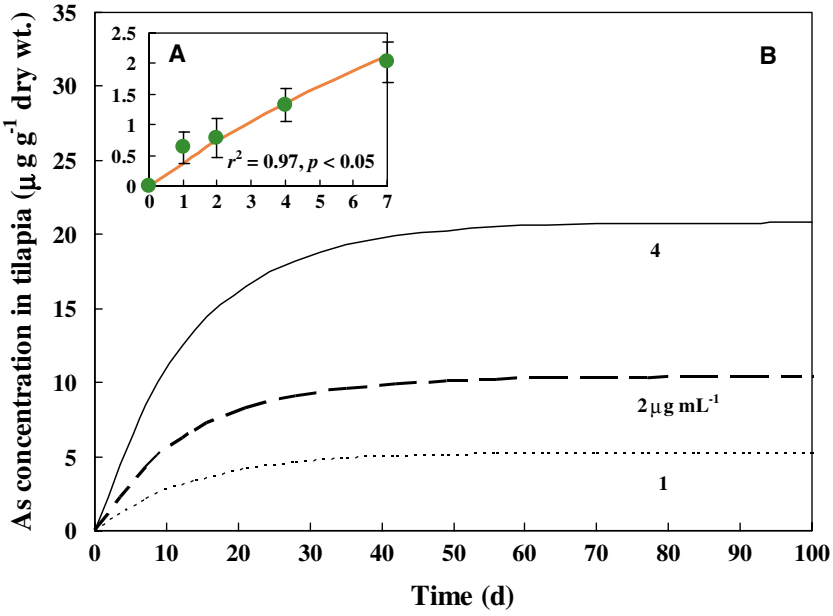

Fig. 1. (**A**) Bioassays of tilapia exposed to 1 μg mL^{-1} waterborne As during a 7-day uptake. Symbols represent mean ± 1 SE (n = 5). The solid line is the best-fit regression curve from the one-compartment bioaccumulation model of tilapia. (**B**) The predicted As concentration in tilapia when exposed to 1, 2, and 4 μg mL^{-1} As during a 100-day uptake

birth (g) of tilapia, respectively, in uncontaminated water (www.fishbase.org/home.htm).

When maintenance energy cost increases, chemicals are likely to increase maintenance costs to compensating for the effects of exposure (Beyers *et al*. 1999). Because maintenance cost has priority over growth, such an increase leads to decreased growth rate. We multiplied body weight by [1+$S(t)$] to account for an increase in the maintenance costs, resulting in the decreased time-dependent body weight (Kooijman *et al*. 1996):

$$W(t)[1+S(t)] = 1130\left\{1-\left[1-\left(\frac{0.05}{1130}\right)^{1/4}\right]e^{-A_0 t/4\times 1130^{1/4}}\right\}^{1/4}. \quad (6)$$

In contrast, when feeding decreases, a growth decrease acts by decreasing incoming energy. The maximum assimilation rate does not appear in the West growth model, yet it can be captured by maximum weight (W_{max}) (Kooijman and Bedaux 1996). Here, maximum weight is defined analogously to the definition of maintenance cost to account for the growth decrease effect on assimilation as $W_{max} = W_{max0}$[1-$S(t)$], and substituting that relation into the West growth model (Equation 3) leads to:

$$W(t) = W_{max}\left\{1-\left[1-\left(\frac{0.05}{W_{max}}\right)^{1/4}\right]e^{-A_0 t/4W_{max}^{1/4}}\right\}^4. \quad (7)$$

Equations 5, 6, and 7 describe the modes of action that lead to effects on either coefficient of growth cost (A), time-dependent body weight ($W(t)$), or ultimate body weight (W_{max}). Parameters of A_0, $S(t)$, and W_{max} are estimated by fitting the West growth model (Equation 3) and three effect models (Equations 5, 6, and 7) to concentration-specific growth data using iterative nonlinear regression. A standard analysis of variance test (ANOVA; Scheffe's t test) was employed to determine the significance of differences between model values and mean actual data on body weight in different groups. In addition, goodness-of-fit was evaluated using the sum of squares (SSs) between the description and data, computed from $SS = \sum_{i=1}^{N}(x_i - X_i)^2$, where N denotes the number of measurements, x_i is the predicted data, and X_i is the measured result corresponding to data point i.

