Bioenergetics-Based Matrix Population Modeling Enhances Life-Cycle Toxicity Assessment of Tilapia *Oreochromis mossambicus* Exposed to Arsenic

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ABSTRACT: The objective of this study was to integrate a bioenergetics-based modeling approach into a population stage structure to enhance life-cycle toxicity assessments of the effects of waterborne arsenic (As) on the population dynamics of the tilapia *Oreochromis mossambicus*. The proposed mathematical model links a Leslie matrix population model and a universal ontogenetic growth model embedding the population-level growth rate and stage-specific modes of toxic action. We present data analyses of key parameters and distributions and discuss the processes of data capture and analysis and the impact of acute/chronic As toxicity responses on population-level effects. We employed a three-parameter Hill equation model to describe the relationship between tilapia whole-body burden and mortality in order to estimate the probability of stage-specific vital rate of survival. Using the DEB_{tox} theory, we distinguished three modes of toxic action (MOA): direct effects on growth and indirect effects via maintenance and food consumption on inhibition by arsenic of the growth of a tilapia population. The asymptotic population growth rate decreased from $\lambda = 1.0027$ for the control group to $\lambda = 0.9935$ for tilapia population exposed to 4 μg mL^{-1} As, indicating a potential risk of population intrinsic growth rates for tilapia exposed to higher levels of waterborne As. Our results estimated that an As concentration of 1.02 μg mL^{-1} would cause a 50% reduction in the tilapia population. We found that the interplay between external stressors of waterborne As concentration and internally generated modes of action decreasing feeding in the juvenile stage and increasing the maintenance cost in the adult stage had a pronounced influence on the population stage structure of tilapia. Environ Toxicol 21: 154–165, 2006.

Keywords: bioenergetics; matrix population model; tilapia; arsenic; life-cycle assessment; ecotoxicology

INTRODUCTION

In recent years the concept of assimilated energy utilization has been extensively employed to determine the growth of organisms and the productivity of ecosystems and has been further extended to assess life-cycle toxicity (Kooijman and Bedaux, 1996; Beyers et al., 1999; Sherwood et al., 2000; Jager et al., 2004; Nichols et al., 2004). The basic insight gained is that exposure to toxicants can be understood as a change in energetic parameters, such as an increase in the costs of maintaining life (including of detoxification) or a decrease in the assimilation of energy from food (Congdon et al., 2001; Pery et al., 2003; Jager et al., 2004). Fish constantly consume energy in order to maintain life and offset

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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 physiological and ecological approach to the assessment of toxicity.

A mode of action (MOA) is defined as a common set of physiological and behavioral signs that characterize a type of adverse biological response (Landis and Yu, 1999). According to Escher and Hermens (2002), elucidating detailed knowledge of chemical-specific modes of toxic action of a metal could enhance the predictive power of models by providing mechanistic explanations for chemical risk assessments in ecotoxicology. Barata and Baird (2000) further suggested that the ecotoxicological modes of action of different chemicals could be determined bioenergetically by studying sublethal effects on food acquisition and hence on growth and reproduction rates.

A rich body of ecotoxicological work today aims to develop mathematical tools for broadening analysis of the effects of individual contaminants to population levels by using demographic models (Chaumot et al., 2003; Spromberg and Meador, 2005; Spromberg and Birge, 2005; Raimondo and McKenney, 2005). The most popular demographic model is the matrix population model, characterized by a population projection matrix (referred to as the Leslie matrix) to project a population from time t to time $t + 1$ (Caswell, 2001). A stage-classified matrix model classifies individuals by an appropriate set of real biological stages of unequal duration (Chandler et al., 2004). Because of its biological relevance, the stage-classified matrix model has been widely applied in addressing the relationships among toxicants, in life-table response experiments, and in assessing population growth rates in ecological applications (Chandler et al., 2004; Lin et al., 2005; Spromberg and Birge, 2005; Smith et al., 2005; Louda et al., 2005; Griffith and Forseth, 2005).

The tilapia *Oreochromis mossambicus*, a traditional food fish for the people of Taiwan, is appreciated for its delicacy and is the first most important farmed fish in Taiwan. For decades, the tilapia industry ranked first in the aquaculture industry of Taiwan. Furthermore, tilapia was the most consumed fish in Taiwan (Lung et al., 2003). Therefore, aquaculture of tilapia is a promising business. Most tilapia ponds are on the southwest coast of Taiwan, whose inhabitants used to suffer from blackfoot disease (BFD) because of long-term ingestion groundwater contaminated by inorganic arsenic (As; Chen et al., 2001). Today most of the people in this region do not have to use groundwater as a source of drinking water because tap water has been made available. However, groundwater is still used for aquaculture (Lin et al., 2001). Liao et al. (2003) reported that As concentrations in BFD-area pond water ranged from 8.1 to 251.7 μg mL^{-1}. Arsenic content in several farming ponds exceeded the water quality criteria for total As in the freshwater ecosystems (150 μg mL^{-1}), documented as the criterion for continuous concentrations (U.S. EPA, 2002). A significant increase in As levels in pond water could produce severe effects on the health of farmed fish and even pose a carcinogenic risk to people who consume the farmed tilapia.

Liao et al. (2003, 2004) and Tsai (2005) conducted 14-day bioaccumulation, 7-day acute, and 28-day chronic toxicity bioassays to determine the toxicokinetic parameters LC_{50} and IEC_{10}, respectively, of tilapia exposed to As at different concentrations. Both acute and chronic end points (i.e., mortality rate and growth rate) were also examined to investigate the toxic effects of As on individual tilapia. Compared with these individual end points, however, population-level effects are of particular concerns for the development of ambient water quality criteria because of their superior importance for ecological functions (U.S. EPA, 1991). From this point of view, the data obtained from traditional toxicity tests should be incorporated into a population model to predict effects of As on tilapia populations.

How do we describe mechanistically the population-level response to a toxic substance in order to overcome the consequences of restrictions of space and time in a population in testing that is too complicated and too expensive for routine application? Simple mathematical analyses can provide some insight. We used a mathematical model that links a Leslie matrix population model and a bioenergetics-based modeling method embedding the population-level growth rate and life-stage-specific MOAs in order to enhance the life-cycle toxicity assessment in the context of waterborne metals management in aquacultural ecosystems. In this article, we present data analyses of key parameters and distributions and discuss the processes of data capture and analysis and the impact of acute/chronic As toxicity responses to population-level effects.

