

# Demographic Responses to Multigeneration Cadmium Exposure in Two Strains of the Freshwater Gastropod, *Biomphalaria glabrata*

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**Abstract** A life table response experiment (LTRE) was used to quantify the population-level effects of continuous, multigeneration cadmium exposure on two strains of the freshwater gastropod, *Biomphalaria glabrata*: the parasite-resistant BS90 and parasite-susceptible NMRI strains. Snails were exposed to waterborne cadmium for three consecutive generations. Survival, growth, and reproduction were measured empirically and incorporated into a stage-based, deterministic population model. Cadmium significantly affected hatching success, time to maturity, and juvenile and adult survival in both strains. There were significant effects of generation on fecundity, hatching success, time to maturity and juvenile survival in NMRI, and time to maturity and adult survival in BS90. Cadmium significantly affected the population growth rate,  $\lambda$ , in BS90. Cadmium, generation, and the cadmium  $\times$  generation interaction had significant effects on  $\lambda$  in NMRI. At the high cadmium exposure,  $\lambda$  for NMRI showed a decrease from generation 1 to generation 2, followed by an

increase from generation 2 to generation 3. The  $\lambda$  value in high-cadmium BS90 steadily decreased over the three generations, while NMRI at this same concentration was similar to the controls. The results indicate that strain-specific differences in response to multigeneration cadmium exposure are evident in *B. glabrata*. Moreover, effects seen in the first generation are not necessarily indicative of effects in subsequent generations. Changes in  $\lambda$  over the course of the three-generation exposure suggest that acclimation and/or adaptation to cadmium may have occurred, particularly in NMRI at the high cadmium exposure level.

There is growing interest in incorporating population-level approaches into both ecotoxicology and ecological risk assessment (Barnthouse et al. 2008). Population-level responses to toxicant exposure reflect the integration of toxicant-induced effects on individual vital rates that may directly relate to population dynamics (e.g., growth rate, survival, reproduction). Quantifying the changes to individual vital rates alone may offer little insight into the functioning of the population, as changes in one vital rate may be counteracted or ameliorated by changes in another (Forbes and Calow 1999). Accordingly, to understand the population-level effects, models or experiments that quantify the combined effects of changes in vital rates are required. Life table response experiments (LTREs) (Caswell 1996b) seek to quantify the consequences of experimentally applied treatments on the entire life history (e.g., Levin et al. 1996; Rao and Sarma 1986). Additionally, population models that integrate individual-based responses may offer insights into higher-order, demographic processes. From a toxicological perspective, population models are useful in

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placing individual-based, toxicant-induced effects into a population (and life history) context. It has been argued that such an approach is an improvement over individually focused endpoints (Caswell 1996b; Forbes and Calow 1999) and may prove useful for environmental management decisions.

While there are numerous examples of the use of population models to analyze and interpret toxicity data (Kuhn et al. 2000; Raimondo et al. 2006), there are very few examples where exposures were continued for several generations and the responses analyzed using a population model. Given that environmental exposures are likely to continue through several generations for many species, multigenerational studies can provide valuable insight into the long-term response of organisms to chemically induced stressors (Janssen et al. 2000). Using population models and associated demographic analyses may further enhance the utility of these types of studies.

Implicit in studies on multigeneration toxicant exposure is the potential for acclimation or selection-induced (adaptation) changes in population-level responses (Klerks and Weiss 1987). Most studies that have incorporated multigeneration exposures have focused on alterations of the genetic profile or performance endpoints of the exposed population, which could result in or indicate acclimation or adaptation (Guan and Wang 2006a, b; Muysen and Janssen 2004; Postma and Davids 1995). In addition to potential adaptation as a result of multigeneration exposure, genetic differences within a given species would likely contribute to differences in magnitude and pattern of response through the course of the exposure (Barata et al. 2002; Salice and Roesijadi 2002). Characterization of such genotype-specific differences would contribute to a better generalized understanding of population-level responses to long-term, multigeneration exposure and may provide additional insights into contaminant tolerance. Studies designed to explore the differences in population-level responses within a species can provide insight into the variability in this response and would likely contribute to the applicability of such studies to a broader range of scenarios.

The tropical, freshwater planorbid snail *Biomphalaria glabrata* is an attractive model for studying population-level responses to multigeneration toxicant exposures. This species is an important intermediate host for schistosome parasites and has been used extensively to study host-pathogen relationships (Richards et al. 1992). As such, several independent strains are maintained for their susceptibility or resistance to parasitic infection. Because this species is an important and well-studied host for schistosome parasites, it offers the opportunity to study the effects of toxicants superimposed over any preexisting traits related to parasite susceptibility or resistance. Previously, we described strain-specific differences in sensitivity to

acute cadmium exposures, whereby the parasite-resistant BS90 strain, was significantly more sensitive to cadmium toxicity than the parasite-susceptible NMRI strain (Salice and Roesijadi 2002). In a related study, a LTRE was used to analyze the population-level consequences for both strains of a single-generation exposure to Cd (Salice and Miller 2003). Population growth rates ( $\lambda$ ) were significantly affected by exposure to Cd, but the life history stages most responsible for the changes in population growth rate differed between the two strains (Salice and Miller 2003).

The objective of this study was to evaluate the response to multigeneration Cd exposure in the BS90 and NMRI strains of *Biomphalaria glabrata* using a LTRE in which the population-level consequences were derived from a stage-based deterministic population model.

## Materials and Methods

### Study Organism

The NMRI and BS90 strains of *B. glabrata* used for this project were provided by the Biomedical Research Institute, Rockville, MD. BS90 is a dark-pigmented, parasite-resistant strain, whereas NMRI is an albino, parasite-susceptible strain. *B. glabrata* are hermaphroditic and can undergo both self-fertilization and sexual reproduction. Full details of animal care procedures were reported previously (Salice and Miller 2003). Snails were reared in artificial culture medium (1 mM CaCl<sub>2</sub>, 1 mM MgSO<sub>4</sub>, 0.10 mM K<sub>2</sub>SO<sub>4</sub>, 0.1  $\mu$ M FeCl<sub>3</sub>•6H<sub>2</sub>O, and 0.5 mM NaHCO<sub>3</sub> dissolved in deionized water). Water changes were conducted two or three times per week. Cadmium was added to the water immediately after the water change. Snails were fed romaine lettuce ad libitum: approximately half a leaf every other day per 40 adult snails. Lettuce was cooked for 1 min in a microwave to facilitate consumption by snails. All experiments were conducted in a constant-temperature room at 26°C.

