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POPULATION-LEVEL RESPONSES TO LONG-TERM CADMIUM EXPOSURE IN TWO STRAINS OF THE FRESHWATER GASTROPOD *BIOMPHALARIA GLABRATA*: RESULTS FROM A LIFE-TABLE RESPONSE EXPERIMENT

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Abstract—A life-table response experiment was conducted to ascertain the demographic effects of low-level cadmium exposure on two strains, BS90 and NMRI, of the freshwater gastropod *Biomphalaria glabrata*. Snails were exposed to cadmium continuously from the embryonic stage through adulthood. Results indicated that cadmium significantly affected a number of individual-based parameters, including %hatch, juvenile survival, and adult survival in both strains. Also, fecundity and time to maturity were significantly affected in the NMRI strain. A stage-based, deterministic, population model indicated that population growth rate (λ) was significantly affected by cadmium. Elasticity analysis indicated that juvenile survival, in general, had the greatest contribution to λ. Decomposition analysis indicated that the effects of cadmium on the juvenile stage in BS90 and the embryonic stage in NMRI contributed most to cadmium-induced changes in λ. The BS90 strain was more sensitive to cadmium toxicity than NMRI. Moreover, the two strains differed in pattern of response with different aspects of their respective life histories contributing to cadmium-induced changes in λ. Comparisons were also made between the main model, based on a *Z* transformation of the life-cycle graph, and more commonly used matrix models.

Keywords—Population level Gastropod Cadmium Genetic differences Model

INTRODUCTION

Processes that determine the persistence or extinction of a particular species in a chemically polluted habitat occur, for the most part, at the population level. However, studies designed to improve our understanding of how organisms respond to chemically induced stress or to support ecological risk assessments are primarily individually based. While much of the individual-based research has provided invaluable insight into toxicological effects and may, in some cases, be adequate for risk assessment [1], an ecologically relevant context is generally lacking. Indeed, a widespread criticism of ecotoxicological research and ecological risk assessment has been the apparent lack in establishing and incorporating an understanding of the ecological impacts of chemical exposure [2–4]. One means of placing toxicological studies in an ecological context is to focus on population-level effects [4].

Although efforts to address population-level responses to contaminants have become more common [5–7], for many studies several issues remain that may confound interpretation. Many population-level studies are based on exposure regimes where different life-cycle stages are exposed separately. Yet in natural populations experiencing contaminant-induced stress, exposure is likely to be continuous throughout the life cycle. Accordingly, the focus on single stages in isolation precludes an understanding of the role that residual effects from previous life stages have on the population-level effects of contaminants. Longitudinal studies, in which exposure, for example, is continuous through the life cycle, would likely produce different results compared to exposing different stages separately.

In addition, some studies that have addressed population-level responses have actually emphasized effects of a contaminant on individual-based responses such as growth and reproduction. While these responses do contribute to population-level changes, in and of themselves they offer little insight into what may occur at the population level [8]. Importantly, it has been shown that for chemically stressed organisms, the most important parameter for population growth rate, is not necessarily the most chemically sensitive [9]. Hence, it is difficult to make statements about the population solely on the basis of data from individuals. Addressing toxicological responses in the population-level context requires the use of population-level approaches that integrate individual-based parameters such as survival, growth, and reproduction into a value that represents the health or fitness of the population, such as population growth rate (λ) or the intrinsic rate of increase ($r = \ln[\lambda]$). From a toxicological perspective, it has been argued that λ (or *r*) provides more insight into response to toxicants than do individual-based responses such as survival or reproduction [10]. In addition, population growth rate provides a tractable value for managers and can serve as a convenient and meaningful means to compare populations, habitats, and organisms.

Population models have been applied in a toxicological context and are seeing more widespread use in ecotoxicology [11]. Life-table response experiments, for example, are experiments that explicitly incorporate population models [12]. The primary use of population models in ecotoxicology has been to determine the effects of a contaminant on population growth rates or extinction probabilities. A historically neglected use of population modeling in ecotoxicology is perturbation analyses [12]. Perturbation analyses provide insight into the

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relationships between toxicant-induced changes in population growth and life-history traits. It is this last point that may provide the greatest benefit to management by contributing to a theoretical understanding for how populations respond to toxicant-induced stress.

Several approaches are available for developing models for determination of the population-level responses to contaminants. Recently, Caswell [12] and Forbes et al. [1,10] have explored the use of stage-based projection models in ecotoxicology. Stage-based models are discrete, probabilistic models that allow the impacts of contaminant exposure on vital rates to be quantified and analyzed. They are based on describing the probability of transition between the different stages in the species' life cycle. Many stage-based models can be expressed and solved analytically by either closed-form solution of the fundamental projection equation or the eigenanalysis of the related projection matrix. In this paper we explore both approaches.

Our objective was to develop and compare population models for the planorbid snail *Biomphalaria glabrata*. Models were used to explore the population-level effects of low-level cadmium exposure over the entire life cycle in two strains of this species known to differ in their sensitivity to acute cadmium toxicity [13]. Understanding strain-specific variation in response to toxicants has important implications for toxicity testing [5,14] and aids in characterizing population-level responses across a range of genotypes. Further, elasticity analyses and demographic decomposition were used to analyze and compare the relationship between life-history traits, responses to cadmium, and strain-related effects. The ultimate goal is that studies of this nature will provide a general understanding for how populations respond to toxicant-induced stress that can then be applied to environmental research and management.

MATERIALS AND METHODS

Biomphalaria glabrata is a tropical, freshwater snail that is an intermediate host for the trematode parasites that cause schistosomiasis. Common habitat for this species includes seepage ponds, canals, water tanks, springs, rice fields, and fishponds. *Biomphalaria glabrata* has been used extensively to study host–pathogen interactions, and hence numerous strains are available that differ in traits conferring parasite resistance or susceptibility [15]. The NMRI strain is laboratory derived and highly susceptible to infection by the trematode *Schistosoma mansoni* [16], while the BS90 strain is a parasite-resistant line descended from a Brazilian field isolate [17]. These strains represent phenotypes that can be present in natural habitats, as populations in the wild often are composed of parasite-susceptible and -resistant individuals. In addition to differences in parasite susceptibility, the two strains differed in their sensitivity to cadmium toxicity; the parasite-resistant strain, BS90, was significantly more sensitive to acute cadmium toxicity than the parasite-susceptible NMRI [13]. The NMRI and BS90 strains were chosen for this research because they also differ in genetically determined patterns of pigmentation, a trait useful for easy identification. Both strains can be reared under the same laboratory conditions and exhibit similar behaviors.

Model development

The life cycle of *B. glabrata* can be divided into three stages: embryonic, juvenile, and adult (Fig. 1). Snails of this species live approximately one year. Embryonic development occurs within the egg, and juvenile snails emerge approximately one week after deposition of eggs. The juvenile stage is approximately two months in duration but can be extended or shortened, depending on rearing temperature and food level (F.A. Lewis, Biomedical Research Institute, Bethesda, MD, USA, personal communication). The adult stage is characterized by the onset of reproduction, but otherwise no physical changes are noticeable. Adult *B. glabrata* produce an egg mass every 1.5 to 2.0 d and reach sexual maturity in one to three months, depending on laboratory conditions. Average adult size is 10- to 15-mm shell diameter.