The objectives of this study were twofold: (1) to develop a stage-structured population growth method to investigate the effects of increased concentrations of waterborne As on the population dynamics of tilapia and (2) to integrate a bioenergetics-based modeling approach into a population stage structure in order to enhance the life-cycle toxicity assessment of the effects of As on reproduction in each age class. Data obtained from our previous published bioaccumulation and acute and chronic toxicity bioassays were reanalyzed to reconstruct concentration–response profiles and to examine the survival and growth performance. These data provided stage-specific schedules of vital rates that were used to parameterize a projection matrix model for tilapia. Simulations were carried out to produce changes in population over time under different scenarios of varied exposure to arsenic. Asymptotic population growth rates were also estimated from matrix population models along with stage-specific MOAs to provide additional population-level ecotoxicological end points.

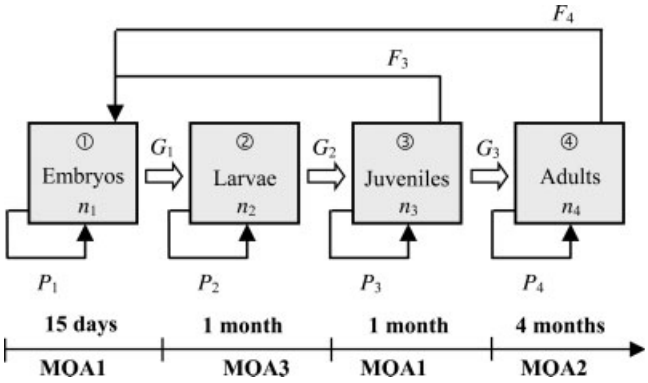

Fig. 1. A four-stage life-cycle graph of an individual tilapia *Orechromis mossambicus*. An explanation of the symbols is provided in the text.

MATERIALS AND METHODS

Stage-Structured Population Growth Model

To develop the stage-structured population growth model of tilapia exposed to waterborne As, a four-stage (embryos to larvae to juveniles to adults) matrix model was used to project offspring production through two generations based on body weight. The estimated durations of these four life stages were defined as suggested by the Taiwan Fisheries Research Institute (http://www.tfrin.gov.tw/friweb/index.php?func=techbook&act=ShowForm&num=8): 0.5, 1, 1, and 4 months, respectively, for a total life span of 6.5 months.

On the basis of the stage-specific survival and life-stage transition rates and fecundity through juveniles and adults, a four-stage matrix population growth model could be constructed and expressed by a state–space representation (Fig. 1) as follows:

$$\{n(t+1)\} = [A]\{n(t)\}, \tag{1}$$

where $n_i(t)$ is the number of tilapia in stage i at time t and matrix $[A]$ is a population projection matrix of

$$[A] = \begin{bmatrix} P_1 & 0 & F_3 & F_4 \\ G_1 & P_2 & 0 & 0 \\ 0 & G_2 & P_3 & 0 \\ 0 & 0 & G_3 & P_4 \end{bmatrix}, \tag{2}$$

where P_i is the probability of surviving and staying in stage i, G_i is the probability of surviving and growing from stage i to stage $i + 1$, and F_i is the per capita fertility of stage i within each projection interval, in that P_i, G_i, and F_i are referred to as the life-cycle parameters or transition probabilities. Matrix $[A]$ can be used to estimate an asymptotic population growth rate, λ (the dominant eigenvalue of $[A]$), reflecting the temporal trend in population abundance (Caswell, 2001). When λ exceeds 1.00, the population is projected to increase over time, whereas the population is projected to decline when λ is less than 1.00. Table I summarizes the essential mathematical equations of the life-cycle parameters of P_i, G_i, and F_i.

Concentration–Response Model

We employed a three-parameter Hill equation model to express the relationship between tilapia whole-body burden and mortality in order to estimate the stage-specific vital rate of survival probability in eq. (TI-4) (Table I)

$$M(t) = \frac{M_{\max} C_f^n(t)}{[\mathrm{CL}_{50}(t)]^n + C_f^n(t)}, \tag{3}$$

where $M(t)$ is the time-dependent mortality of tilapia (%), $M_{\max}$ is tilapia maximum mortality (%), $\mathrm{CL}_{50}(t)$ is the internal body burden of As in tilapia that causes 50% mortality (μg g^{-1}), n is the Hill coefficient, and $C_f(t)$ is the whole-body burden of As in tilapia at time t (μg g^{-1}). The latter can be expressed as $C_f(t) = \mathrm{BCFC}_w(1 - e^{-k_e t})$, which is calculated from the first-order bioaccumulation model $dC_f(t)/dt = k_1 C_w - k_2 C_f(t)$, where C_w is the waterborne As concentration (μg mL^{-1}), BCF is k_1/k_2, and k_1 (mL g^{-1} d^{-1}) and k_2 (d^{-1}) are the uptake and depuration rate constants, respectively.

TABLE I. Mathematical expressions of transition probabilities in population projection matrix [*A*][a]

Probability of surviving and staying in stage i, P_i	
$P_i = \sigma_i (1 - \gamma_i)$,	(TI-1)[b]
Probability of surviving and growing in stage i, G_i	
$G_i = \sigma_i \gamma_i$,	(TI-2)[b]
Fertility of stage i within each projection interval, F_i	
$F_i = f_{ei}\, EF_{mi}$,	(TI-3)[c]
where	
$\sigma_i = \frac{S_i(t+T)(1-M_i(t))}{S_i(t)T}$,	(TI-4)
$\gamma_i = \frac{W_i(t+T) - W_i(t)}{W_i(t)T} \times 100$,	(TI-5)
$f_{ei} = \frac{dF_{ei}(t)}{F_{e0}dt} \times 100$,	(TI-6)
$F_e(t) = m_0[W(t)]^{n_0}$,	(TI-7)[d]
$S(t) = \left(\frac{1-at}{1+bt}\right)^k$,	(TI-8)[e]

[a] σ_i and γ_i, probabilities of stage-specific vital rates of survival and growth (d^{-1}), respectively; f_{ei}, number of eggs per mature female per unit time in stage i (d^{-1}); E, egg eclosion rate, F_{mi}, percentage of mature female in stage i; $S(t)$, intrinsic survival rate of tilapia at age t; T, projection interval (d); $M_i(t)$, mortality rate in stage i; $W(t)$, body weight at age t(g); $F_e(t)$, age-dependent fecundity of tilapia at age t; F_{e0}, control fecundity of tilapia; m_0 and n_0, fitted coefficients; and a, b, and k, constants.