### Experimental Design

Our experiments involved a longitudinal design in which individual cohorts were exposed to a range of cadmium concentrations for three generations and analyzed for survival and reproduction. The duration of the entire experiment was approximately 1 year and used an experimental design similar to that already described (Salice and Miller 2003). The relatively cadmium-resistant NMRI strain was exposed to nominal concentrations of 0.0, 0.025, 0.05, and 0.10  $\mu$ M Cd added to the culture medium, while the cadmium-sensitive BS90 strain was exposed to nominal

concentrations of 0.0, 0.0125, and 0.025  $\mu\text{M}$  Cd. A pilot study indicated that BS90 snails could not survive one generation at 0.05  $\mu\text{M}$  Cd. The overall design allowed between-strain comparisons at the common concentration of 0.025  $\mu\text{M}$  and for assessment of population responses at strain-specific cadmium exposures.

Initially, and at the start of each new generation, 1-day-old egg masses were collected from stock populations or experimental groups of BS90 and NMRI (from previous generation). Time-to-hatch and survival were determined for each egg mass. Once all eggs had hatched (at  $\sim 1$  week), juveniles were transferred to 3-L plastic containers, which contained 2 L culture medium. After 3–4 weeks of growth, density was reduced to 20 snails per liter, the approximate final density in experiments. Each treatment consisted of three replicates, with 40 juvenile/adult snails in each replicate. In all cases, eggs from a specific treatment and replicate were used to start the next generation, maintaining the same treatment and replicate designation (e.g., BS90 0.025  $\mu\text{M}$  Cd replicate A). Observations were taken at least twice weekly on number of snails alive, number dead, and egg masses produced. The number of eggs per egg mass was counted throughout exposures. Hatching success, determined as percentage hatch, was determined at the start of the experiment for eggs from snails naive to cadmium and at the end of each generation for eggs from both control and cadmium-exposed replicates.

#### Estimation of Vital Rates

##### Reproduction

As snails were cultured collectively in treatment groups of up to 40 individuals, it was impossible to determine the onset of reproduction on a per individual basis. Based on observations on control snails, we determined a basal level of reproduction was about 0.6 egg mass per day. The number of egg capsules produced by the entire replicate was recorded, from which was determined the average number of egg masses per snail per day, which was used to estimate the average time to reproductive maturity. Fecundity,  $f$ , for the model represents the number of eggs produced per snail per time step not taking into account hatching success, which appears separately as survival,  $\sigma_1$ , through development.

##### Survival

Estimates of embryo survival were determined by following egg masses through the course of development and determining the percentage that successfully hatched. Dead juvenile and adult snails were counted and survival rate since the last observation (2–4 days) was determined. Average survival from one observation time to the next

was used for survival rate in the model. The demarcation of juvenile and adult stages was based on the time to maturity of juveniles; all snails younger than the average time to maturity were considered juveniles.

#### Population Model

The *B. glabrata* life cycle was represented as three stages: embryonic, juvenile, and adult (Salice and Miller 2003). The embryo stage is approximately 1 week and transitions to the juvenile stage through hatching. The juvenile stage is 1–3 months long transitioning to the adult stage, which is characterized by the onset of egg laying. There are no noticeable external morphological changes associated with transition from the juvenile to the adult stage. Average adult size is 10–15 mm. Adults produce an egg mass every 1.5–2.0 days. Snails of this species live approximately 1 year.

Each life history stage was represented directly in a stage-based, deterministic model (Caswell 1996a; Levin et al. 1996) to quantify the impacts of empirically estimated vital rates on the population-level response to cadmium exposure. The modeling approach used in this study was based directly on the life cycle graph of *B. glabrata* and closely followed previously reported methods (Salice and Miller 2003). This model construct was chosen in order to gain insight into the potential population-level effects as they relate to particular stage-specific vital rates.  $Z$  transformations of the graph result in transitions that ultimately yield the following characteristic equation:

$$\lambda^{\alpha+2} - \sigma_1 \sigma_2^\alpha F - \sigma_3 \lambda^{\alpha+1} = 0 \quad (1)$$

$\sigma_i$  represents survival,  $\lambda$  is population growth rate, and  $\alpha$  is stage duration (in model time steps) of the juvenile (time to maturity). The stage-specific probabilities of survival were calculated from estimates of the probability of an individual in stage  $i$  ( $1 = \text{embryo}$ ,  $2 = \text{juvenile}$ ,  $3 = \text{adult}$ ) surviving one time step, which was set at 2 days. In a previous study, embryonic stage duration was included as an explicit parameter, as is time to maturity in the current model (Salice and Miller 2003). Perturbation analyses indicated that development time was not contributing to population growth rate or changes in population growth rate as a result of cadmium exposure (Salice and Miller 2003) and, for simplicity, was excluded from the current model. Population growth rate,  $\lambda$ , was solved explicitly by substituting values of  $\lambda$  that satisfy the characteristic equation. When  $\lambda > 1$ , it indicates that the population is growing, when  $\lambda < 1$ , the population is declining, and when  $\lambda = 1$ , the population size is stable.

Sensitivity analysis of population models is used to determine the change in  $\lambda$  resulting from a change in a given model parameter while holding the other parameters

constant (Caswell 1996b). Sensitivity of  $\lambda$  to infinitesimal changes in each of the vital rates was determined for each model parameter by implicit differentiation of the characteristic equation (Caswell 1996a, b). The formulas for sensitivities were

$$\frac{\partial \lambda}{\partial \sigma_1} = \frac{\sigma_2^\alpha f}{(\alpha + 2)\lambda^{\alpha+1} - \sigma_3(\alpha + 1)\lambda^\alpha} \quad (2)$$

$$\frac{\partial \lambda}{\partial \sigma_2} = \frac{\sigma_1 \alpha \sigma_2^{\alpha-1} f}{(\alpha + 2)\lambda^{\alpha+1} - \sigma_3(\alpha + 1)\lambda^\alpha} \quad (3)$$

$$\frac{\partial \lambda}{\partial \sigma_3} = \frac{\lambda^{\alpha+1}}{(\alpha + 2)\lambda^{\alpha+1} - \sigma_3(\alpha + 1)\lambda^\alpha} \quad (4)$$

$$\frac{\partial \lambda}{\partial f} = \frac{\sigma_1 \sigma_2^\alpha}{(\alpha + 2)\lambda^{\alpha+1} - \sigma_3(\alpha + 1)\lambda^\alpha} \quad (5)$$

$$\frac{\partial \lambda}{\partial \alpha} = \frac{-\lambda^{\alpha+2} \ln \lambda + \sigma_1 \sigma_2^\alpha f \ln \sigma_2 + \sigma_3 \lambda^{\alpha+1} \ln \lambda}{(\alpha + 2)\lambda^{\alpha+1} - \sigma_3(\alpha + 1)\lambda^\alpha} \quad (6)$$

