Hazard/Risk Assessment

EVALUATING THE ECOLOGICAL SIGNIFICANCE OF LABORATORY RESPONSE DATA TO PREDICT POPULATION-LEVEL EFFECTS FOR THE ESTRUARINE AMPHIPOD AMPELISCA ABDITA

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Abstract—Ten-day acute mortality of the benthic amphipod Ampelisca abdita is used in a number of regulatory, research, and monitoring programs to evaluate chemical contamination of marine sediments. Although this endpoint has proven to be valuable for characterizing the relative toxicities of sediments, the significance of acute mortality with respect to population viability has not yet been established. In this study, population modeling along with empirical extrapolation were used to describe a relationship between acute mortality and population-level response of A. abdita. The research involved the performance of a standard 10-d sediment toxicity bioassay and a 70-d full life-cycle chronic population (including reproduction) bioassay exposing A. abdita to sediments spiked with concentrations of the divalent metal cadmium (normalized to acid volatile sulﬁde) expected to produce a range of biological effects. These data provided age-speciﬁc schedules of survival and fecundity that were used to parameterize an age-classiﬁed projection matrix model for A. abdita. Measured exposure data and population growth rate estimates, obtained using the demographic information collected during the 70-d assay, were used to develop exposure–response models. These data were also used to develop an empirical relationship between population growth rate (λ) and acute mortality. This relationship describes how acute data may be used to predict concentrations that produce population-level effects. Model manipulations permit extrapolation of early life-stage mortality (the acute endpoint) to changes in population growth rate. These relationships were used to evaluate a range of ecologically acceptable acute mortality for A. abdita.

Keywords—Ampelisca Population model Exposure–response models Cadmium

INTRODUCTION

The ﬁeld of sediment toxicity testing has expanded during the last 15 years in response to concerns over the assessment and remediation of dredged material and its suitability for open-water disposal. The objective of a sediment toxicity test is to evaluate the potential adverse effects and time-dependent availability of the contaminant mixtures in the sediment. Toxicity testing of sediment can be used to determine the relationship between toxic effects and bioavailability, investigate interactions among contaminants, determine the spatial and temporal distribution of toxicity, evaluate hazards of dredged materials, and monitor the effectiveness of remediation and management actions [1,2]. Effective management of contaminated sediments requires the incorporation of both qualitative and quantitative sediment toxicity data into an ecological risk assessment framework. These risk assessment data provide both chemical characterization and biological information concerning the relationship between chemical concentrations in the sediment and bioavailable concentrations in the organism.

The standard 10-d sediment toxicity test with the benthic amphipod Ampelisca abdita [3] has been used extensively in a number of regulatory, research, and monitoring programs [4–7] to evaluate the effects of chemical contamination of marine sediments. In this assay, toxicity is deﬁned as producing less than 80% survival as compared with a control reference sediment. This 10-d acute mortality endpoint is useful in identifying hot spots and characterizing the relative toxicities of sediments, but its relationship to chronic ﬁeld response or population viability is unknown. A better understanding of the sublethal effects of chemicals in sediment is needed to identify contaminants in sediment that may be interfering with the ability of organisms within a population to develop, grow, or reproduce effectively. Information regarding the effects of long-term exposures, sublethal effects, and ﬁeld population dynamics for A. abdita would reduce uncertainty of extrapolations from a 10-d mortality test performed in the laboratory to chronic and population-level effects [8]. Recent studies [9,10] have begun to explore the ecological relevance of the acute toxicity data by comparing those results with measures of abundance and diversity in benthic assemblages in the field. In these studies, Long et al. [9,10] provide a synoptic review and analysis of the data using multiple ﬁeld monitoring data sets and laboratory data to determine whether measures of chronic benthic health and acute survival correlate signiﬁcantly over both spatial and temporal scales.

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Another method that can be used to explore the links between laboratory responses and population-level field effects for *A. abdita* is the matrix population model. Matrix population models can evaluate the effectiveness of using acute toxicity data for indicating potential effects and for predicting population-level responses (population growth or decline).

In this study, an existing age-classified matrix model [11] for *A. abdita* was refined and parameterized with survival and fecundity probabilities calculated from age-specific schedules of benthic health (e.g., benthic index, infaunal abundance, amphipod abundance, species richness, and biomass) collected from the same field sites. This comparison will provide a measure of the uncertainty associated with extrapolating population-level field effects from acute laboratory tests. In this context, population models may be used as predictive tools both for indicating potential effects and for predicting population-level responses (population growth or decline).

In this study, an existing age-classified matrix model [11] for *A. abdita* was refined and parameterized with survival and fecundity probabilities calculated from age-specific schedules obtained during a 70-d chronic population assay. This chronic assay provided measures of survival and reproduction of *A. abdita* exposed to sediments spiked with cadmium concentrations expected to produce a range of biological effects. Using demographic information collected during the long-term assay, population growth rate estimates (λ) and exposure data were used to develop an exposure–response model. This exposure–response model describes population-level effects produced by a range of cadmium concentrations.