[b] Adapted from Caswell (2001).

[c] Adapted from Simas et al. (2001).

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

[e] Adapted from Klok and deRoos (1996).

Bedaux and Kooijman (1994) have developed a biologically based damage assessment model (DAM) to investigate the relationships among body residues, cumulative damage, and survival rate in order to describe time-dependent survival probability. DAM depicts the modes of action, including rapid reversible binding to a target site as well as target site binding that is irreversible (Lee et al., 2002). DAM assumes that death occurs when the cumulative damage reaches a critical level (DL) and is described by a combination of both first-order toxicokinetic and toxicodynamic models. Damage is assumed to accumulate in proportion to the accumulated residue and damage recovery in proportion to the cumulative damage when damage is reversible. Time-dependent LC_{50} data are determined by both a damage recovery rate and an elimination rate, suggesting that the critical cumulative damage is the determinant of the time–concentration response relationship.

If $D(t)$ can be denoted by DL_{50} for damage level that causes 50% mortality, then the damage-based lethal body concentration [$CL_{50}(t)$] in eq. (3) can be derived from the first-order damage accumulation model (Lee et al., 2002) as

$$CL_{50}(t) = \frac{DL_{50}/k_a}{\left(\frac{e^{-k_r t} - e^{-k_2 t}}{k_r - k_2} + \frac{1 - e^{-k_2 t}}{k_r}\right)}(1 - e^{-k_2 t}), \qquad (4)$$

where k_a is the uptake accumulation rate (g μg^{-1} h^{-1}), k_r is the damage recovery rate constant (h^{-1}), and DL_{50}/k_a is a coefficient that reflects the compound equivalent toxic damage level required for 50% mortality (μg h g^{-1}).

Bioenergetics-Based Model

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

$$W(t) = W_{\max 0}\left\{1 - \left[1 - \left(\frac{W_0}{W_{\max 0}}\right)^{1/4}\right] e^{-A_0 t/4W_{\max 0}^{1/4}}\right\}^4, \qquad (5)$$

where $W_{\max 0}$ and W_0 are the maximum biomass (g) in uncontaminated water and the mass at birth (g), respectively. Also, 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 the taxon-specific constant (W), m_c is the mass of a cell (g), and E_{c0} is the metabolic energy required to create a cell (J). A_0 can be estimated by optimal fitting of eq. (5) to the body growth profile in control–exposure conditions.

On the basis of the DEB_{tox} theory, we distinguished three modes of toxic action in the model of As growth inhibition of the tilapia population (Fig. 1; Jager et al., 2004): (1) decrease assimilation (feeding), designated MOA1, during the embryonic and juvenile stages; (2) increase the costs of maintaining life (MOA2) during the adult stage; and (3) increase the cost of growth (MOA3) during the larval stage. The basic assumption of the DEB_{tox} theory is that chemicals first have to be taken up by the organism before they can exert an effect. Once the chemical gets inside the target tissues, it increases the probability of adverse response and affects a parameter of the general ontogenetic growth model (e.g., the assimilation rate). Kooijman and Bedaux (1996) and Pery et al. (2003) introduced a stress function, $s(t) = b[C_f(t) - IEC_{10}(t)]$, to describe the extent of the adverse effect, where IEC_{10} is the 10% effect threshold for chronic growth inhibition and b is the level of toxicity (g g^{-1}) once C_f exceeds IEC_{10}. Mechanistic descriptions of MOA1, MOA2, and MOA3 follow.

MOA1. When feeding is decreasing, growth reduction acts by reducing incoming energy. The maximum assimilation rate does not appear in the West growth model, yet it can be captured by the parameter maximum weight ($W_{\max}$), as suggested by Kooijman and Bedaux (1996), and the West growth model leads to

$$W(t) = W_{\max}\left\{1 - \left[1 - \left(\frac{0.05}{W_{\max}}\right)^{1/4}\right] e^{-A_0 t/4W_{\max}^{1/4}}\right\}^4, \qquad (6)$$

where $W_{\max}$ is $W_{\max 0}\{1 - b[C_f(t) - IEC_{10}]\}$ and the constant 0.05 is the mass at birth (g) of tilapia in uncontaminated water (www.fishbase.org/home.htm).

MOA2. When maintenance energy cost is increasing, chemicals are likely to increase maintenance cost for compensating for the effects of exposure (Beyers et al., 1999). Because maintenance cost has priority over growth, such an increase leads to a reduction in growth rate. We multiplied body weight by $[1 + s(t)]$ to account for an increase in maintenance costs, resulting in reduction of time-dependent body weight, as suggested by Kooijman and Bedaux (1996),

$$W(t)[1 + s(t)] = 1130\left\{1 - \left[1 - \left(\frac{0.05}{1130}\right)^{1/4}\right] e^{-A_0 t/4\times 1130^{1/4}}\right\}^4, \qquad (7)$$

where the constant of 1130 is the maximum biomass (g) of tilapia in uncontaminated water (www.fishbase.org/home.htm).

MOA3. When growth energy cost increases, that is, when C_f exceeds IEC_{10}, we assumed that the metabolic energy (E_c) required to create a new cell would be multiplied by $[1 + s(t)]$, which can be expressed as $E_c = E_{c0}[1 + s(t)]$, where E_{c0} is the growth energy cost in the control condition. A $C_f \leq IEC_{10}$ leads to $s(t) = 0$ and $E_c = E_{c0}$. We substituted the effect function into West growth model, obtaining the mode of action on growth cost:

$$W(t) = 1130\left\{1 - \left[1 - \left(\frac{0.05}{1130}\right)^{1/4}\right] e^{-At/4\times 1130^{1/4}}\right\}^4, \quad (8)$$

where A is $B_0 m_c\{E_{c0}[+ s(t)]\}^{-1} = A_0[1 + s(t)]^{-1}$.