Elasticity, like sensitivity, is useful for comparing the relative contribution of changes in a parameter to changes in  $\lambda$  and is calculated by multiplying the sensitivity of a given parameter by the parameter value divided by  $\lambda$  (Caswell 1996b, 2000). However, where sensitivity is a measure of the absolute contribution, elasticity is a measure of proportional contributions and therefore accounts for differences in scale between vital rates such as survival and fecundity. Elasticity can also be interpreted as a measure of the contribution of each of the vital rates to population growth rate (De Kroon et al. 1986). Elasticities were reported in order to compare the relative contribution of the vital rates to population growth rate in control and cadmium-exposed snails.

Demographic decomposition was used to determine the relative contribution of the cadmium-affected vital rates to differences in population growth rate (Caswell 1989). Sensitivity and elasticity are considered prospective perturbation analyses and generally provide insight into how  $\lambda$  changes in response to a small change in a given vital rate (Caswell 2000). 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 change in vital rates to differences in  $\lambda$ . For cadmium-exposed snails, the contribution of each of the parameters to treatment-induced changes in  $\lambda$  can be calculated from the following expression:

$$\lambda^{(t)} - \lambda^{(c)} = \left( a_{ij}^{(t)} - a_{ij}^{(c)} \right) \cdot \frac{\partial \lambda}{\partial a_{ij}} \quad (7)$$

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.

## Statistical Analysis

A two-way analysis of variance (ANOVA) was used to examine the effects of cadmium and generation on both empirically estimated organism-level endpoints (e.g., egg production) and model-derived population-level parameters. Survival estimates were arcsine square root transformed and fecundity was square root transformed to better accommodate the assumptions of ANOVA. Additional transformations are noted in the results when used. Post hoc tests to compare cadmium treatments and/or generation effects were conducted using the Bonferroni *t*-test. If a cadmium treatment did not last the entire three generations, the treatment was removed for the two-way ANOVA to keep a balanced design. The two strains were analyzed independently, as the treatment levels were not equal for the two strains. The level of statistical significance was set at  $\alpha \leq 0.05$ . Results for BS90 and NMRI were compared at the common concentration of 0.025  $\mu\text{M}$  and were based on significant difference from the respective controls.

## Results

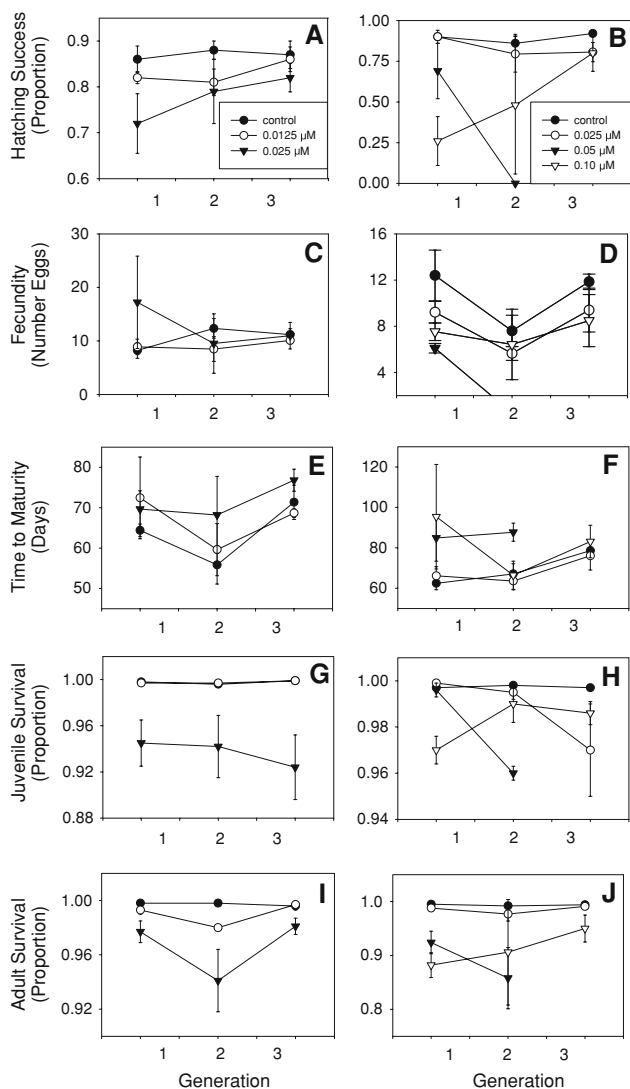
### Reproductive Effects of Cadmium

#### Fecundity

Exposure to elevated cadmium caused a significant decrease in the number of eggs produced in NMRI ( $p \leq 0.003$ ; Fig. 1b) although not in BS90 (Fig. 1a). BS90 exposed to 0.025  $\mu\text{M}$  had produced more eggs in the first generation compared to both the control and the 0.0125  $\mu\text{M}$  treatments and, in fact, was the highest reported for the entire experiment (Fig. 1a), although there were no significant effects of cadmium ( $p \leq 0.252$ ) or generation ( $p \leq 0.757$ ) on fecundity in BS90. In NMRI (Fig. 1b), cadmium exposure resulted in lower numbers of eggs produced than the control NMRI for the 0.025  $\mu\text{M}$  ( $p \leq 0.017$ ) and the 0.10  $\mu\text{M}$  ( $p \leq 0.005$ ) treatments. Separate statistical analysis including the 0.05  $\mu\text{M}$  treatment showed that it differed significantly from the control ( $p \leq 0.001$ ). There was a significant effect of generation on the number of eggs produced in NMRI ( $p \leq 0.001$ ), although the cadmium  $\times$  generation interaction was not significant ( $p \leq 0.460$ ). Post hoc comparison indicated a significant difference between the first and the second generations ( $p \leq 0.003$ ) and between the second and the third generations ( $p \leq 0.003$ ) for NMRI.