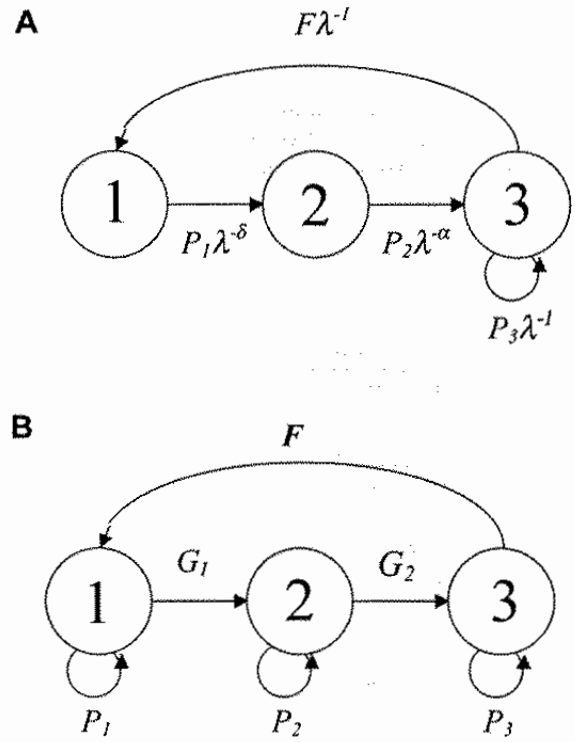

Fig. 1. Life-cycle representation for *Biomphalaria glabrata*. (**A**) *Z* transformation of the *B. glabrata* life-cycle graph. P_i is survival, and F is reproduction. Stage 1 is the embryonic stage, stage 2 is juvenile, and stage 3 is adult. (**B**) Life-cycle graph used to conceptualize matrix models. P_i represents probability of surviving and remaining in the stage, and G_i represents probability of surviving and growing to the next stage.

The modeling approach used in this study was based directly on the life-cycle graph (Fig. 1). Since an individual snail produces an egg capsule about every other day, the time step of the model was set at 2 d. The transitions between the various stages are shown in Figure 1A and can be estimated by combinations of P_i, the probability of survival from stage i to stage $i + 1$. Application of the *Z* transformation [18] produced a characteristic equation given by

$$\lambda^{\delta+\alpha+1} - P_1P_2F - P_3\lambda^{\delta+\alpha} = 0 \quad (1)$$

where F is fecundity, λ is population growth rate (per 2 d), δ is stage duration of the embryo (development time), and α is stage duration of the juvenile (time to maturity). It was assumed that survival through the stage results from surviving the necessary time steps with a per-time-step survival probability of σ_i; hence, $P_1 = \sigma_1^\delta$, $P_2 = \sigma_2^\alpha$, and $P_3 = \sigma_3$. Population growth rate, λ, is solved explicitly by substituting for values of λ that satisfy the characteristic equation.

Sensitivity analysis of population models is used to determine the influence on population growth rate of a change in a model parameter while holding the other parameters constant. The sensitivity of λ to infinitesimally small changes in each of the vital rates was determined by implicit differentiation of the characteristic equation. The sensitivities are as follows:

$$\frac{\partial\lambda}{\partial\sigma_1} = \frac{\sigma_2^{\alpha}\delta\sigma_1^{\delta-1}F}{(\alpha+\delta+1)\lambda^{\alpha+\delta} - \sigma_3(\alpha+\delta)\lambda^{\alpha+\delta-1}} \tag{2}$$

$$\frac{\partial\lambda}{\partial\sigma_2} = \frac{\sigma_1^{\delta}\alpha\sigma_2^{\alpha-1}F}{(\alpha+\delta+1)\lambda^{\alpha+\delta} - \sigma_3(\alpha+\delta)\lambda^{\alpha+\delta-1}} \tag{3}$$

$$\frac{\partial\lambda}{\partial\sigma_3} = \frac{\lambda^{\alpha+\delta}}{(\alpha+\delta+1)\lambda^{\alpha+\delta} - \sigma_3(\alpha+\delta)\lambda^{\alpha+\delta-1}} \tag{4}$$

$$\frac{\partial\lambda}{\partial\alpha} = \frac{-\lambda^{\alpha+\delta+1}\ln\lambda + \sigma_1^{\delta}\sigma_2^{\alpha}F\ln\sigma_2 + \sigma_3^{\beta}\lambda^{\alpha+\delta+1-\beta}\ln\lambda}{(\alpha+\delta+1)\lambda^{\alpha+\delta} - \sigma_3(\alpha+\delta)\lambda^{\alpha+\delta-1}} \tag{5}$$

$$\frac{\partial\lambda}{\partial F} = \frac{\sigma_1^{\delta}\cdot\sigma_2^{\alpha}}{(\alpha+\delta+1)\lambda^{\alpha+\delta} - \sigma_3(\alpha+\delta)\lambda^{\alpha+\delta-1}} \tag{6}$$

$$\frac{\partial\lambda}{\partial\delta} = \frac{-\lambda^{\alpha+\delta+1}\ln\lambda + \sigma_1^{\delta}\sigma_2^{\alpha}F\ln\sigma_1 + \sigma_3\lambda^{\alpha+\delta}\ln\lambda}{(\alpha+\delta+1)\lambda^{\alpha+\delta} - \sigma_3(\alpha+\delta)\lambda^{\alpha+\delta-1}} \tag{7}$$

Elasticities, which quantify the change in λ to equal proportional changes in transitions in the life-cycle graph, are calculated by multiplying the sensitivity of a given parameter by the parameter value divided by λ. Thus,

$$e_{ij} = \left(\frac{a_{ij}}{\lambda}\right)s_{ij} \tag{8}$$

Elasticity, like sensitivity, is useful for comparing the relative contribution of changes in a parameter to changes in λ. However, elasticity accounts for differences in scale between vital rates (such as survival and fecundity) and provides a measure of the contribution of each of the vital rates to population growth rate [19]. Elasticities were reported for comparing the relative contribution of the vital rates to population growth rate in this study.

Sensitivity and elasticity are considered prospective perturbation analyses and generally provide insight into how λ changes in response to a small change in a given parameter [20]. Demographic decomposition, a retrospective perturbation analysis, can yield valuable insights into the population-level responses of toxicant-exposed organisms by providing the relative contribution of the vital rates to differences in population growth rate [21]. For cadmium-exposed snails, the contribution of each of the parameters to treatment-induced changes in λ can be calculated from the following expression:

$$\lambda^{(t)} - \lambda^{(c)} = (a_{ij}^{(t)} - a_{ij}^{(c)})\cdot\frac{\partial\lambda}{\partial a_{ij}} \tag{9}$$

where sensitivity is determined at the mean of the treatment and control parameters and a_{ij} are the vital rates of treatment (t) and control (c) snails.