Input Parameters and Simulation Scheme

All parameters used to calculate the vital rates of individual tilapia, including point values and maximum likelihood estimates, are summarized in Table II. The experiments carried out by Liao et al. (2003, 2004) and Tsai (2005) were adapted to represent the actual culture operations and management of tilapia farms. Therefore, all parameters listed in Table II were obtained under comparable experimental conditions. To manipulate the simulation of the bioenergetics-based stage-structured population growth model, a projection interval of 1 day was used. Caswell (2001) pointed out that the initial condition has no influence on stable age distributions as well as on the population growth rate. Therefore, the initial number of tilapia at each stage (n_1, n_2, n_3, n_4) was arbitrarily assumed to be 500, 0, 0, and 0, respectively, yielding an initial population density of 500 individuals per unit area. Model simulations and the determination of asymptotic population growth rate under different scenarios were performed using the MATLAB® software (Mathworks Inc., Natick, MA, USA).

Uncertainty arises from estimation of both exposure and effect. To quantify this uncertainty and its impact on the estimation of population growth rate, we implemented a Monte Carlo simulation that included input distributions for the parameters of the vital rates of a tilapia population. Largely because of limitations in the data used to derive model parameters, inputs were assumed to be independent. The result showed that 5000 iterations were sufficient to ensure stability of the results. The 95% confidence interval (CI) was defined as the 2.5th and 97.5th percentiles obtained from the Monte Carlo simulation.

We also performed a sensitivity analysis to identify the most significant exposure-specific life-cycle parameters that influenced the tilapia population growth rate. We assessed

TABLE II. Input parameters represented as point values used to calculate vital rates of individual tilapia, and values represented as random variables [lognormal distribution (LN)] used in the uncertainty and sensitivity analyses

Exposure concentration (μg As mL^{-1})	A_0 ($g^{1/4}$ d^{-1})	$s(t)$	W_{max} (g)
	Growth Probability[a]		
0	LN(0.023, 1.28)	LN(0.022, 1.29)	LN(0.022, 1.24)
1	0	LN(0.025, 1.83)	LN(0.11, 1.31)
2	LN(1100.61, 1.02)	LN(921.37, 1.08)	LN(418.69, 1.12)
4	1.91	1.91	1.91
	Survival Probability		
a	0.0051		
b	0.07		
k[b]	0.176		
n[a]	4.07		
BCF (ml g^{-1})[a]	5.03		
k_2 (d^{-1})	0.075		
M_{max}[a]	100		
DL_{50}/k_a (μg h g^{-1})[a]	0.0051		
k_r (h^{-1})[a]	0.07		
IEC_{10} (μg g^{-1})	1.91		
	Fertility		
F_m (%)	0.5		
E (%)	0.9		
m_0[c]	LN(82.55, 1.53)		
n_0[c]	LN(0.75, 1.11)		

[a] Adapted from Tsai (2005).
[b] Adapted from Brown et al. (2001).
[c] Adapted from Coward and Bromage (1999).

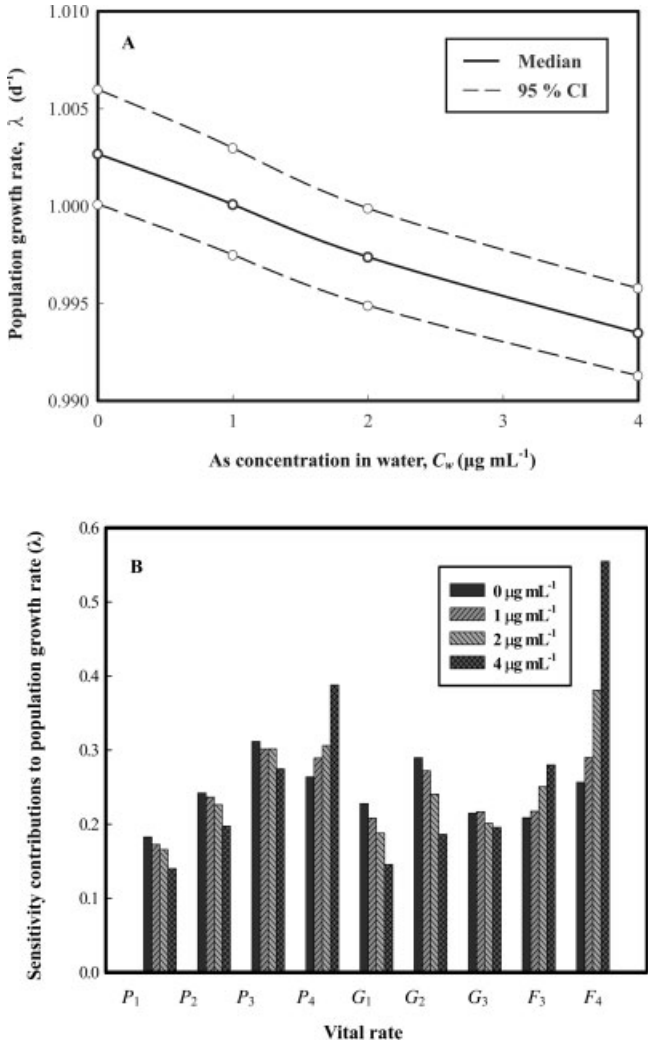

Fig. 2. (A) Effects of increasing waterborne arsenic concentrations on the asymptotic population growth rate of tilapia and (B) sensitivity of the asymptotic population growth rate to life-cycle vital rates subject to different waterborne arsenic concentrations.

the sensitivity of each variable relative to one another by calculating Spearman rank correlation coefficients between each input and output during simulations and then estimating each input contribution to the output variance by squaring the output variance and normalizing to 100% (Zar, 1999). The Monte Carlo simulation and sensitivity analysis were implemented using Crystal Ball® software (Version 2000.2, Decisioneering, Inc., Denver, CO, USA).