#### Hatching Success

Hatching success in BS90 was significantly affected by cadmium ( $p \leq 0.001$ ; Fig. 1c) but not generation ( $p \leq 0.05$ );



**Fig. 1** Individual parameters in BS90 (a) and NMRI (b) exposed to cadmium for three consecutive generations. NMRI exposed to 0.5  $\mu\text{M}$  did not progress to generation three. Note that the scale for the y-axis may differ between strains for a given parameter. Error bars are standard deviations

the cadmium  $\times$  generation interaction was not significant ( $p \leq 0.304$ ). Hatching success showed an exposure-dependent decrease to cadmium exposure (Fig. 1c). This general relationship persisted for all three generations, although it belied an increase in hatching success at the 0.025  $\mu\text{M}$  concentration in the second and third generations (Fig. 1a). Post hoc comparisons indicated a significant difference in BS90 hatching success between the control and the 0.025  $\mu\text{M}$  treatment ( $p \leq 0.001$ ). In NMRI there were significant effects of cadmium (Fig 1d; squared transformation,  $p \leq 0.001$ ), no significant effects of generation ( $p \leq 0.110$ ), and a significant cadmium  $\times$  generation interaction ( $p \leq 0.010$ ). Hatching success in NMRI showed a clear concentration-response relationship in the first generation (Fig. 1d). In the second

generation, hatching success of the 0.05  $\mu\text{M}$  treatment dropped to zero, while hatching success of the 0.10  $\mu\text{M}$  treatment increased from 25% in the first generation to nearly 50% in the second. This trend in the 0.10  $\mu\text{M}$  treatment continued through the third generation, with a hatching success of almost 80%, near that of the 0.025  $\mu\text{M}$  treatment. Post hoc comparisons indicated that the 0.10  $\mu\text{M}$  treatment was significantly different from the control in the first ( $p \leq 0.001$ ) and second ( $p \leq 0.012$ ) generations but not the third ( $p \leq 0.373$ ). NMRI at 0.025  $\mu\text{M}$  had a lower percentage hatch than the control; the difference increased with generation but was not significant for any generation (Fig. 1d).

#### Time to Maturity

Cadmium significantly affected estimated time to maturity in BS90 ( $p \leq 0.038$ ; Fig. 1e) and NMRI (ln transformed,  $p \leq 0.038$ ; Fig. 1f). There was also a significant effect of generation on time to maturity in BS90 ( $p \leq 0.003$ ) and NMRI ( $p \leq 0.023$ ) but the interaction was not significant in either strain. In BS90, each generation showed a mixed response among the different cadmium treatments, with the only apparent exposure-response seen in generation 2 (Fig. 1e). Post hoc comparisons indicated a significant difference between the BS90 control and the 0.025  $\mu\text{M}$  treatment ( $p \leq 0.036$ ) and a significant difference between the first and the second ( $p \leq 0.039$ ) and between the second and the third generations ( $p \leq 0.002$ ). Time to maturity in first-generation NMRI increased with increasing cadmium concentration (Fig. 1f). This relationship did not hold through the second and third generations; time to maturity in the high-cadmium treatment (0.10  $\mu\text{M}$ ) was similar to that of the control in the second generation, and all three remaining treatments in generation 3 had similar times to maturity. The only post hoc comparison that was significantly different for NMRI was between generation 2 and generation 3 ( $p \leq 0.021$ ).

#### Effects of Cadmium on Survival

##### Juvenile Survival

Survival during the juvenile stage was significantly affected by cadmium in BS90 ( $p \leq 0.001$ ; Fig. 1g) but not generation. In BS90 exposed to 0.025  $\mu\text{M}$ , juvenile survival was significantly reduced for all three generations compared to the control ( $p \leq 0.001$ ) and 0.0125  $\mu\text{M}$  ( $p \leq 0.001$ ) treatments (Fig. 1g). The effect of cadmium on juvenile survival in NMRI was also statistically significant ( $p \leq 0.042$ ; Fig. 1h). There were also significant effects of generation ( $p \leq 0.005$ ) and the cadmium  $\times$  generation interaction ( $p \leq 0.023$ ) in NMRI. Generally, effects on juvenile survival were not quite as severe in NMRI

compared to BS90, though survival was particularly low (0.96) at the 0.05  $\mu\text{M}$  treatment in generation 2 (Fig. 1h). When the 0.05  $\mu\text{M}$  treatment in NMRI is included in statistical comparisons, results show a significant difference compared to control ( $p \leq 0.039$ ). Juvenile survival at 0.10  $\mu\text{M}$  was lower than control for all three generations; however, this difference decreased with each subsequent generation.