Although the model based on the Z-transformed, life-cycle graph was chosen as the primary model, matrix models have also been used to assess population-level impacts of toxicants [7]. Two matrix models, based on different parameterization methods, were used to compare against the model derived from the Z-transformed life-cycle graph. The purpose of the comparison was to determine the validity of the model derived from the life-cycle graph and to provide a comparison of different modeling approaches that may be useful in promoting the use of models in ecotoxicology.

For both matrix models, the projection matrix was determined from the life-cycle graph (Fig. 1B) and was

$$A = \begin{bmatrix} P_1 & 0 & F \\ G_1 & P_2 & 0 \\ 0 & G_2 & P_3 \end{bmatrix} \tag{10}$$

where P_i, for a given stage, represents the probability of surviving and remaining in that stage over one time step and G_i represents the probability of surviving and moving to the next stage over one time step. Fecundity (F) was the same value as that used for the model described previously.

The two matrix models have the same basic projection matrix but differ in the estimates of the survival and growth parameters, P_i and G_i, respectively. Estimation of P_i and G_i were based on survival and life-history data. In this first model, P_i and G_i were obtained from the lower-order parameter, γ_i, which is an approximate constant probability of an individual moving from stage i to stage $i+1$ over the given time interval. For each stage, γ_i is calculated from the following expression:

$$\gamma_i = \frac{(\sigma_i/\lambda_{init})^{T_i} - (\sigma_i/\lambda_{init})^{T_i-1}}{(\sigma_i/\lambda_{init})^{T_i} - 1} \tag{11}$$

where T_i is stage duration, σ_i is survival, and λ_{init} is a value of population growth rate required for this parameterization. Both P_i and G_i were obtained from

$$P_i = \sigma_i\cdot\gamma_i \qquad G_i = \sigma_i\cdot(1-\gamma_i)$$

Population growth rate is the dominant eigenvalue of the projection matrix. The model was iterated until values of λ_{init} and λ_{final} were equal. This model will be referred to as the gamma model, after the parameter γ_i used to generate the vital rates used in the projection matrix.

In a second parameterization method for matrix models, P_i and G_i were calculated as

$$P_i = \left(\frac{1-\sigma_i^{T_i-1}}{1-\sigma_i^{T_i}}\right)\sigma_i \qquad G_i = \frac{\sigma_i^{T_i}(1-\sigma_i)}{1-\sigma_i^{T_i}} \tag{12}$$

This method of parameterization is based on a geometric series representing survival through a stage and will be referred to as the geometric model.

The stable stage distribution for both matrix models (w_i) and the reproductive values (v_i) are the right and left eigenvectors of the projection matrix. Sensitivities, s_{ij}, can be calculated using the following formula:

$$s_{ij} = \frac{\partial\lambda}{\partial a_{ij}} = \frac{\langle v_i\cdot w_j\rangle}{\langle v,\ w\rangle} \tag{13}$$

where $\langle\ \rangle$ indicates a scalar product.

Elasticity was calculated as previously mentioned (Eqn. 8). Likewise, the formula for demographic decomposition was the same, with sensitivity evaluated at the mean of the matrices for treatment (cadmium) and control.

Experiments to estimate model parameters

We employed a longitudinal experimental design that enabled us to follow each cohort and measure survival, growth, and reproduction under different cadmium concentrations. The

cadmium exposure regime was strain specific and determined by comparing the median effective concentration (EC50) and no-observed-effect level for the developmental stage, which was the most sensitive in both strains [13]. The criteria for selection of cadmium concentrations for the experiment was to use concentrations that caused stress and mortality but not total mortality. Since NMRI is more tolerant to cadmium then BS90, this strain was exposed to 0.0, 0.025, 0.05, and 0.10 μM cadmium, and BS90 was exposed to 0.0, 0.0125, and 0.025 μM cadmium. Pilot studies indicated that in BS90, concentrations above 0.025 μM cadmium resulted in 100% mortality prior to the onset of reproduction. The greater number of concentrations used for NMRI is related to the greater variation in response to cadmium seen in this strain. The overall design allowed for between-strain comparisons at the common concentration of 0.025 μM and also allowed for assessment of population responses at strain-specific cadmium exposures. All cadmium concentrations were nominal.

All exposures were conducted in a constant temperature room (26°C). Strains of *B. glabrata* used for this study were obtained from the Biomedical Research Institute (Rockville, MD, USA). All strains were reared for one generation before use in the experiments. In general, snails were fed romaine lettuce cooked in a microwave. Snails were kept in 3-L plastic containers at a density of approximately 20 per liter. Artificial spring water was used for all experiments and snail maintenance. The composition of the snail media was 1 mM NaCl, 1 mM $MgSO_4$, 0.10 mM K_2SO_4, 0.1 μM $FeCl_3 \cdot 6H_2O$, and 0.5 mM $NaHCO_3$. Water changes (100%) were completed two to three times per week. Cadmium exposure concentrations were obtained by supplementing the artificial spring water from a stock solution of 1 mM cadmium chloride in metal-free deionized water.

Each treatment consisted of three replicates with 40 snails in each replicate. To initiate a replicate, 1-d-old egg masses were collected from stock populations of BS90 and NMRI and placed into the treatments. Egg masses were observed for time to hatch and %hatch (survival). Once all eggs had hatched, juveniles were transferred to 3-L plastic containers. When juveniles reached a size large enough to survive handling (two to three weeks), the density was lowered to approximately 30 snails per liter. On growing another two to three weeks, density was reduced to 20 snails per liter, the final density. Water volume was reduced as snails died so that a relatively constant density could be maintained. Observations were taken at least biweekly on number of snails alive, number dead, and egg masses produced. Periodically, the number of eggs per egg mass was counted. Hatching success was determined both at the start of the experiment (with eggs from snails naive to cadmium) and at the end of the experiment (with eggs from snails that had been exposed to cadmium for their life cycle).

The parameters for the population model were derived from direct observation of snails in the long-term exposures. As snails were in populations of up to 40, it was impossible to determine the onset of reproduction on a per individual basis. Instead, it was assumed that adult snails produce about 0.6 egg masses per day, a number derived from observations on control snails. Number of egg capsules produced by the entire replicate was recorded and used to determine the average number of egg masses per snail per day. When the number of egg masses per snail per day reached 0.6, it was assumed all snails were reproductive. By back calculating, the number of mature snails at a given time could be determined. The number of new adults at a given time was used to determine the average time to reproductive maturity. This method, although not as precise as following isolated individuals, provided a realistic measure based on experimental conditions. Estimates of survival for embryos, σ_1, were determined by following egg masses through the course of development and determining percentage that successfully hatched. Estimates of survival through development used in the model were based on %hatch of embryos derived from snails that had experienced the cadmium exposure for their life cycle as opposed to using %hatch of embryos derived from parental snails naive to cadmium. It was hypothesized that long-term exposure to cadmium would influence the hatching success of embryos and therefore that the chosen estimate of survival would allow an accurate projection of the population-level responses. Survival of juvenile and adult snails was determined by counting dead snails and calculating the survival rate since the last observation (2–4-d interval). Average survival from one observation time to the next was used for survival rate in the model. Juvenile (σ_2) and adult (σ_3) survival rates were differentiated based on the time to maturity of juveniles; all snails prior to average time to maturity were considered juveniles.