We employed the nonlinear option of Statistica software (StatSoft, Tulsa, OK) to perform all curve fittings in this study. Statistica software was also used to calculate the coefficient of determination (r^2) and statistical analyses (ANOVA and Student t test). Statistical significance was judged at $p < 0.05$.

Results

Toxicokinetics

The 7-day water-exposure bioassay of As in tilapia was significantly correlated with nonlinear regression profiles (r^2 = 0.97, $p < 0.05$) resulting from the best fit of the first-order bioaccumulation model (Fig. 1A). The estimated uptake rate constant (k_1), depuration rate constant (k_2), and BCF were 0.39 mL g^{-1} d^{-1}, 0.075 d^{-1}, and 4.70 mL g^{-1}, respectively. The BCF was >1, showing that the tilapia accumulated waterborne As. The toxicokinetic parameters not only described As kinetics in tilapia but also could be applied to predict As residue in tilapia. We assumed that toxicokinetic parameters were independent of As concentration in chronic-exposure conditions. We employed Equation 2, cooperating with experimental-derived toxicokinetic parameters, to predict the profiles of As kinetics when tilapia were exposed to 1, 2, and 4 μg mL^{-1} waterborne

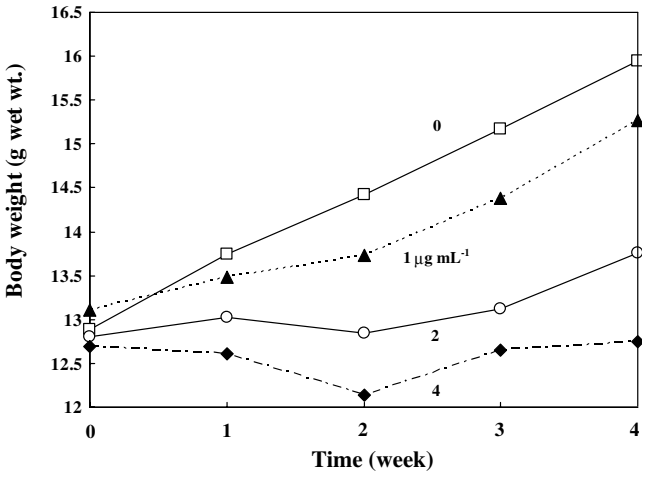

Fig. 2. Growth curves of tilapia *O. mossambicus* exposed to waterborne As concentrations during 4-week bioassays. Symbols (open squares, closed triangles, open circles, and closed diamonds) are the measured data.

As, respectively (Fig. 1B). The corresponding steady-state As concentrations in the tilapia were 5.2 μg g^{-1} in 110 days, 10.4 μg g^{-1}in 85 days, and, 20.8 μg g^{-1}in 77 days, respectively (Fig. 1B).

Chronic Toxicity

Our bioassays revealed that all of the exposure concentrations (1, 2, and 4 μg mL^{-1}) affected the growth of tilapia (Fig. 2). In the control groups, the tilapia grew progressively from 12.89 to 15.95 g with the extension of duration, and the specific growth rate (SGR) was calculated as 0.76 % d^{-1} (Fig. 2). The SGR of tilapia exhibited high variation in different experimental settings. The SGR of our control group fell within the reported values, which range from 0.35% to 1.8% d^{-1} (Balasubramanian and Bai 1996; Uchida *et al*. 2003).