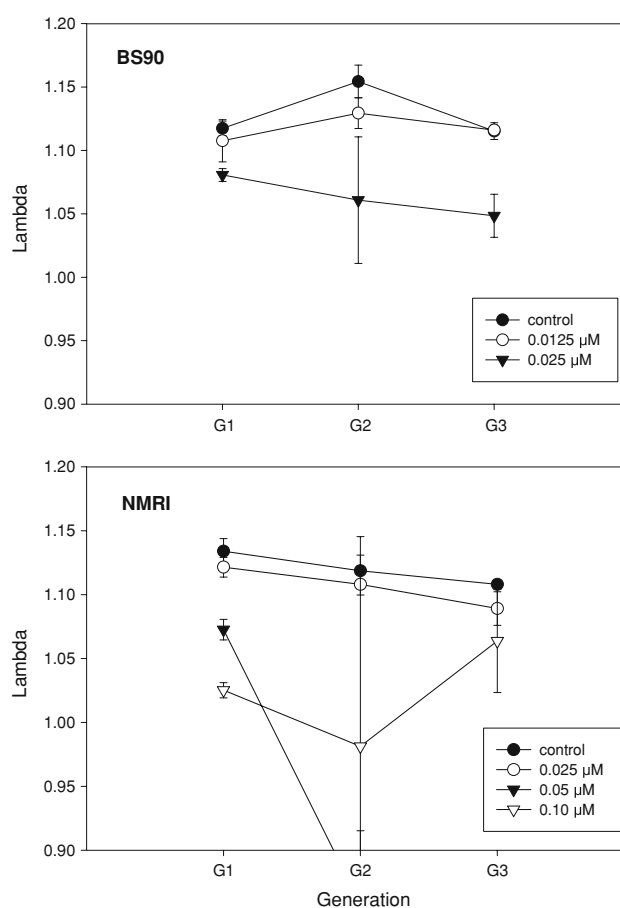
#### Adult Survival

Adult survival was significantly affected by cadmium in BS90 ( $p \leq 0.001$ ; Fig. 1i) and NMRI ( $p \leq 0.001$ ; Fig. 1j). There was a significant effect of generation in BS90 ( $p \leq 0.007$ ) though not in NMRI. Also, the cadmium  $\times$  generation interaction for adult survival was significant in BS90 ( $p \leq 0.001$ ) but not in NMRI ( $p \leq 0.365$ ). In BS90, there was a strong difference in adult survival in the second generation among cadmium treatments though the difference was less in the first and third generations (Fig. 1i). In all three generations of BS90, adult survival was lowest in the 0.025  $\mu\text{M}$  treatment, ranging from 0.97 to 0.94. Post hoc comparisons showed that the 0.025  $\mu\text{M}$  treatment was significantly different from controls in generations 1 ( $p \leq 0.02$ ) and 2 ( $p \leq 0.001$ ) but not 3 ( $p \leq 0.341$ ). In NMRI, adult survival was inversely related to cadmium concentration in the first generation (Fig. 1j). Adult survival in the first-generation NMRI exposed to 0.10  $\mu\text{M}$  Cd was 0.88 and steadily increased in the second (0.91) and third (0.95) generations (Fig. 1j) and, overall, was statistically different from controls ( $p \leq 0.001$ ). Adult survival in the 0.025  $\mu\text{M}$  treatment in NMRI was similar to the control. Adult survival at 0.05  $\mu\text{M}$  was 0.92 ( $p \leq 0.008$ ) in the first generation and only 0.86 ( $p \leq 0.001$ ) in the second generation; a lack of reproduction prevented progress of this treatment to the third generation.

#### Model-Derived Parameters

##### Population Growth Rate

Cadmium significantly affected population growth rate,  $\lambda$ , in BS90 ( $p \leq 0.001$ ; Fig. 2a). Control BS90 had the highest  $\lambda$  (1.12–1.15), while  $\lambda$  values for BS90 at 0.025  $\mu\text{M}$  cadmium were significantly lower than values for both the control ( $p \leq 0.001$ ) and the 0.0125  $\mu\text{M}$  ( $p \leq 0.001$ ) cadmium treatments. The highest value of  $\lambda$  for BS90 at 0.025  $\mu\text{M}$  was 1.081 and occurred in the first generation of exposure. Although there were no significant effects of generation on  $\lambda$ , there were fluctuations between generations with the second generation having the highest  $\lambda$  in both the control ( $\lambda = 1.15$ ) and the 0.0125  $\mu\text{M}$  ( $\lambda = 1.13$ ) cadmium treatments (Fig. 2a). In the 0.025  $\mu\text{M}$  cadmium



**Fig. 2** Population growth rate,  $\lambda$ , in BS90 (a) and NMRI (b) exposed to cadmium for three consecutive generations. Note that NMRI exposed to 0.5  $\mu\text{M}$  did not progress to generation 3. Error bars are standard deviations

treatment,  $\lambda$  decreased steadily from the first to the third generation (Fig. 2a), from 1.08 to 1.04.

In NMRI, cadmium significantly affected  $\lambda$  ( $p \leq 0.001$ ; Fig. 2b). Also, unlike BS90, there was a significant effect of generation ( $p \leq 0.015$ ) and the cadmium  $\times$  generation interaction ( $p \leq 0.025$ ) on  $\lambda$ . NMRI exposed to 0.025  $\mu\text{M}$  cadmium had average values of  $\lambda$  that were 1.11 for all three generations, only slightly lower than control values (Fig. 2b), which averaged 1.12. The  $\lambda$  values for snails at 0.05 and 0.10  $\mu\text{M}$  cadmium concentrations showed wider variation between the generations compared to control snails and snails exposed to 0.025  $\mu\text{M}$  cadmium (Fig. 2b). In the first generation, cadmium concentration and  $\lambda$  were inversely related, with the highest cadmium concentration of 0.10  $\mu\text{M}$  corresponding to the lowest  $\lambda$  of 1.025. In the second generation, NMRI at 0.05  $\mu\text{M}$  cadmium failed to successfully reproduce, effectively ending this treatment (Fig. 2b). Snails at 0.10  $\mu\text{M}$  cadmium had a value of  $\lambda$  during the second generation that went below 1.0 (0.981), primarily due to zero survivorship in one of the replicates.

In the third generation, snails at 0.10  $\mu\text{M}$  had the highest  $\lambda$  (1.064) of the three generations of snails at this cadmium concentration (Fig. 2b).

### Elasticity Analysis

The pattern of elasticity values in BS90 was affected slightly by cadmium and did not appear to change between generations (Fig. 3). In general, juvenile survival ( $\sigma_2$ ) had the highest elasticity followed by adult survival ( $\sigma_3$ ). Elasticity of survival through development ( $\sigma_1$ ) and reproduction ( $f$ ) were both low and approximately equal. Time to maturity ( $\alpha$ ) had negative elasticity values, indicating a negative effect of increasing time to maturity on  $\lambda$ . The only noticeable effect of cadmium occurred at the highest concentration, 0.025  $\mu\text{M}$ . In this group, the elasticity of juvenile survival decreased and the elasticity of adult survival increased compared to control (Fig. 3). This effect was most apparent in the third generation.