RESULTS

Effects of As on Asymptotic Population Growth Rate

Incorporating survival and fecundity rates into the probabilistic population model revealed substantial differences in the asymptotic population growth rate (λ) in each scenario. Figure 2(A) shows that the asymptotic population growth rate decreased with increasing As concentrations. Used as the reference value was the rate of $\lambda = 1.0027$ (95% CI: 1.0001–1.0060), which was the asymptotic population rate obtained in the absence of As exposure, indicating potential population growth. Stable growth of the population [$\lambda = 1.0001$ (95% CI: 0.9975–1.0030)] was found at As concentrations of 1 μg mL^{-1}, shown in Figure 2(A), in which it also can be seen that the asymptotic population growth rate decreased from $\lambda = 1.0027$ for the control group to $\lambda = 0.9935$ (95% CI: 0.9913–0.9958) for tilapia exposed to an arsenic concentration of 4 μg mL^{-1}. This indicates a potential risk of intrinsic growth rates of a tilapia population exposed to higher levels of waterborne As.

To understand the effect of waterborne As on the population growth rate (λ), we conducted a sensitivity analysis to determine the contributions of life-cycle parameters P_i, G_i, and F_i to λ, shown in Figure 2(B), which indicates that stage 4 fertility and probability of survival (F_4 and P_4) as well as stage 3 probability of survival (P_3) were the parameters most sensitive to the population growth rate in that on average they contributed 37%, 31%, and 27%, respectively, to λ.

Effect of As on Body Weight and Bioaccumulation of Tilapia

We employed the West growth model to simulate the growth trajectories of tilapia life stages with different exposure scenarios [Fig. 3(A)]. With exposure to 1 μg As mL^{-1}, maximum body weight (W_{max}) of the control tilapia was nearly 4.5 g, whereas for groups exposed to 2 and 4 μg As mL^{-1}, the corresponding W_{max} was around 3.5 g [Fig. 3(A)]. This is because in uncontaminated conditions, when fish feed *ad libitum*, individuals store surplus metabolic energy in reserve, causing increased biomass even after having already reached mature body size; in contrast, when fish are consistently exposed to higher concentrations for longer durations, they translate a large amount of the assimilated energy from growth or maintenance in order to compensate for the stress of toxicants, inducing the inhibition or cessation of growth (Beyers et al., 1999; Sherwood et al., 2000).

With all exposure scenarios, accumulated As concentrations in tilapia increased rapidly during the first month and reached equilibrium after 50 days [Fig. 3(B)]. The equilibrium whole-body burden for tilapia exposed to 1, 2, and 4 μg mL^{-1} waterborne As was 2.2, 5.8, and 11.8 μg g^{-1} wet wt, respectively [Fig. 3(B)].

Survivorship and Fecundity

Figure 4(A) depicts a survival curve for tilapia not exposed to arsenic. The time-dependent mortality profiles for tilapia

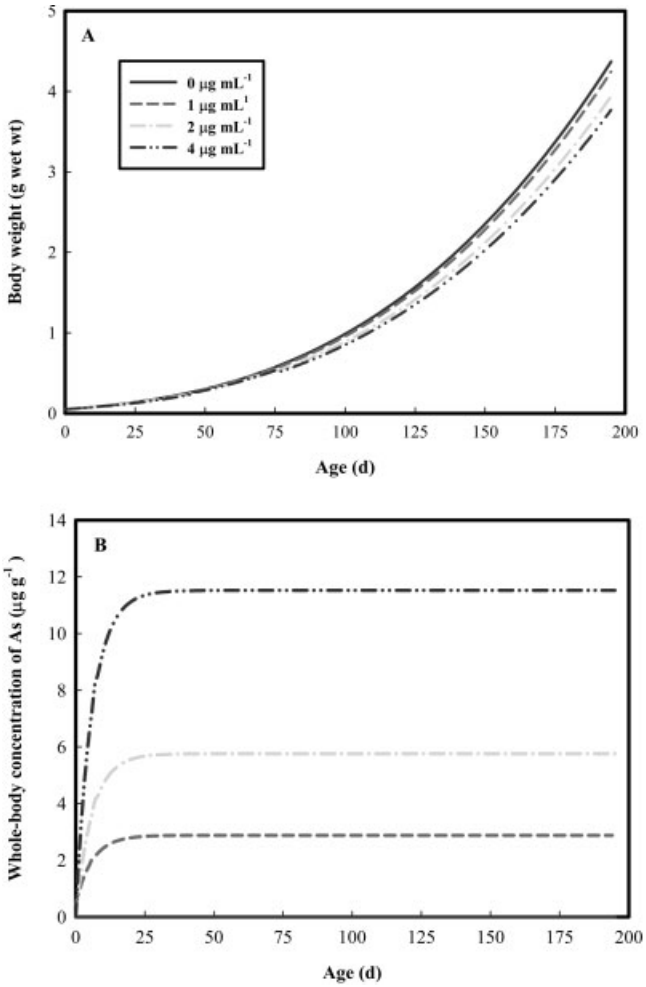

Fig. 3. (A) Simulations of growth trajectory of tilapia life stage with different waterborne arsenic levels and (B) predicted time-varying whole-body arsenic concentrations of tilapia with exposure to concentrations ranging from 1 to 4 μg mL^{-1}.

exposed to different concentrations of As [Fig. 4(B)] were incorporated into the daily survival probabilities (σ_i) of the stage-classified projection matrix model in order to simulate the stage-specific survival proportions of tilapia subject to waterborne As concentrations [Fig. 4(C)].

Significant changes in tilapia survival occurred at all life stages when the waterborne As concentration exceeded 1 μg mL^{-1} [Fig. 4(C)]. For stages 2–4, exposure was long enough for As concentrations to increase in tilapia since equilibrium was reached after 50 days [Fig. 3(B)]. Therefore, similar survival profiles for these stages were obtained because tilapia accumulated identical whole-body concentrations of As. Liao et al. (2003) suggested that As is lethal to tilapia at concentrations larger than 1 μg mL^{-1}. However, we showed a potential risk of lethality for larval tilapia when the waterborne As concentration exceeded 1 μg mL^{-1} because of additional As accumulation from the dietary route.