At the intermediate concentrations, between 1 and 4 μg mL^{-1} As, a clear growth inhibition was observed from the second week. Biomass loss even occurred in the second week in 2 μg mL^{-1} As and in the second and third weeks in 4 μg mL^{-1} As. The SGRs of exposure tilapia were negatively correlated with As concentrations and calculated as 0.57 % d^{-1} in 1 μg mL^{-1}, 0.25 % d^{-1} in 2 μg mL^{-1}, and 0.04 % d^{-1} in 4 μg mL^{-1} As, respectively. The tilapia almost tended to stop growing in 4 μg mL^{-1} As, the SRG approximately 19 times lower than that in the control, showing that the fish were suffering sublethal effects rather than chronic growth inhibitions (Fig. 2). Liao *et al*. (2004) indicated maximum mortality up to 70% when the tilapia were exposed to 4 μg mL^{-1} waterborne As.

We used the concentration-specific growth coefficients to establish the regression equations (Table 1 and Fig. 3). We calculated EC_{10} values and then estimated IEC_{10} values using the CBR approach. The IEC_{10}s were used as an internal threshold concentration. The EC_{10}s did not change significantly with time and ranged from 0.38 to 0.41 μg mL^{-1} (Fig. 3). The constant EC_{10} value demonstrated that the CBR approach is applicable to estimate the corresponding internal threshold concentration (*i.e.*, IEC_{10}) in chronic As toxicity assessment.

Table 1. Estimated toxic effects of regressive equations of As on growth for tilapia *O. mossambicus* after 1 to 4 weeks

Time (wk)	Regression equation	r^2	EC_{10} (μg mL^{-1})	IEC_{10} (μg g^{-1})[a]
1	Y =1.051–0.016X	0.75	0.38	1.79
2	Y = 1.084–0.033X	0.71	0.43	2.02
3	Y = 1.159–0.041X	0.84	0.40	1.88
4	Y = 1.221–0.055X	0.95	0.41	1.93

[a] $IEC_{10} = EC_{10} \times BCF$, where BCF is 4.70 $mL^{-1}g^{-1}$.
BCF = Bioconcentration factor.
EC_{10} = Effect concentration for 10% growth inhibition.
IEC_{10} = Internal effect concentration choosing 10% response.

Mode of Action

Figure 4 shows the optimal fits of the feeding-decrease, growth-cost, and maintenance-cost models to the experimental data. Table 2 lists the estimates of model parameters. In the growth-cost model, the species-specific growth coefficient (A) seems not to depend on the exposure As concentration, revealing that the metabolic energy required to create a cell does not change significantly in different exposure conditions. Our results indicated that the growth-cost model could not discriminate the mode of action of As toxicity well (Fig. 4). In the maintenance-cost model, the values of toxic stress ($S(t)$) increased slightly with increasing As concentrations, revealing that this model can describe the decrease in fish body weight with increases in As concentration. In the feeding-decrease model, the estimated maximum body weight (W_{max}) was negatively correlation with As concentrations, which means that the As has direct effects on maximum body weight by decreasing the appetite.

Statistical analyses indicated that no significant differences ($p > 0.05$ with Student t tests) were observed between the means of data and the descriptions of the three models in As concentrations of 0 and 1 μg mL^{-1}, resulting in difficulties of assessing As toxicity in lower exposures ($\leq$ 1 μg mL^{-1}). However, significant differences existed between descriptions of the maintenance-cost and growth-cost models and the measured data in the 2- and 4-μg mL^{-1} experiments. We further employed SSs of the differences between model predictions and mean actual data to assess the performance between three effect models (Table 3). Obviously, the fits of the feeding-decrease model were more accurate than the others. The values of SSs increased with the gradient of the concentrations, *e.g.*, the feeding-decrease model, from 0.009 to 0.245 g^2. Similar results also occurred in the fitting of the other two models. Kooijman and Bedaux (1996) indicated that there might not be only one mode of action accounting for the toxic effect at higher concentrations, that several other physiologic processes might be responsible, indicating that the proposed DEB_{tox}–based toxicity models may be restricted to describe the growth