Fecundity for the model represents the number of eggs produced per snail per time step and is estimated for each replicate from the product of the number of eggs per egg mass and average number of egg masses produced per snail per time step for that replicate. Importantly, F did not take into account hatching success, which appears separately as survival through development (σ_1).

Statistical analyses

Analysis of variance was used for all comparisons of individual-based and population-level responses unless variances were nonhomogeneous, in which case an analysis of variance was conducted on log-transformed data. If variances were still nonhomogeneous, the nonparametric Kruskal–Wallis test was used. All replicates were treated as distinct populations and followed accordingly. Elasticity values were reported as averages (± standard deviation) of a given treatment. Demographic decomposition was conducted using the mean of a given cadmium exposure compared to the mean control; thus, no estimates of variance were reported.

RESULTS

Individual-level responses to cadmium

The individual-based responses to complete life-cycle exposure to cadmium are presented in Figure 2. In BS90, cadmium had varied effects on reproduction; hatching success (Kruskal–Wallis, $p < 0.027$) was significantly less in cadmium-exposed snails while fecundity actually increased (see *Discussion*). Time to maturity for juvenile BS90 was not significantly affected by cadmium. However, both juvenile ($p \leq 0.002$) and adult (Kruskal–Wallis, $p < 0.027$) survival per time step were significantly reduced in BS90 snails exposed to cadmium, though survival was reduced to a greater degree in juveniles than adults.

In NMRI, the individual-level responses to long-term cadmium exposure were somewhat different than in BS90 (Fig. 2). Cadmium significantly reduced hatching success ($p \leq 0.0001$) and fecundity ($p \leq 0.002$). A positive correlation was observed between time to maturity and cadmium concentration; however, the effect was not significant. Both juvenile (p

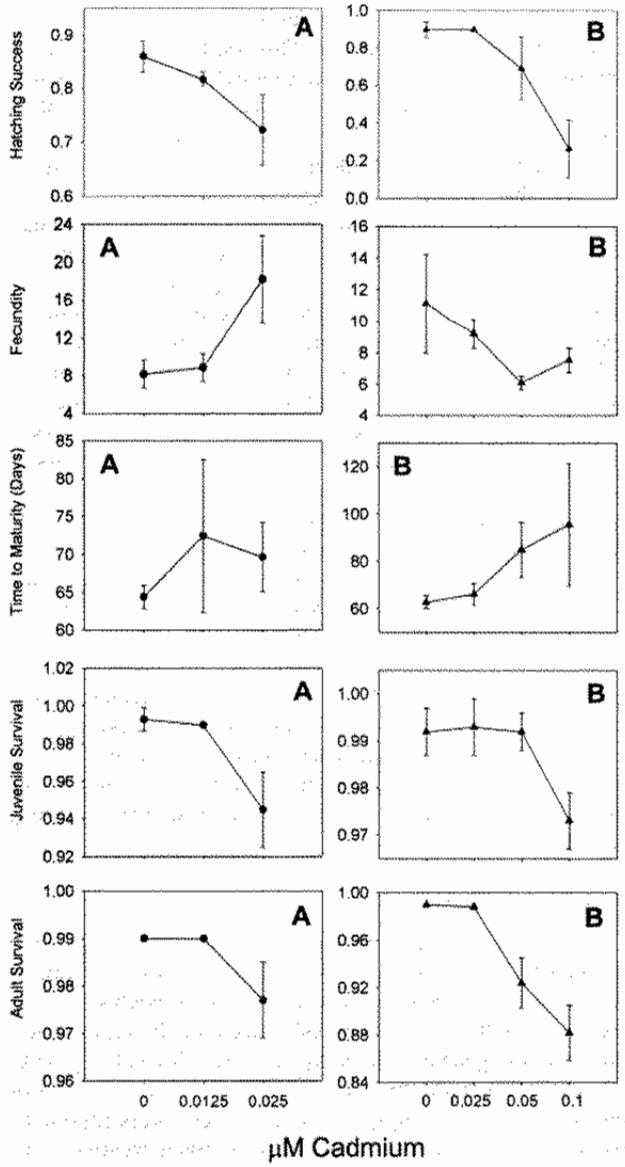

Fig. 2. Effects of cadmium on individual-based parameters in BS90 (**A**) and NMRI (**B**). Error bars are standard deviation.

≤ 0.0001) and adult survival (Kruskal–Wallis, $p < 0.019$) per time step was significantly reduced by cadmium. In contrast to BS90, adult survival at all cadmium concentrations was reduced more than juvenile survival in NMRI.

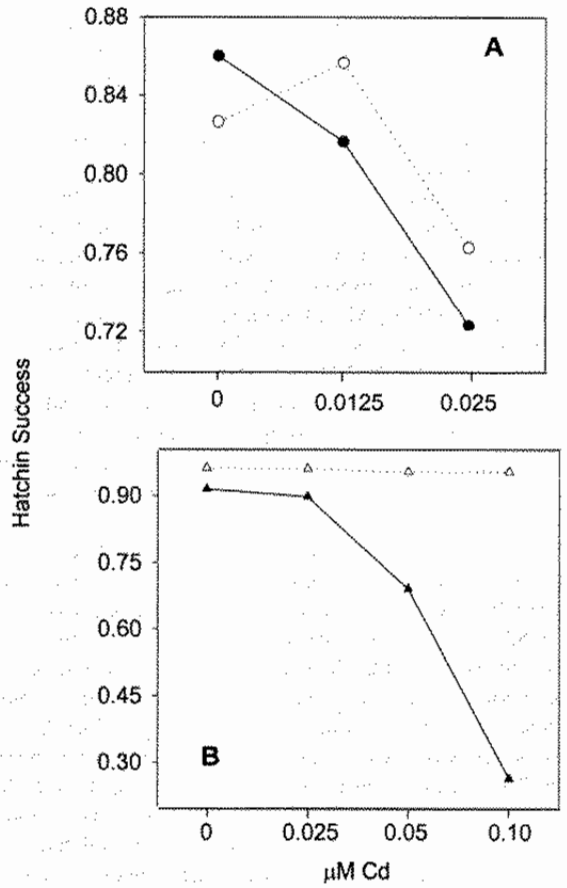

Fig. 3. Comparison of hatching success of BS90 (**A**) and NMRI (**B**) embryos from adults naive to cadmium (open symbols, dashed lines) and embryos from adults exposed to cadmium for one life cycle (closed symbols, solid lines).