We applied a bioenergetics-based approach to model the effects of As on tilapia fecundity. Because of the mathematical characteristics of eq. (TI-7) (Table I), tilapia fecundity is an MOA-dependent (expressed by body weight) function subject to various exposure scenarios. Figure 5 shows that the stage-specific effects of waterborne As on fecundity varied with body weight in stages 3 and 4, indicating different stages produced a pronounced change in fecundity density. The simulation results indicated that at the end of stage 3 ($t = 45$th day), body weight decreased from 0.27 to 0.26 g, and fecundity density decreased from 27.7 to 26.5 egg/female; whereas at the end of stage 4 ($t = 195$th day), body weight decreased from 4.4 to 3.8 g, and fecundity density decreased from 340.8 to 298.2 egg/female, subject to waterborne As increasing from 0 to 4 μg mL^{-1}. The contrast in the population stage structure between externally forced (i.e., waterborne As concentration) and internally generated

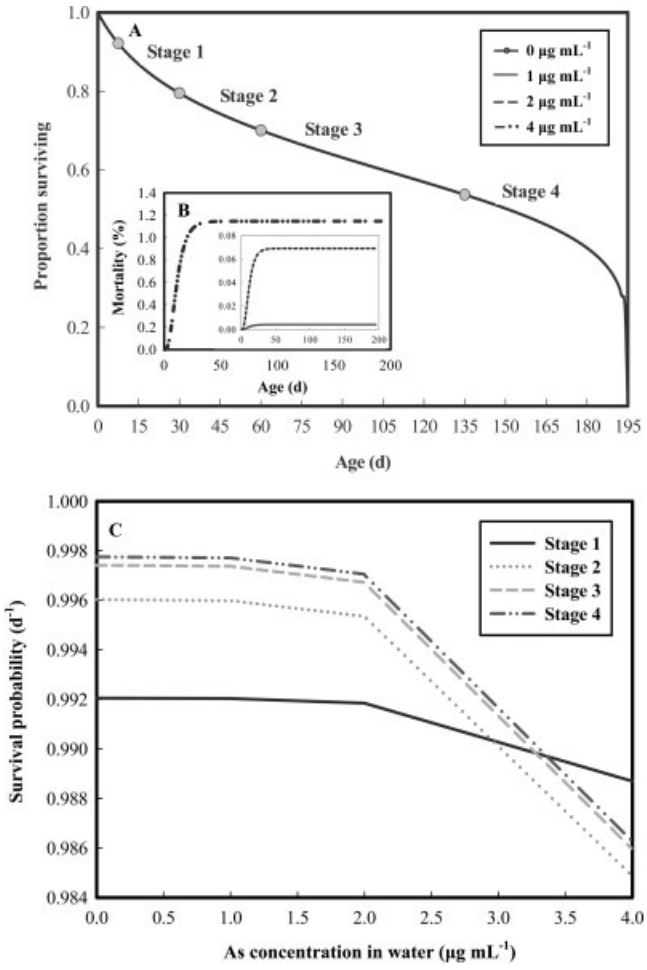

Fig. 4. (A) Stage-specific survival of tilapia (A) in natural circumstances; (B) with exposure to different arsenic concentrations, showing time-dependent mortality profiles; and (C) with exposure to different waterborne arsenic concentrations.

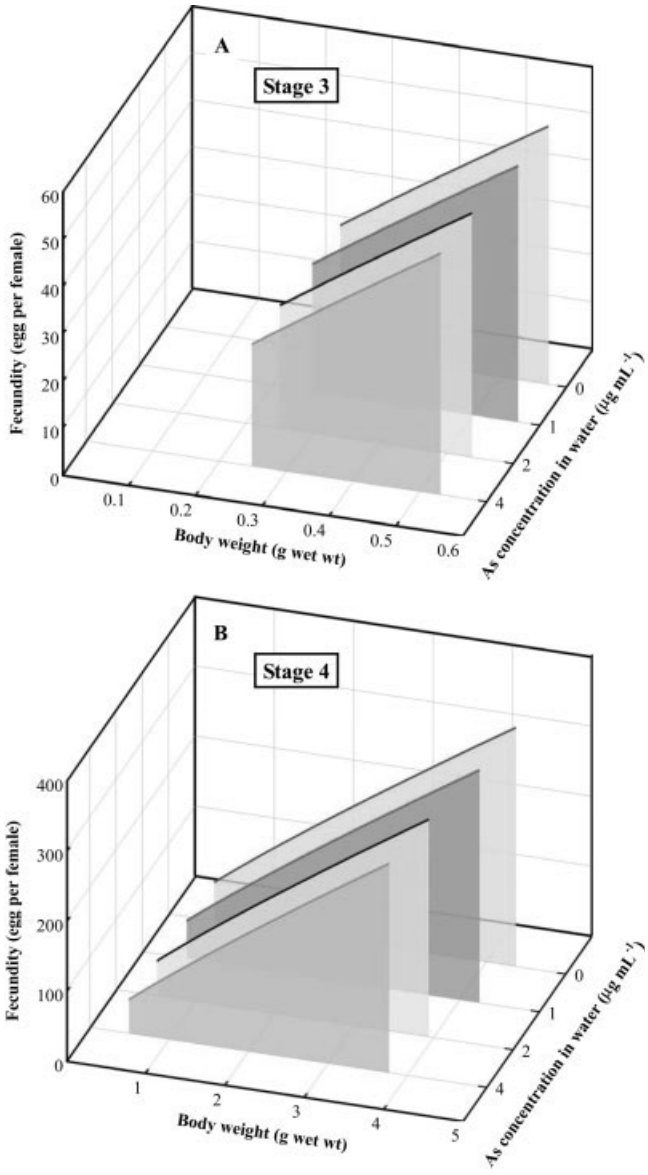

Fig. 5. Stage-specific effects of waterborne arsenic concentration on fecundity varied with body weight at (A) stage 3 and (B) stage 4.

[i.e., MOA1 (decreasing feeding) in the juvenile stage and MOA2 (increasing maintenance cost) in the adult stage] environments thus provides an explanation.