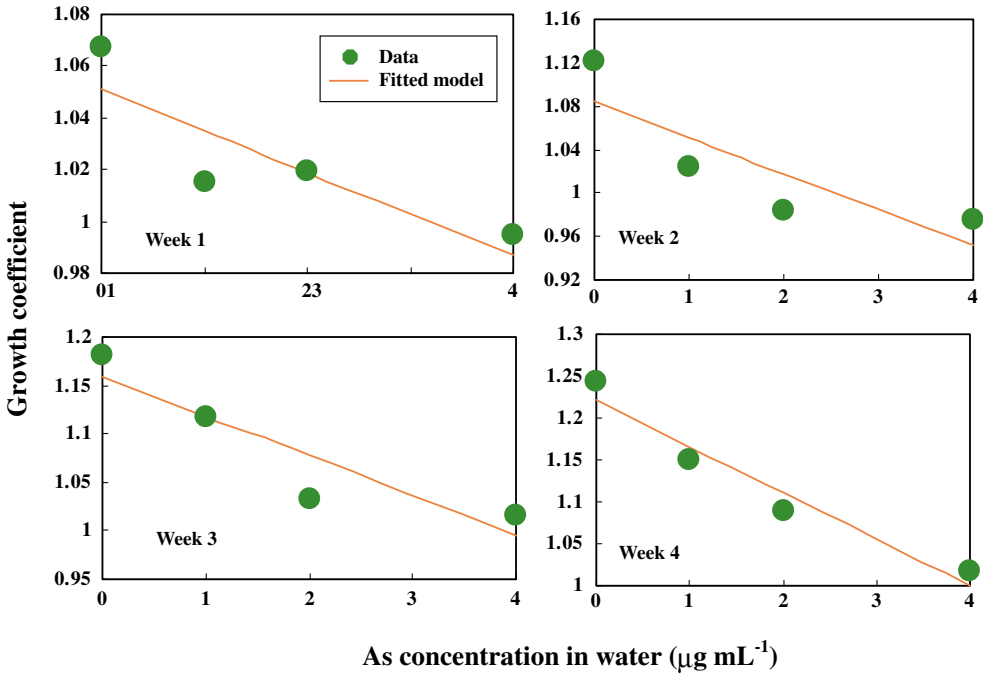

Fig. 3. Growth coefficients of tilapia versus nominal As concentrations during different exposure periods

Table 2. The estimated effect parameters of the growth-cost, maintenance cost, and feeding-decrease models[a]

Treatment (μg mL^{-1})	Growth cost model, A (g$^{1/4}$ d^{-1})[b]	Maintenance cost model, $S(t)$[c]	Feeding decrease model, W_{max} (g)[d]
Control	0.024 ± 0.006	0.00 ± 0.00	1100.82 ± 21.49
1	0.023 ± 0.006	0.03 ± 0.02	924.00 ± 69.79
2	0.023 ± 0.005	0.11 ± 0.03	421.54 ± 49.23
4	0.022 ± 0.005	0.16 ± 0.05	352.13 ± 52.74

Data are expressed as mean ± SD.
[a] Estimated from Eq. (5).
[b] Estimated from Eq. (6).
[c] Estimated from Eq. (7).

trajectories of organisms when they are suffering sublethal or acute toxic effects. Our results support that the mode of action of As growth inhibition acts through decreased feeding, *i.e.*, the growth decrease acts through decreasing incoming energy.

Prediction of Tilapia Growth

We employed the feeding decrease model to illustrate the growth trajectories of tilapia from birth to natural death in different exposure scenarios (Fig. 5). The reported life span of tilapia *O. mossambicus* is 11 years (approximately 4000 days) (www.fishbase.org/home.htm). The maximum body weight (W_{max}) of the control tilapia and the 1 μg mL^{-1} As group were 1038.75 and 872.97 g, respectively, whereas for the groups exposed to 2 and 4 μg mL^{-1} As, the corresponding W_{max}s were 403.06 and 336.65 g, respectively (Fig. 5). Tilapia potentially grow to maximum body weight until their end of life. This is comprehensible because in uncontaminated conditions, when fish are feeding *ad libitum*, individuals store surplus metabolic energy in reserve, which causes an increase in biomass even they have already reached mature body size. In contrast, when fish are consistently exposed to higher concentrations during a longer duration, fish translate large amount of assimilated energy from growth or maintenance to compensate for the stress of toxicants, thus inducing growth cessation or inhibition (Beyers *et al*. 1999; Sherwood *et al*. 2000).