Hatching success was affected by exposure to cadmium over the life cycle. Figure 3 shows hatching success for embryos exposed to cadmium at the start of the long-term exposure and for embryos obtained from adults that had survived the long-term exposure to the point of egg mass collection. No significant effects of cadmium on hatching success of embryos from snails naive to cadmium were observed. Alternatively, cadmium significantly affected hatching success in both BS90 (Fig. 3A; $p \leq 0.018$) and NMRI (Fig. 3B $p \leq 0.0001$) embryos obtained from snails that had experienced the long-term exposure.

Table 1. Comparison of λ from the model based on the *Z* transfromation of the life-cycle graph and the two matrix models[a]

Strain	µM	λ_I	λ_{II}	λ_{III}
BS90	Control	1.097 (0.006)	1.099 (0.005)	1.233 (0.013)
	0.0125	1.086 (0.013)	1.093 (0.005)	1.209 (0.032)
	0.025	1.052 (0.010)*	1.049 (0.011)*	1.109 (0.039)*
NMRI	Control	1.118 (0.009)	1.118 (0.009)	1.316 (0.036)
	0.025	1.108 (0.006)	1.106 (0.008)	1.274 (0.017)
	0.05	1.046 (0.016)*	1.047 (0.016)	1.101 (0.053)*
	0.10	0.947 (0.039)*	0.931 (0.064)*	0.967 (0.016)*

[a] Values in parentheses are standard deviations of mean λ. λ_I obtained from the model based on *Z* transformation of life-cycle graph; λ_{II} obtained from the matrix model, gamma parameterization; λ_{III} obtained from the matrix model, geometric series expansion parameterization. * indicates significantly different than control as determined from Bonferroni post hoc test.

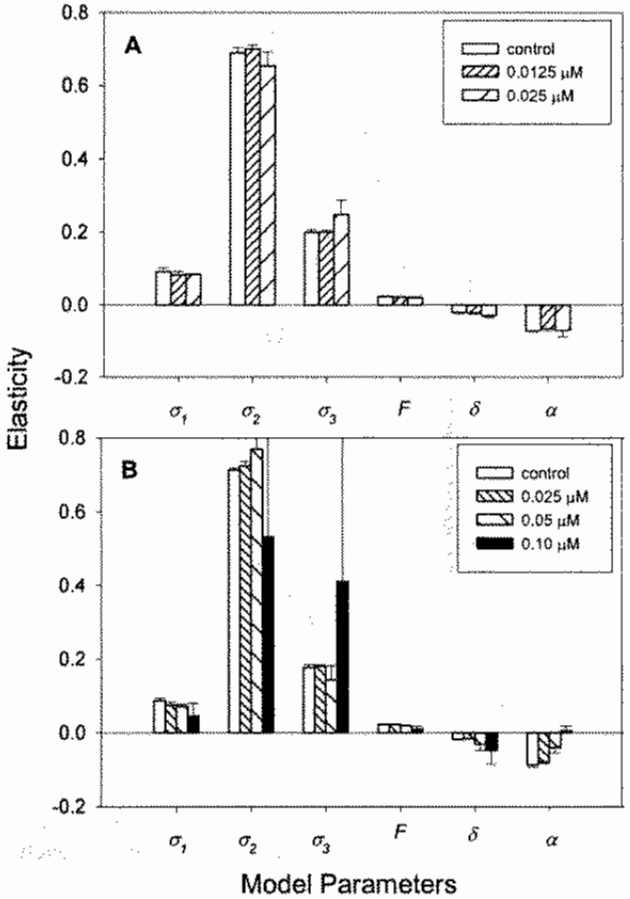

Fig. 4. Effects of cadmium on elasticity patterns in BS90 (**A**) and NMRI (**B**). Error bars are standard deviation.

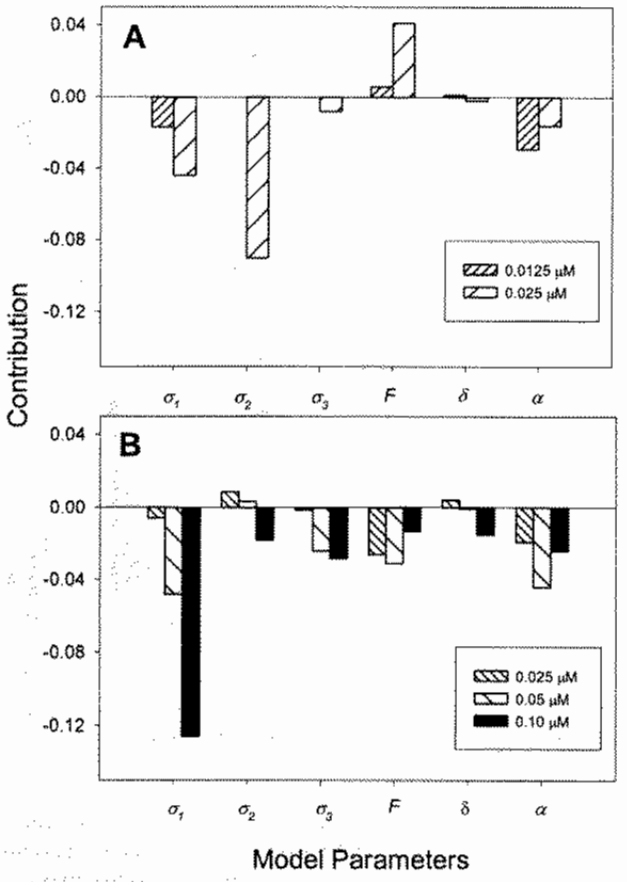

Fig. 5. Effects of cadmium on the contribution of life-history traits to changes in λ for BS90 (**A**) and NMRI (**B**).

Population-level responses

Life-cycle graph model. Population growth rates, λ (per 2 d) for control BS90 and control NMRI were 1.097 (± 0.006) and 1.118 (± 0.009), respectively (Table 1; λ_1). Cadmium significantly reduced population growth rate in both BS90 and NMRI (Table 1; λ_1). Although a decrease in population growth rate from the control to the lowest cadmium concentration in both strains was observed, these differences were not significant. At the higher cadmium concentrations (0.025 µM for BS90 and 0.05 and 0.10 µM for NMRI), cadmium-exposed snails had values of λ significantly different from control and significantly different from the lowest cadmium concentration. The model predicted that all strain/treatment groups were exhibiting positive population growth ($\lambda > 1$) except in NMRI at 0.10 µM cadmium, for which λ was 0.947 (± 0.039).

The pattern of elasticity values in BS90 and NMRI control and low-cadmium-exposed populations indicated that λ is most sensitive to changes in juvenile survivorship, σ_2, followed by changes in adult survivorship, σ_3 (Fig. 4). Increases in time to maturity, α, had the greatest negative impact on population growth rate followed by increases in egg development time, δ. Fecundity, *F*, had low elasticity values, indicating that growth rate of both strains of *B. glabrata* was not very sensitive to changes in fecundity.