Population Abundance

The temporal changes in the stage-specific and overall population of tilapia exposed to different concentrations of waterborne As are illustrated in Figure 6. A concentration–response profile of the relation of reduction in population with waterborne As exposure in a tilapia population after a 1-year simulation was derived using a three-parameter Hill equation model [eq. (3)] by a nonlinear regression technique (Fig. 7), in that the simulation time was determined from a preanalysis of the control population to determine its stable age distribution. The optimal fits of eq. (3) to the predicted percent reduction in population of tilapia versus waterborne As concentration resulted in an estimated effect concentration (concentration that would cause a 50% reduction in

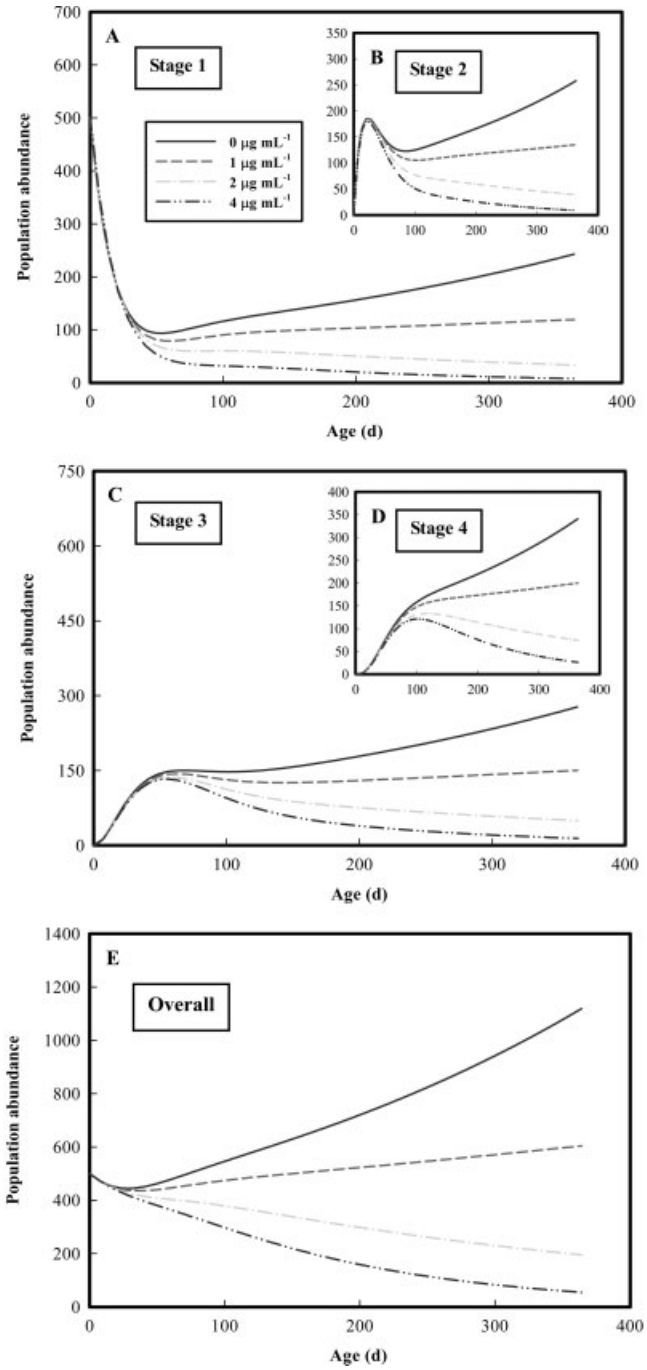

Fig. 6. Temporal changes in life stages 1–4 and overall population abundance of tilapia exposed to concentrations of waterborne ranging from 0 to 4 μg As mL^{-1}.

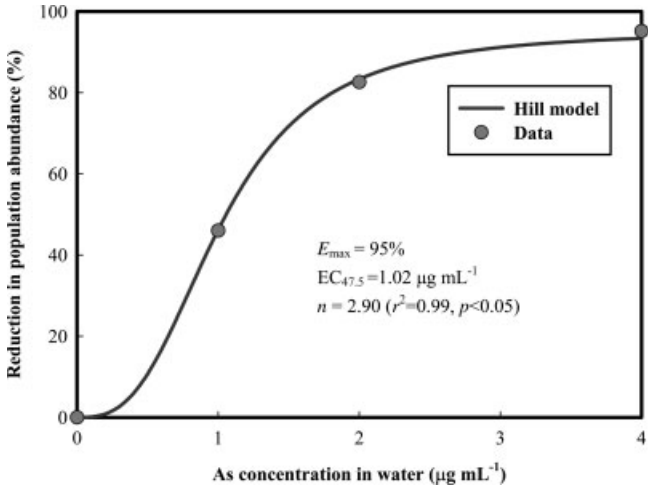

Fig. 7. Concentration–response profile of reduction in population abundance with waterborne arsenic level for a tilapia population after a 1-year simulation. The profile is based on a three-parameter Hill equation model and was constructed using a nonlinear regression technique.

population) of $EC_{50} = 1.02$ μg mL^{-1} and a Hill coefficient of $n = 2.02$ ($r^2 = 0.96$, $P < 0.05$).

In the control group, tilapia abundance in all life stages followed a monotonic increase pattern after 1 year, with embryo, larval, juvenile, and overall of 243, 258, 277, 341, and 1119, respectively (Fig. 6). The differences in the number of tilapia at each stage mainly resulted from the unequal durations of the stages.

Figure 7 shows a monotonic and sigmoid concentration–response profile of the effect of waterborne As on the population abundance of tilapia. Liao et al. (2003) reported that As concentrations in aquacultural waters in southwestern Taiwan, where farming tilapia is one of the most promising aquatic products, ranged from 18 to 49 μg L^{-1}. According to the concentration-response profile shown in Figure 7, the population of tilapia may decrease in these field circumstances. Figure 7 therefore can be used to improve the derivation of environmental quality criteria and to support the establishment of an ecological risk assessment in the management of toxic chemicals in aquacultural ecosystems.

DISCUSSION

MOA on Population Growth Rate

Rankin and Dxion (1994) reported observing an immediate reduction in feed intake in response to both waterborne and dietary As exposure in freshwater fish species. Health (1995) pointed out that food consumption reduction frequently occurs with chemical exposure, especially during the early days of the exposure. When organisms are exposed to chemical toxicants, the effects of this exposure disturb the homeostasis of the organism. As the organism's physiological systems adjust to compensate for specific effects from the mode of action of the chemical, a number of nonspecific homeostatic mechanisms are also induced in order to reestablish equilibrium. This stage may be associated with loss of feeding appetite, loss of equilibrium, and behavioral changes, according to Beyers et al. (1999), who also pointed out that the mechanism for suppression of feeding is unknown but that it may be related to the physiological effects of the general adaptation syndrome. Physiological changes that induce repair mechanisms may reduce the ability or desire to process food (Health, 1995). Pedlar and Klaverkamp (2002) showed that impairment of chemoreception may be a mechanism for food refusal.