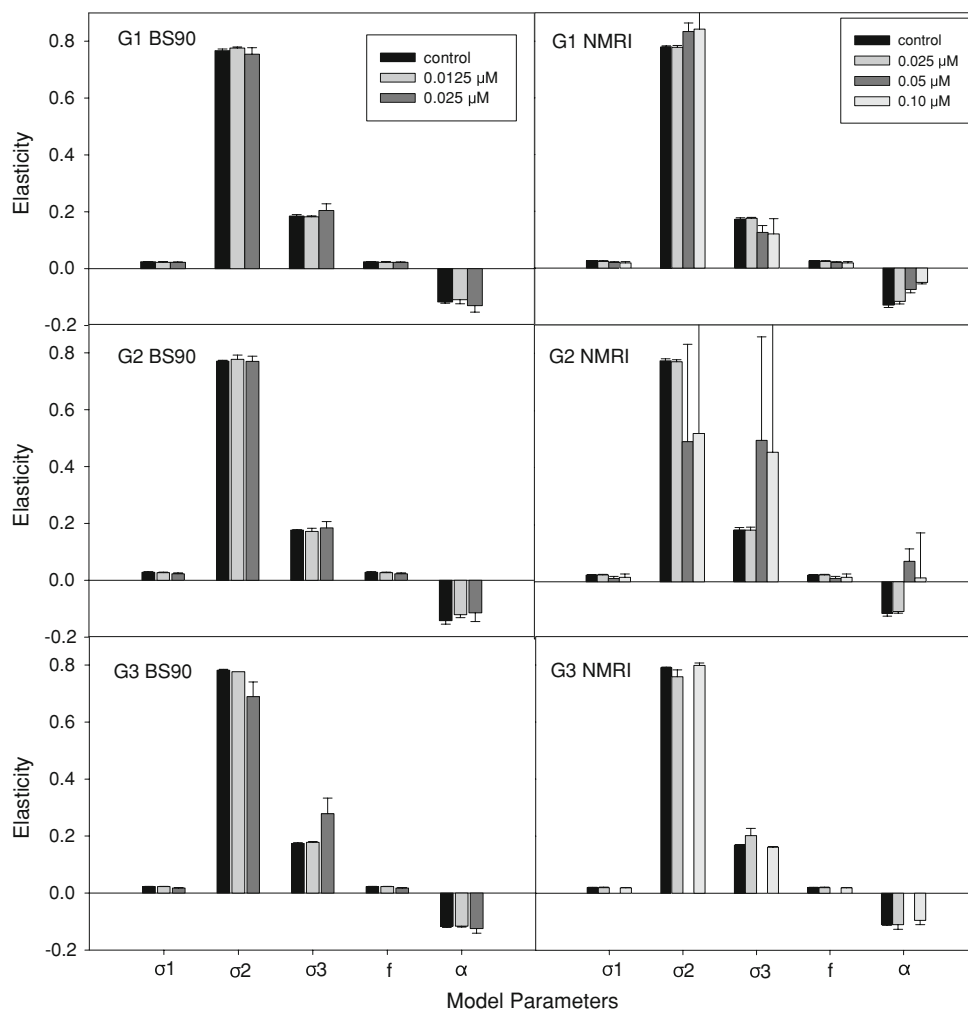
In NMRI, general elasticity patterns were similar to those of BS90, with juvenile survival having the highest

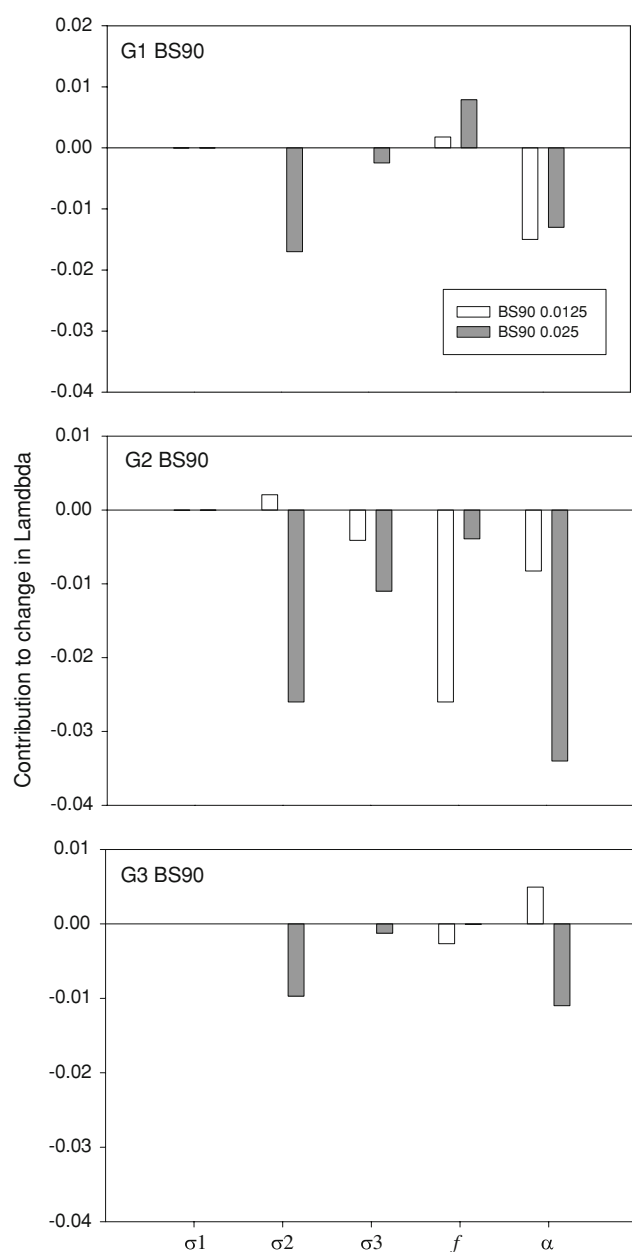
elasticity (Fig. 3). There were apparent effects of both cadmium and generation on elasticity patterns, particularly evident in generation 2 (Fig. 3). Time to maturity showed a concentration-dependent decrease (absolute value) in elasticity. In the second generation, juvenile and adult survival had nearly equal elasticity values in NMRI snails exposed to 0.05 and 0.10  $\mu\text{M}$  cadmium. Time to maturity in both of these treatments showed a positive elasticity, unlike in the previous generation or in comparison to controls. In the third generation, surviving treatment groups (control, 0.025  $\mu\text{M}$ , and 0.10  $\mu\text{M}$ ) had very similar elasticity patterns; juvenile survival was highest followed by adult survival, time to maturity, survival through development, and reproduction (Fig. 3).