Cadmium altered the elasticity patterns in both BS90 and NMRI but had the largest impact in NMRI. The high cadmium exposure concentration (0.025 µM) in BS90 caused the elasticity of adult survivorship to increase, while the elasticity of both egg survival and juvenile survival decreased compared to control (Fig. 4A). For NMRI, the higher cadmium concentrations (0.05 and 0.10 µM) caused a shift in the elasticity pattern as well. Similar to BS90, the elasticity of NMRI adult survival increased with increasing cadmium, while both egg survival and juvenile survival decreased compared to control (Fig. 4B).

Decomposition analysis indicated that the vital rates that were altered when populations were exposed to Cd were not well predicted by elasticities. For BS90 exposed to 0.025 µM cadmium, decomposition analysis showed that a decrease in juvenile survival contributed most to the decrease in λ, followed by a decrease in survival in the developmental stage (Fig. 5A). The increase in time to maturity also contributed to negative effects on population growth in cadmium-exposed BS90, particularly in the lower cadmium concentration (0.0125 µM). In NMRI, a decrease in survival through development contributed most to lower values of λ seen in cadmium-exposed snails (Fig. 5B). An increase in time to maturity and a decrease in fecundity also contributed to the lower value of λ in cadmium-exposed NMRI (Fig. 5B). In NMRI exposed to 0.10 µM cadmium, juvenile and adult survival also contributed to the lower value of λ.

Model comparison

The λ estimates projected by the stage-based matrix models were different than those produced by the model based on the Z-tansformed life-cycle graph (Table 1). The projections also differed between the two different matrix model formulations. The model based on the gamma parameterization produced values of λ (Table 1, λ_{II}) most similar to those of the model

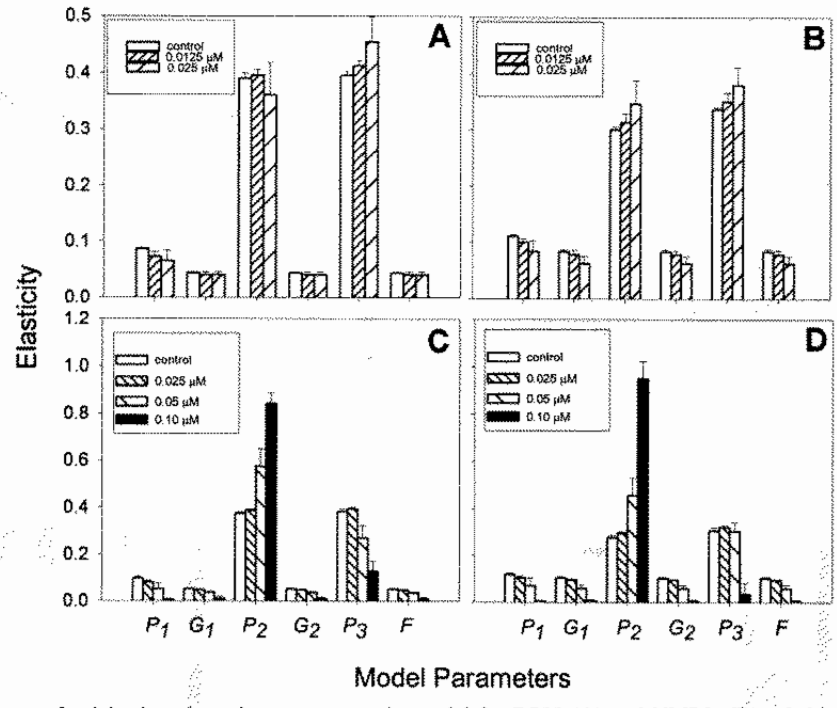

Fig. 6. Effects of cadmium on elasticity based on the gamma matrix model for BS90 (**A**) and NMRI (**C**) and the geometric matrix model for BS90 (**B**) and NMRI (**D**). Error bars are standard deviation.

derived from the life-cycle graph for NMRI and BS90. Values of λ provided by the model based on the geometric parameterization (Table 1, λ_{III}) were higher than the other two estimates. Demographic decomposition indicated that for the model based on the geometric parameterization, G_2, contributed most to the differences in population growth rate between the two matrix modeling methods. That is, the geometric parameterization yielded higher estimates of the probability of juveniles surviving and growing into the next stage, which translated into higher values of λ.

Patterns of elasticity were similar between the two matrix models (Fig. 6) and differed slightly from the elasticity patterns generated by the model based on the *Z* transformation of the life-cycle graph (Fig. 4). Direct comparison of the elasticities between the matrix models and the model based on the life-cycle graph is complicated because of the different parameterizations of the two basic approaches. However, elasticity values generated by the matrix models indicated a high elasticity for the adult stage (Fig. 6). In comparison, the elasticity of adult survival was relatively less in the model based on the life-cycle graph (Fig. 4). For BS90, elasticity analysis of the gamma matrix model (Fig. 6A) and the geometric matrix model (Fig. 6B) indicated that P_2 and P_3 had the highest elasticity.

Cadmium significantly affected both matrix model estimates of λ in BS90 and NMRI in a pattern similar to that observed in the *Z* transformation model (Table 1). Elasticity patterns from both matrix models changed following exposure to cadmium. The BS90 adult survival (P_3) was associated with relatively higher elasticity values with increasing cadmium concentration and the elasticity values of the other parameters decreasing with increasing cadmium (Fig. 6A and B). For NMRI, elasticity patterns of the two matrix models were again similar to each other with P_2 and P_3 having the highest values. Cadmium had a marked influence on elasticity values with a relative increase in P_2 and a relative decrease in all other parameters with increasing cadmium concentration (Fig. 6C and D). Overall, only minor differences were observed in the elasticity patterns produced from each of the matrix modeling approaches.

Decomposition of the effects of cadmium on λ for BS90 is shown for both the gamma (Fig. 7A) and the geometric matrix (Fig. 7B) model. The lower λ in BS90 exposed to 0.0125 µM cadmium was due to cadmium-induced effects on primarily the embryonic and juvenile stages. Minor differences were observed between the two matrix models. The parameter, P_1, had a greater contribution to λ than G_1 in the gamma model, while the values were reversed in the geometric model. Also, P_2 was slightly positive in the geometric model though negative in the gamma model. For BS90 exposed to 0.025 µM cadmium, the differences between demographic decomposition of the two matrix models were somewhat more extreme. In the gamma model, G_1 contributed most to the change in λ followed by P_2, G_2, P_3, and P_1. Summing the contributions to the change in λ, the developmental and juvenile stages were roughly equal (Fig. 7A). In the geometric model, the juvenile stage (G_2 and P_2) contributed more to the change in λ than the developmental stage (G_1 and P_1) (Fig. 7B). Fecundity for BS90 at both cadmium concentrations and in both models contributed positively to λ.