Discussion

Chronic Toxicity and Toxicokinetics

Because of the scarcity of chronic toxicity data, acute-to-chronic ratios often are traditionally employed to derive quality standards for prolonged exposure to toxicants; however, changes in toxicity with long-term exposure might be attributed to a change in the mode of action and the induction of physiologic acclimation or genetic adaptation to local contaminant regimes (Forrester *et al*. 2003). This would cause limitations in assessing the long-term chemical effects with acute toxicity data because it seems plausible that organisms might somehow become weakened after enduring long-term chemical loading, and, nonspecifically, initially sublethal effects might worsen with time.

We assumed that chronic toxicity is initiated when the accumulated chemical exceeds the internal threshold concentration, represented by IEC_{10}. The magnitude-of-toxicity effect can be expressed as being proportional to the difference between accumulated chemicals and IEC_{10} and can be formulated as a stress function as shown in Equation 4. IEC_{10} can be accurately derived from the chronic bioassays data using sta-

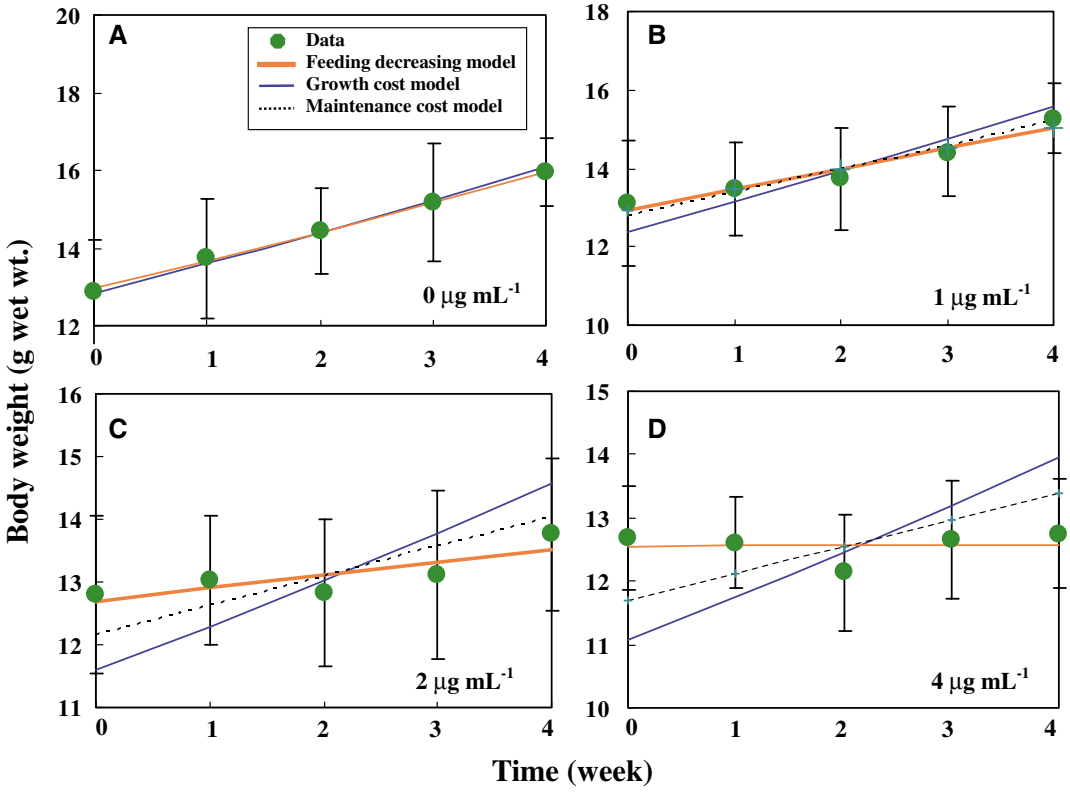

Fig. 4. Optimal fittings of the feeding-decrease, growth-cost, and maintenance-cost models. Error bar represents mean ± 1 SD