### Decomposition Analysis

In BS90, decomposition analysis of the 0.0125  $\mu\text{M}$  cadmium treatment showed that a decrease in reproduction (second and third generation) and an increase in time to maturity (second generation) were largely responsible for

**Fig. 3** Elasticity of BS90 and NMRI exposed to cadmium for three consecutive generations (G1–G3). Error bars are standard deviations

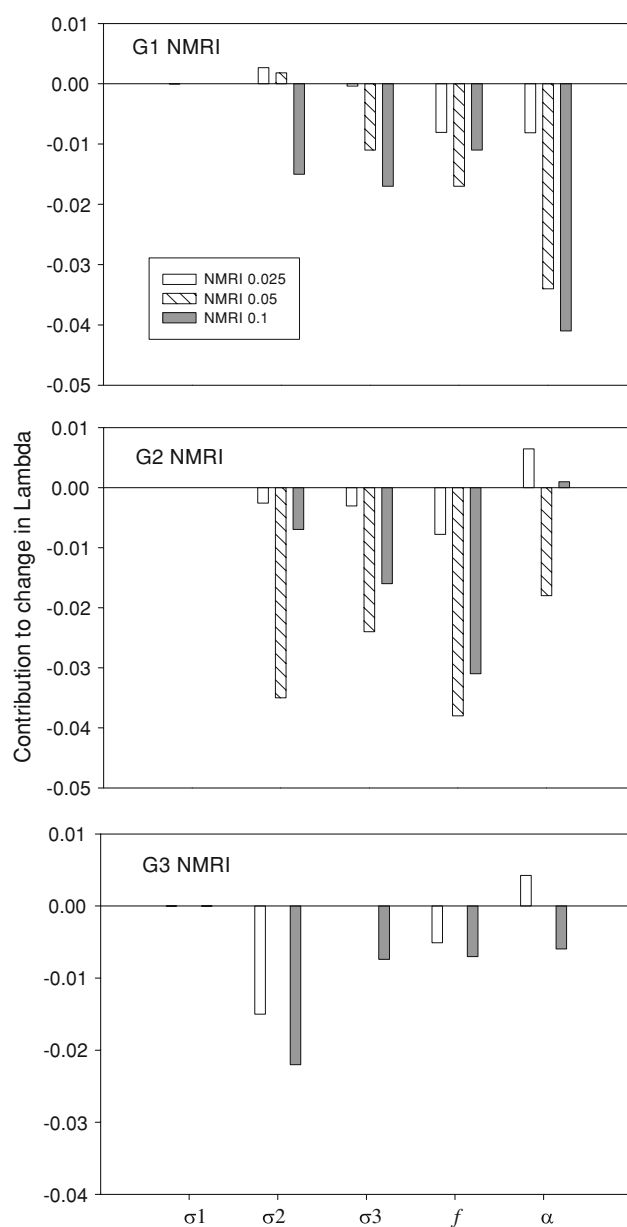




**Fig. 4** Contribution of cadmium-induced changes in model parameters to changes in  $\lambda$  for BS90 exposed to 0.0125  $\mu\text{M}$  cadmium and 0.025  $\mu\text{M}$  cadmium for three consecutive generations (G1–G3)

changes in  $\lambda$  among generations (Fig. 4). For BS90 at 0.025  $\mu\text{M}$ , overall decreases in juvenile survival and increases in time to maturity were responsible for effects on  $\lambda$  (Fig. 4). While the pattern of decomposition was similar for all three generations, magnitude varied, with the largest negative values associated with the greatest decrease in  $\lambda$  (generation 3). In the third generation, adult survival also contributed somewhat to the lower value of  $\lambda$  seen in this generation (Fig. 4).

Decomposition analysis in NMRI indicated that, for each generation, the demographic parameters responsible



**Fig. 5** Contribution of cadmium-induced changes in model parameters to changes in  $\lambda$  for NMRI exposed to 0.025, 0.05, and 0.10  $\mu\text{M}$  cadmium for three consecutive generations (G1–G3)

for changes in  $\lambda$  varied (Fig. 5). For snails exposed to 0.025  $\mu\text{M}$  cadmium, a decrease in reproduction consistently contributed to the lower values of  $\lambda$  (Fig. 5). Increased time to maturity also contributed to changes in  $\lambda$ , especially in the first generation, while juvenile survival was important in the third generation (Fig. 5). In snails exposed to 0.05  $\mu\text{M}$  cadmium, increased time to maturity had the greatest effect on  $\lambda$  followed by reproduction and adult survival in the first generation. In the second generation, snails exposed to 0.05  $\mu\text{M}$  cadmium failed to successfully reproduce and reproduction had the greatest contribution to decreases in  $\lambda$  (Fig. 5). For other



parameters, the order of contribution to the decrease in  $\lambda$  was juvenile survival > adult survival > time to maturity. Population growth rate for NMRI snails exposed to 0.10  $\mu\text{M}$  cadmium in the first generation was less than the control  $\lambda$ , due in large part to an increase in time to maturity, followed by decreases in juvenile and adult survival and reproduction (Fig. 5). In the second generation, reproduction contributed most to the change in  $\lambda$ , followed by adult and then juvenile survival. Third-generation snails exposed to 0.10  $\mu\text{M}$  cadmium had the highest value of  $\lambda$  for the three generations, although it was still lower than control  $\lambda$ . For this treatment/generation, decreases in juvenile survival contributed most to the lower value of  $\lambda$ , followed by adult survival, reproduction, and time to maturity (Fig. 5).

## Discussion

Our results suggest that exposure history can be important in understanding the population-level impacts of toxicant exposure. The results of the first-generation exposure to cadmium in both strains of *B. glabrata* supported the typical toxicological response pattern observed in other studies on metal toxicity in snails (Allah et al. 1997; Münzinger and Guarducci 1988), where survival and/or reproduction generally decrease with increasing toxicant concentration. However, population-level responses in subsequent generations diverged somewhat from this pattern. The exposure-response relationship did not hold in subsequent generations in NMRI. In BS90, the pattern of response was more similar across generations, although snails at the highest concentration appeared to be in steady decline in comparison to BS90 controls.

There are no examples of multigeneration toxicity studies in mollusks; however, several multigeneration studies using *Daphnia* species have shown an increased ability in cadmium-exposed daphnids to tolerate acute cadmium exposure (Guan and Wang 2006a, b; Muysen and Janssen 2004). *Daphnia* in two of the studies (Guan and Wang 2006b; Muysen and Janssen 2004) showed a biphasic pattern in cadmium tolerance whereby tolerance increased to a maximal level by generation 4 (reproductive performance (Guan and Wang 2006b) and generation 2 (48 h  $\text{EC}_{50}$  [Muysen and Janssen 2004]). Estimates of the intrinsic rate of increase,  $r_m$  ( $r_m = \ln\lambda$ ), over seven generations of cadmium exposure (Muysen and Janssen 2004) showed that cadmium had nonlinear effects, with  $r_m$  sometimes higher and sometimes lower than control values. Indeed, some of the highest estimates of  $r_m$  were obtained in the parental generation of the medium cadmium concentration (0.48 nM  $\text{Cd}^{2+}$ ) and the fifth generation of the second-to-highest cadmium concentration

(1.11 nM  $\text{Cd}^{2+}$ ) (Muysen and Janssen 2004). These last results support results obtained in this study where responses to multigeneration cadmium exposure can depart from typical exposure-response relationships observed under single-generation exposures.