In NMRI, the results of the demographic decomposition of the two matrix models differed. In the model based on the gamma parameterization, G_2, juvenile probability of surviving and moving to the next stage, contributed positively to population growth rate in cadmium-exposed snails (Fig. 7C). In the geometric parameterized matrix model, G_2 contributed negatively to population growth rate in cadmium-exposed snails (Fig. 7D). In both models for NMRI, the probability of surviving and remaining in the developmental stage had the greatest negative effect on population growth rate of cadmium-exposed snails, followed by negative values in all other parameters (except G_2 of gamma model as noted). Another difference between the demographic decomposition in the two

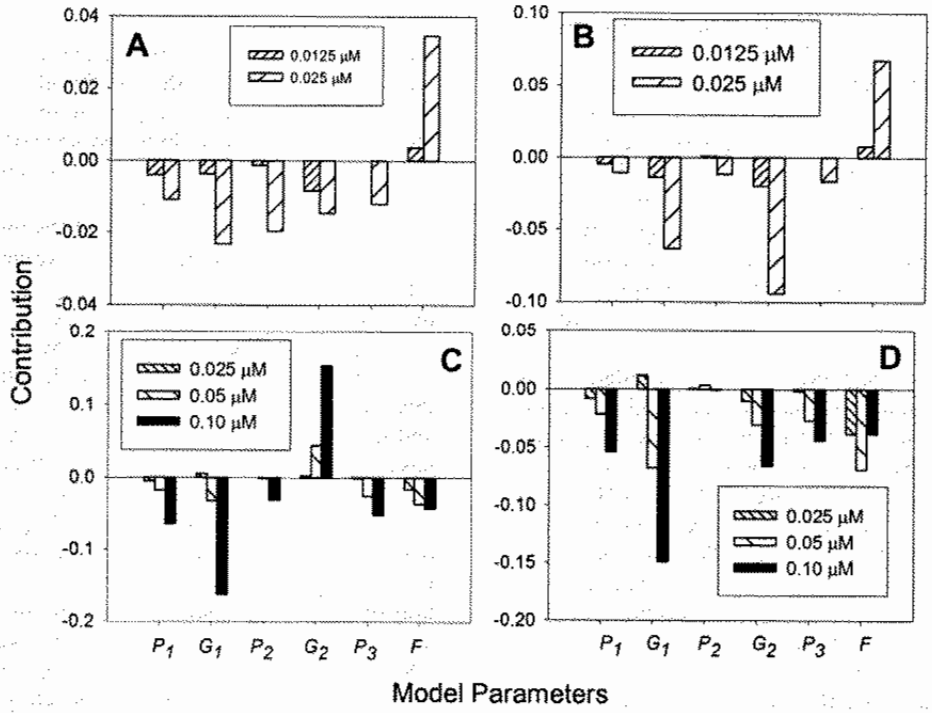

Fig. 7. Effects of cadmium on the contribution of life-history traits to changes in λ for the gamma matrix model for BS90 (**A**) and NMRI (**C**) and the geometric matrix model BS90 (**B**) and NMRI (**D**).

matrix models was in fecundity. In the gamma model, the contribution of fecundity to changes in λ increased with cadmium concentration (Fig. 7C). In the geometric model, the contribution of fecundity to changes in λ and cadmium concentration was not correlated (Fig. 7D).

DISCUSSION

A central objective of this study was to develop stage-based population models for *B. glabrata* that could be used to examine the population-level effects of continuous, long-term, and low-level cadmium exposure. The use of population modeling places toxicant-induced effects in a robust population-level context that can be explored further. Without the use of population models, it is difficult to understand the demographic consequences of effects, such as reduced reproduction. Frequently, population-level effects are considered any individual-level response that could be related to population viability. The application of population modeling in ecotoxicology and conservation biology, however, has shown that life-history traits that appear to be most important to the population, can, in fact, play a relatively minor role in the actual dynamics of the population [9,12,21]. To base the response of the population on only one or a few parameters, without consideration for their interaction, would likely lead to an inaccurate assessment of population-level effects. This is true even if the response of the most chemically sensitive parameters are known [9].

Population models have been used in ecotoxicology [7,22,23]; however, only recently have perturbation analyses been conducted [5,6]. Often, λ has been the major parameter of interest and the only model output reported. Detailed demographic information, including perturbation analyses, is available for polychaetes exposed to several organic enrichment/contaminant treatments [6]. For polychaetes exposed to hydrocarbon contaminants, a decrease in λ was driven primarily by reductions in fecundity and an increase in time to maturity [6]. Similarly, cadmium-induced decreases in λ were driven in large part by an increase in time to maturity in nematodes [9]. A study on the demographic effects of cadmium on clones of the gastropod *Potamopyrgus antipodarum* showed that λ was most sensitive to changes in survival parameters, while cadmium-induced changes in λ were driven by effects of cadmium on time to first reproduction and reproductive output [5]. In the study reported here, cadmium-induced reductions in λ were driven mostly by effects on mortality, although increased time to maturity did contribute to the changes in λ but primarily in NMRI. *Biomphalaria glabrata* has a relatively long time to maturity and life span; hence, changes in these parameters are less likely to contribute strongly to changes in λ. As an example, the polychaete *Streblaspio benedicti* exposed to hydrocarbons exhibited a 167% increase in age at first reproduction from 11.3 to 30.2 d [6]. The greatest change in time to maturity in this study occurred for NMRI at 0.10 µM and was a 52% change.

In this study, comparison of results from the elasticity and decomposition analyses provided insight into which life-history traits contributed most to λ and which contributed most to cadmium-induced changes in λ. In both BS90 and NMRI, reductions in λ were driven largely by effects of cadmium on embryonic survival (hatching success), which had a low elasticity. However, for BS90 at the highest cadmium concentration, effects of cadmium on juvenile survival, which had the highest elasticity, had the greatest impact on λ. In clones of the snail *Potamopyrgus antipodarum*, cadmium-induced alterations in λ were driven by effects on reproductive output and time to first reproduction, both of which had low elasticities compared to juvenile and adult survival [5]. Further characterizing the interplay between the importance of life-history traits to λ and their contribution to toxicant-induced changes in λ may provide extremely useful insight into the application

of toxicity tests toward predicting effects in naturally occurring populations in contaminated habitats.

The differences in response to long-term cadmium exposure in BS90 and NMRI, particularly at 0.025 μM cadmium, indicate that intraspecific variation in response to contaminants can be significant and, therefore, has likely implications for assessing toxicity. Clone-specific differences in response to toxicant-induced stress, including metal exposure, have been documented in *Daphnia magna* [14,24] and the gastropod *P. antipodarum* [5]. The range in EC50 for mortality of *D. magna* clones varied from less than one to more than two orders of magnitude [24]. Moreover, no patterns in rank order of clone resistance between toxicants were observed. Similarly, *P. antipodarum* clones showed differences in magnitude and pattern of response to cadmium [5]. For *B. glabrata* larval, juvenile, and adult stages, NMRI have been shown to be more resistant to acute cadmium exposure compared to BS90 [13]. Sullivan [25] showed that the M-line strain of *B. glabrata* was more tolerant to copper toxicity than the PR-79 strain. In addition, the M-line strain had increased resistance to copper after several generations of selection for copper resistance, while the PR-79 strain showed no differences in resistance to copper under the same selection process [25].