Loss of appetite and reduction of growth suggest that the homeostatic mechanism of exposed tilapia is overwhelmed. Then, if damage occurs, repair/homeostatic mechanisms might be activated to reestablish equilibrium. An increased cost of maintenance would fail to account for the reduction in growth, indicating that these tilapia had yet to compensate for the As stressor. Metabolic rate is a good measure of energy expended for compensation because it integrates all physiological processes. Despite the modified West growth model employed in this study being applicable to the description and prediction of As toxicity, to better assist the accurate assessments of the risks posed by metals in aquatic ecosystems, more studies and experimental data are needed to validate the applications of the proposed models.

Factors affecting various toxicity end points, such as growth, fecundity, behavior, aging, or immune function, alter different demographic traits and have various impacts on a population (Jager et al., 2004; Spromberg and Meador, 2005; Spromberg and Birge, 2005). Thus, factors whose effects are not felt immediately may also be important in determining population growth or decline. Our conclusion was that the complexity added to the model was not warranted, given the quality of data sets in our analysis. However, we believe that it would be interesting to explore carefully the possibility of including behavior, aging, and immune function in future studies.

Bioenergetics and West Growth Model in Population Ecotoxicology

The West growth model has never been employed in population-level ecological risk assessment. Our study provides a novel assessment framework for analyzing the mode of action of metal toxicity in aquatic organisms by incorporating the West growth model and the DEB_{tox} theory in a bioenergetics-based perspective into a population stage-structured model. The DEB_{tox} theory distinguishes three types of effects on growth, including direct effects and indirect effects via maintenance and assimilation. The inherent assumption is that only one effect occurs at any time in the lower effect range of the chemical (Kooijman and Bedaux,

1996). Our bioenergetics-based toxicity model described the trend of growth well at lower concentrations (i.e., $\lambda = 1.0001$ at an As exposure of 1 μg mL^{-1}), yet the bias between the model description and the measured data may increase with the gradient of exposure concentration. We suggest that at higher concentrations, multiple effects might coincide to induce the growth toxicity. The single stage-specific MOA proposed in our model may not be reliable at higher concentrations (e.g., a sublethal exposure condition). Sherwood et al. (2000) attributed the inhibition of the growth of yellow perch in heavy-metal-polluted (Cd, Cu, and Zn) lake to the fish being less efficient energy converters and not just to reduced food intake.

Individual development is fueled by metabolism and occurs primarily by cell division. Incoming energy and material from the environment are transformed into metabolic energy and consequently are 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 conservation of metabolic energy, allometric scaling of the metabolic rate, and the energetic cost of producing and maintaining biomass. The capability of this model to quantitatively predict growth curves from birth to mature body size for all multicellular organisms has been validated. This universal growth model provides a basis for understanding the general and fundamental features governing organism growth, although there has been some criticism 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) stated that this model does not intend to account for all observed variation in the growth rate and life histories but that it does provide a baseline for developing more detailed treatments of ontogenetic growth.

The species-specific growth coefficient (A_0) relates the rate of energy allocation of producing a cell to the rate of the whole-organism metabolic rate that fuels this biosynthesis in terms of normalization (West et al., 2004). Our study showed that the values of A_0 did not change significantly in different exposure concentrations, demonstrating waterborne As exposure does not disturb the translation of energy from life-sustaining activities to new biomass production. Growth inhibition from As exposure is not induced by increasing the energy cost to propagate new body tissue. The concentration–effect tilapia growth trajectories could be well described by MOA1 in the embryonic and juvenile stages of decreasing maximum biomass (W_{max}) in the West growth model. Several studies provided evidence that in many organisms, from fruit flies to humans, severe restriction of the food supply during development can prolong the time to maturity and result in a smaller adult size (Davidovitz et al., 2003; West et al., 2004), which correspond to the basic description of the model of decreased feeding according to the DEB_{tox} theory.

Implications on Life-Cycle Risk Assessment

In the present study we assumed that chronic toxicity was initiated when the accumulated chemical exceeded the internal threshold concentration represented by IEC_{10}. The magnitude of the toxicity effect could be expressed as proportional to the difference between the accumulated chemicals and the IEC_{10} and could be formulated as a maximum weight function, shown in eq. (6) for MOA1. IEC_{10} can be accurately derived from the chronic bioassay data by statistical techniques. Thus, the extent of toxicity was strongly determined by the predicted As residue. Our simulations showed that the As residue in tilapia was proportional to the waterborne concentration. 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 to residue predictions in the lower range of exposures, in which the uptake process is not limiting the rate of uptake. Suhendrayatna et al. (2002) indicated that higher concentrations (>10 μg mL^{-1}) of As(III) are toxic to tilapia, thus affecting accumulation of As by tilapia, and that the total As accumulated in tilapia are proportional to external concentrations under 5 μg mL^{-1}. We confirmed that our predictions of As residues in chronic exposure conditions (≤4 μg mL^{-1}) were almost completely captured by our proposed model.

The Leslie matrix population model we employed is density-independent growth of an age-structured population observed at continuous time intervals. Therefore, the effect of the self-limitation was negligible, that is, we did not consider density-dependent parameters such as carrying capacity in the present model. If the population project matrix [A] in eq. (1) is density dependent, it would render most of the analysis for the Leslie matrix population model inapplicable (Caswell, 2001). Populations no longer grow exponentially, and solutions can no longer be written in terms of eigenvalues and eigenvectors. Even the simplest density-dependent models are capable of complex dynamic behaviors, and experiments have confirmed that real populations exhibit at least some of these complexities. The analysis of nonlinear matrix models can be found in Caswell (2001).

In conclusion, the current study has shown how a mechanistic perspective based on the chemical effects on the fish energy budget can promote life-cycle toxicity assessment. The bioenergetics-based matrix population methodology could be employed in a life-cycle toxicity assessment framework to explore the effect of stage-specific MOAs in a population's response to contaminants. Our bioenergetics-based Leslie model yields population end point along with MOAs allowing a comparison of different environmental stressor scenarios. An important implication of our study is that mathematical models can be used to give a population stage structure and clarity to the analysis of the key

population-level end points (the asymptotic population growth rate and stage-specific mode of toxic action) of population dynamics and to evaluate the effect of bioenergetics-based MOAs in field tilapia population response to waterborne As.

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