Table 3. The SSs of the difference between measured growth data and estimations of the growth-cost, feeding-decrease, and maintenance-cost models

As concentrate	Growth-cost model	Maintenance-cost model	Feeding-decrease model
0 μg mL^{-1}	0.057	0.056	0.009
1 μg mL^{-1}	0.887	0.223	0.165
2 μg mL^{-1}	3.122	0.927	0.205
4 μg mL^{-1}	5.069	1.919	0.245

[a] The feeding decrease model describes the data better than the other two models.
SSs = Sum of squares.

tistical techniques. Thus, the extent of toxicity is strongly determined by predicted As residue. Our simulations elucidated that As residue in tilapia was proportional to waterborne concentrations. The first-order bioaccumulation model has been extensively applied to describe and predict chemical kinetics in aquatic organisms (Reinfelder *et al*. 1998). McGeer *et al*. (2003) pointed out that the first-order BCF-based bioaccumulation model for metals is only applicable for residue predictions in the lower range of exposures, in which the uptake process does not limit the rate of uptake. Suhendrayatna *et al*. (2002) indicated that the higher concentrations (>10 μg mL^{-1}) of As (III) are toxic to tilapia, thus affecting accumulation of As by tilapia, and the total As accumulated in tilapia is proportional to external concentrations <5 μg mL^{-1}. We confirmed that our hypothesis that As residues in tilapia under chronic-exposures conditions (≤ 4 μg mL^{-1}) would be almost completely captured by our proposed model.

Mode of Action of Growth Inhibition

Our study revealed that As toxicity acts by causing a decrease in feeding. Although the mechanism accounting for growth decrease is statistically significant, the biologic meaning of this result remains unclear. Rankin and Dxion (1994) pointed out that an immediate decrease in feeding in response to both waterborne and dietary As exposure has been observed in freshwater fish species. Health (1995) pointed out that decreased food consumption frequently occurs with chemical exposure, especially during the early days of exposure. When organisms are exposed to chemical toxicants, the effects of chemical exposure disturb the homeostasis of the organism. As the organism's physiologic systems adjusts to compensate for specific effects from the mode of action of the chemical, a number of nonspecific homestatic mechanisms are also induced to re-establish equilibrium. This stage may be associated with a loss of feeding, loss of equilibrium, and behavioral changes (Beyers *et al*. 1999). Beyers *et al*. (1999) pointed out that the mechanism for the suppression of feeding is unknown, but it may be related to physiologic effects of the general adaptation syndrome. Physiologic changes that induce repair mechanisms may decrease ability or desire to process food (Health 1995). Pedlar and Klaverkamp (2002) revealed that the impairments of chemoreception may have been a mechanism for food refusal.

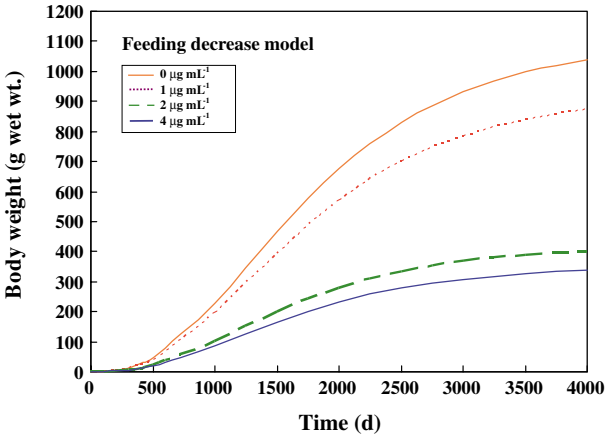

Fig. 5. Predictions of the growth of tilapia *O. mossambicus* during their entire life span in different waterborne As concentrations using the feeding-decrease model

The loss of appetite and decrease of growth suggest that the homeostatic mechanism of exposed tilapia are overwhelmed, resulting in damages and activation of repair and homeostatic mechanisms to re-establish equilibrium. The increased maintenance cost fails to account for the growth decrease, indicating that these tilapia have yet to compensate for the As stressor. No tilapia died during the bioassays, showing that the measured growth data derived from the experimental protocol is suitable for chronic mode of action identification.