Studies of selection for metal tolerance in animals have also been conducted and have incorporated multigenerational exposures (Bengtsson et al. 1985; Postma and Davids 1995). For example, intergenerational differences in response to cadmium exposure have been documented in the midge, *Chironomus riparius* (Postma and Davids 1995). There were no survivors after nine and seven generations at the highest (160 nM Cd) and lowest (17 nM Cd) concentrations, respectively. However, midges at the intermediate cadmium concentration (54.2 nM) survived through 10 generations (Postma and Davids 1995). The lack of a clear concentration-response relationship in later generations (Postma and Davids 1995) mirrors the concentration-specific response of the NMRI strain in our experiment. Other examples of a nonlinear and unpredictable response as a result of multigeneration exposures exist (Lingjaerde et al. 2001). The general expectation is that organisms exposed to higher concentrations of toxicants should exhibit the most severe effects. It seems that exposures over multiple generations can introduce an element of complexity whereby the exposure history impacts the pattern of response in a nonlinear manner. Because this pattern of response is not well studied or even recognized as a generality, no commonly held hypotheses exist to explain it. There are, however, a number of factors that could contribute to a departure from the expected concentration-response pattern. These might include variability in organism tolerance, environmental conditions, or random factors. One hypothesis is that there may exist, in some circumstances, a threshold of stress required to elicit a more pronounced survival response and this response may not manifest under single-generation exposures. Understanding the factors that may contribute to this type of nonlinear response requires further study.

Retrospective analyses (elasticity and demographic decomposition) were used to provide insight into *B. glabrata* demographics in response to cadmium-induced toxicity. Our analysis of elasticity patterns of cadmium-exposed BS90 and NMRI indicated that a few life history parameters (i.e., juvenile and adult survival) were consistently higher compared to other life history parameters through each generation. For NMRI, however, cadmium exposure altered the relative contribution of life history parameters to population growth rate among generations, particularly in the second generation, which was accompanied by greater variation in elasticities as well. It is interesting to note that first- and third-generation NMRI treatments had similar elasticity patterns. Toxicants may alter elasticities but there could perhaps be a drive toward a

baseline or typical elasticity pattern. It is possible that a significant departure from a baseline pattern may indicate that the population is nearing a point of no return.

Demographic decomposition provides an overall sense of how individual trait toxicity might contribute to population-level responses. For example, although elasticity for fecundity was low, the effects of cadmium exposure on reproduction were often responsible for decreases in  $\lambda$ . This was especially true in NMRI at 0.10 and 0.05  $\mu\text{M}$  cadmium, in which effects of cadmium on the number of eggs produced in the latter were responsible for the inability of this treatment to progress to a third generation.

Alternatively, although cadmium significantly affected hatching success, this parameter contributed little to cadmium-induced changes in  $\lambda$ , presumably related to the low elasticity of this value. These results support the hypothesis that fitness reduction or population-level responses are a function of both the life history of the organism and the individual trait sensitivity to toxicants (Kammenga et al. 1996; Raimondo et al. 2006).

It is important to acknowledge that potential population-level effects seen over long-term exposure are not necessarily (1) predicted by effects seen in the first generation, (2) a reflection of the effects of the toxicant on the most sensitive parameter, or (3) predicted by the traits that contribute most to  $\lambda$ . Indeed, a number of factors can play important roles in determining how populations may respond to toxicant-induced stress (Raimondo et al. 2006). It seems difficult, based on results reported here, to know a priori which traits will be most sensitive and which, when impacted, will contribute most to population-level change, especially over several generations. In conservation applications, models are used to determine the population responses to specific conditions (e.g., bycatch in a fishery [Crowder et al. 1994]) or management strategies (e.g., reductions in fishing mortality [Brewster-Geisz and Miller 2000; Miller 2003]). In these cases, it has been assumed that direct effects on life history are not likely to change through time. In the case of toxicants, our study showed that the effects might vary over the course of several generations, with different aspects of the life history contributing to changes in  $\lambda$ .

Intraspecific differences in toxicant tolerance can further complicate efforts to understand and predict population-level responses to long-term exposure. Previously, BS90 was more sensitive to cadmium than NMRI when exposed to acutely toxic levels of cadmium (Salice and Roesijadi 2002). The present study showed that when exposed to the same concentration in a chronic, multigeneration scenario, BS90 is again more sensitive to cadmium than NMRI. Intraspecific differences in toxicant sensitivity are fairly well documented, particularly in *Daphnia* (Barata et al. 2002; Guan and Wang 2006a), and interclonal variation in

sensitivity of *Daphnia* to cadmium toxicity may be related to metallothionein (MT) production differences between the clones (Guan and Wang 2006a). Although no data are available on *B. glabrata* MT production, it is possible that MT or MT-like proteins may account for differences in cadmium sensitivity between BS90 and NMRI. MT is prevalent in many species and can confer increased resistance to some metal exposures in aquatic animals (Roesijadi 1992).

Although the strains were exposed independently and the results have been reported in such a way that suggests a clear delineation between the strains, they likely represent coexisting genotypes. Given that the strain-specific responses to cadmium exposure were different, it is likely that cadmium (or potentially other toxicants) in natural populations could cause significant demographic and genetic changes. Previously, we have suggested that the inverse relationship between parasite resistance and cadmium resistance seen in these strains may reflect an evolutionary tradeoff (Salice and Roesijadi 2002). If that were the case, and the genotypes were to co-occur, our results suggest that selection for cadmium-tolerant snails could result in a corresponding increase in the frequency of parasite susceptibility and hence a concomitant increase in the frequency of schistosome-infected snails. Further study is required to better understand the relationship between resistance to pathogens and exposure/resistance to toxicants.

The nonintuitive nature of the responses seen in the NMRI strain highlights the importance of long-term studies and population models in assessing higher-order effects of toxicants. More studies of this nature may lead to general rules by which outcomes of toxicant exposure can be better characterized, if not predicted. This is particularly pertinent considering that many management decisions rest on results from fairly short-term studies.

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