It is not surprising that differences in response to toxicant exposure may exist between groups of the same species that have different histories, either in the laboratory or in the field. This type of variation, however, has strong implications for the interpretation and application of data obtained from toxicological studies. Ideally, variation due to strain-specific differences in toxicant resistance and response should be considered in environmental assessments. However, this would likely be difficult, time consuming, and expensive. Nonetheless, further research is necessary to determine the level of effort required in elucidating the significance of interstrain (or interpopulation) variation.

In addition to the differences in sensitivity to cadmium, the two strains in this study exhibited differences in the reproductive response to cadmium. All concentrations of cadmium negatively affected reproduction in NMRI, while reproduction in cadmium-exposed BS90 actually increased compared to control BS90. The negative effect of cadmium on reproduction in NMRI is not atypical, as numerous examples of the negative effects of cadmium on reproduction exist in a wide variety of species. The apparent positive effect of cadmium on reproduction in BS90 is somewhat surprising. A possible explanation is that mortality, particularly at 0.025 μM, alleviated some density-dependent effects by allowing the surviving snails greater access to resources. Toxicant-induced effects can have a net positive effect by reducing density-dependent mortality [26]. Although attempts were made to reduce the potential for density-dependent effects by reducing volume of media as snails died, below approximately 20 snails, the volume could not be reduced further. Therefore, as the number of snails went below 20, some relief from density-dependent stresses likely occurred. Adequately addressing the increased reproductive output in BS90 exposed to 0.025 μM cadmium would require further experimentation. Indeed, the relationship between density-dependent effects and toxicant-induced stress requires more study, as this is likely an important interaction. Another explanation for increased reproduction in cadmium-exposed BS90 could be related to a hormetic or stimulatory effect of low toxicant exposure/dose. Although controversy exists concerning the validity of the hormetic response, numerous examples have been noted in the literature [27]. It is difficult to say whether hormesis played a role in the increased reproduction of BS90, though the elevated reproduction at 0.0125 μM cadmium is suggestive of hormetic effects. Hormetic effects occur only in the absence of observable toxicity; thus, BS90 at 0.025 μM cadmium did not have increased fecundity as a result of hormesis.

In addition to the intraspecific differences in effects of cadmium, it is worth noting that λ for control BS90 and NMRI differed with NMRI having the higher value. Demographic decomposition of the control of both strains indicates that the higher population growth rate in NMRI is attributable to, in order of decreasing contribution to the difference in λ, fecundity (0.036), %hatch (0.021), time to maturity (5.1×10^{-3}), and developmental time (9.6×10^{-3}). Simply stated, NMRI has a higher reproductive output than BS90. It is hypothesized that resistance to parasitic infection is accompanied by costs of resistance that can manifest in *B. glabrata* as decreased reproduction [28,29] and/or an increase in time to maturity [30]. This is the first study to attempt a detailed characterization of the demographics of two strains of *B. glabrata* that differ in parasite susceptibility. The lower λ seen in control BS90 compared to control NMRI supports the cost-of-resistance hypothesis. An interesting insight provided by the elasticity analysis is that for control BS90 and NMRI, reproduction had a relatively low elasticity value. Often, costs of parasite resistance have manifested in at least one aspect of reproduction. The low elasticity of this parameter suggests that it would be most susceptible to variation since it is not contributing strongly to λ. Hence, from a life-history perspective, reproduction would be a likely and least damaging trait in which costs might manifest.

From a methodological perspective, this study provides some insight into the use of different models in assessing the demographic effects of toxicant exposure. The principal model developed in this study was based on the *Z*-transformed, life-cycle graph [18,31], which has the advantage of incorporating stage duration as an explicit parameter in the model. Stage duration, particularly time to maturity, is an important demographic parameter [32] and has been shown to vary in response to exposure to metals [9] and organic compounds [6]. In addition, the sensitivities and elasticities of the vital rates were expressed directly as opposed to stage-based matrix models, where sensitivities and elasticities were obtained for matrix entries that are themselves functions of the vital rates (P_i and G_i).

The purpose behind constructing the two matrix models was to corroborate results obtained from the model derived from the *Z*-transformed, life-cycle graph and to provide a comparison of several different approaches that may be useful in designing and implementing models. The matrix model based on the calculation of the lower-order parameter, γ_i, produced values of λ similar to those of the life-cycle graph model. The estimates of λ produced by the matrix model based on the geometric expansion of survival were higher. In the latter model, estimates of λ similar to those produced by the other methods can be obtained by dividing survival, σ_i, in the estimation of P_i and G_i by λ_{init} and iterating until λ_{init} and λ are equal [31]. The matrix model based on γ_i has been used to evaluate the population-level effects of dioxin and polychlorinated biphenyls in estuarine fish, *Fundulus heteroclitus* [7]. Munns et al. [7] concluded that dioxin and polychlorinated biphenyl exposure in this fish can result in a reduction in λ of 2% per 14-

d time step, resulting in a 40% decline in *F. heteroclitus* over the course of one year. The parameterization based on the geometric series of survival has not been applied in an ecotoxicological context but has seen application in the conservation of loggerhead sea turtles [33] and sandbar sharks [34,35].

In comparing the models, the life-cycle graph approach has the advantage that vital rates are directly incorporated into the model. As a result, perturbation analyses, also in terms of the vital rates, are more easily interpretable. The disadvantages are an increase in analytical complexity, particularly with the estimation of sensitivities (requiring implicit differentiation). The matrix models are relatively simple to construct, and perturbation analyses are quite elegant. The disadvantage to matrix approaches is that matrix entries are functions of the vital rates; hence, interpretation is slightly more complicated. In addition, stage duration is not normally an explicit parameter and hence not directly addressed in perturbation analyses, although knowledge of the relationship between duration, P_i and G_i can remedy this. As a bottom line, the matrix models are extremely useful for assessing λ and the perturbation analyses, while the model based on the Z transformation or the life-cycle graph provides a more clearcut interpretation and allows incorporation of demographically important data on stage duration. The choice of model must balance the data available and the project objectives.

CONCLUSION

Population models are useful for understanding population-level responses to contaminant-induced stress. In particular, the model based on the Z transformation of the life-cycle graph allowed the explicit inclusion of development time and time to maturity, which provided more detailed insight into the population-level effects of cadmium. However, this model is somewhat more difficult than the matrix-based models, and the math can become quite complex as the number of life-cycle stages increases. Hence, modeling effort should be driven by the information identified as important. For many cases, an assessment of λ may be all that is required; however, perturbation analyses have the advantage of providing detailed insight into population-level effects.

Intraspecies differences in response to contaminants can manifest as differences not only in the magnitude of response but also in the pattern of response. For example, while life-history traits of both BS90 and NMRI had similar elasticities, the relative contribution to cadmium-induced changes in λ differed. Further understanding of these patterns of response will contribute to our ability to predict the effects of contaminants on natural populations. Moreover, characterizing differences in response within a species is invaluable for interpreting and using toxicity information for environmental management and regulation.

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