Metabolic rate is a good measure of energy being expended for compensation because it integrates all physiologic process. Fits of three effect models revealed that apparent growth decreases occurred because of decreased feeding. Despite this, the modified West growth model employed in this study is applicable to the description and prediction of As toxicity. Nevertheless, to better assist accurate risk assessment posed by metals in aquatic ecosystems, more studies and experimental data are needed to validate applications of the proposed models.

Application of DEB_{tox} and West Growth Model in Ecotoxicology

The West growth model has never been employed in an ecotoxicologic study. Our study provided a novel assessment framework to analyze the mode of action of metal toxicity to aquatic organisms by linking the West growth model and the DEB_{tox} theory in a bioenergetics-based approach. The DEB_{tox} theory distinguishes three types of effects on growth, including direct effects and indirect effects by way of maintenance and assimilation. The inherent assumption is that only one of these effects occurs at the same time in the lower effect range of the chemical (Kooijman and Bedaux, 1996). Our bioenergetics-based toxicity model well describes the trend of growth in lower concentrations (*i.e.*, 1 $\mu g\ mL^{-1}$), yet the bias between the model description and the measured data increases with the gradient of exposure concentration. We inferred that multiple effects might work together to induce growth toxicity in higher concentrations. Our single-mode-of-action–based effect models may not be reliable in higher concentration (*e.g.*, sublethal exposure conditions). Sherwood *et al*. (2000) indicated that the growth inhibition of yellow perch in a heavy-metal (Cd, Cu, and Zn)–polluted lake was attributable to decreased conversion efficiency of the fish and not simply just to decreased food intake.

Individual development is fuelled by metabolism and occurs primarily by cell division. Incoming energy and material from the environment are transformed into metabolic energy and consequently transported through hierarchical branching network systems for life-sustaining activities and production of new tissue (West *et al*. 2001). The West growth model describes the universal properties of individual growth based on the first principles of the basic of the 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 mature body size for all multicellular organisms. This universal growth model provides a basis for understanding the general and fundamental features governing organism growth. Although some criticisms indicate that the conceptual foundation of this model is not applicable to the growth of birds and their life-history properties (Ricklefs 2003). West *et al*. (2004) indicated that 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.

The species-specific growth coefficient (A_0) relates the rate of energy allocation to produce a new cell to the rate of the whole organism's metabolic rate, which fuels this biosynthesis in terms of normalization (West *et al*. 2004). Our study shows that the values of A_0 do not change significantly in different exposure concentrations (Table 2), demonstrating that waterborne As exposures do not disturb the energy translations between life-sustenance activities and new biomass production. The growth inhibition by As exposure is not induced by increasing the energy cost to propagate new body tissues. The concentration-effect tilapia growth trajectories could be well described by decreasing the values of maximum biomass (W_{max}) in the West growth model (Table 2), *i.e.*, the feeding-decrease model. Several studies have shown that in many organisms, from fruit flies to humans, severe restriction of food supply during development can prolong time to maturity and result in smaller adult size (Davidovitz *et al*. 2003; West *et al*. 2004), which corresponds with the basic description of the feeding-decrease model in the DEB_{tox} theory.

In conclusion, the proposed bioenergetics-based growth-effect model allows us to make a comprehensive survey of growth effects during the entire life cycle of an organism when stressed by chemicals. Different modes of action can have similar effects, but very different consequences, at the individual level when the data are integrated at the population, community, or ecosystem levels (Barata and Baird 2000). We believe that a mechanistic-based study to understand the mode of action would improve any attempt to create predictive models for ecotoxicologic assessment.

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