One of the main objectives of this study was to describe the relationship between a commonly evaluated sediment bioassay endpoint (acute 10-d survival) and an indicator of population response, λ. This objective was accomplished by comparing 10-d acute assay results with population model projections (λ) based on data obtained from the 70-d assay (both bioassays used the same acid volatile sulfide [AVS]-normalized cadmium-amended sediments). The resulting relationship allows extrapolation of acute mortality, resulting from stressors measured in the standard 10-d bioassay, to population-level effects. This relationship is important for the development of guidance for the interpretation of standard toxicity data (10-d sediment bioassay) as it relates to population-level effects. This type of analysis provides insight concerning the ecological relevance (population-level effect) of changes observed in endpoints measured in the laboratory and at the individual level of biological organization.

A valuable tool for evaluating the influence of life history traits (survival or reproduction) on the population growth rate of a species is elasticity analysis [12]. Elasticity analysis measures the degree to which λ changes with small proportional changes in each matrix element (survival or reproduction). In this study, elasticity analysis was used to evaluate the effects of different model manipulation scenarios on population growth rate for *A. abdita*. This has become an important tool for population management in conservation biology because it allows quantification of the relative importance of different life history stages. Identification of important stages in a species’ life history can provide environmental managers with a focus for setting meaningful protection limits based on these relatively sensitive stages.

**METHODS**

**Sediment collection and spiking**

Surface sediment (114 L) was collected from the Pettaquamscutt River (locally and henceforth known as the Narrow River), a small estuary flowing into Narragansett Bay, Rhode Island, USA. Stainless steel sieves were used to collect the sediment, which was placed into plastic 18.9-L buckets. Sediment was transported to the laboratory and press sieved through a 2.0-mm sieve to remove indigenous organisms, grass, rocks, and organic matter. The sediment was then press sieved through a 0.3-mm sieve and homogenized in a 450-L tank using a power drill and Teflon®-coated paddle. Samples were taken to determine AVS content and sediment density. The sediment was then placed back into plastic 18.9-L buckets and stored in the dark at 4°C until amendment.

Sediment was amended with CdCl₂ salt based on sediment density, AVS content, fraction solids, and desired exposure treatment concentrations and volumes. Sediment concentrations were determined based on the desired simultaneously extracted metal (SEM):AVS ratios of 0.6, 1.2, 1.6, 2.0, 4.0. (SEM is the simultaneously extracted metal obtained while determining AVS.) A primary stock was made by adding 126 g CdCl₂ to 2,500 ml deionized water and mixing it into solution on a magnetic stir plate. Appropriate volumes of stock were added to sediment for each concentration in plastic 18.9-L buckets while being stirred with a power drill equipped with a Teflon-coated stir paddle. Following stock additions and homogenization, spiked sediment treatments were placed in labeled 4.5-L glass jars (four per concentration) with overlying air purged of oxygen using nitrogen. Jars were then sealed with Teflon-lined caps and electrical tape and placed on a roller (<4 rpm) in the dark (4°C) for 2 h to mix thoroughly. Sediments were then returned to an upright position under 4°C refrigeration in the dark until test initiation.

**Organism collection and holding**

Amphipods, *A. abdita*, were collected on May 5, 1997, from tidal flats in the Narrow River. Surface sediment containing the amphipod tubes was collected using a pool net and sieved in the field through a 0.5-mm mesh screen. Amphipods, their tubes, and remaining sediment were placed in covered 5-gallon buckets and transferred to the laboratory within one-half hour. Overlying water was removed from the buckets and the tube material was vigorously sprayed with a hose supplying ambient-temperature seawater. Amphipods were collected from the water surface within the buckets. Gravid females were removed and placed in shallow trays (61 × 30 × 13 cm) containing Narrow River sediment (press sieved to 0.3 mm) under flow-through, filtered seawater. Amphipods were fed daily a mix of laboratory-cultured phytoplankton, *Tetraselmus* sp. and *Isochrysis* sp. The amphipods were maintained on flow-through filtered seawater at 20°C with a 16:8-h light:dark cycle. The gravid females were kept under these conditions until the beginning of the chronic population bioassay, which began May 20, 1997.

**Acute 10-d mortality bioassay**

The 10-d acute mortality bioassay was conducted following the guidelines described in the American Society for Testing and Materials standard methods document [3]. Sediments were removed from refrigeration (one gallon jar/treatment) and rehomogenized using a power drill and Teflon paddle. Two hundred milliliters of sediment were added to five replicate test chambers per treatment (labeled treatments A–E). Test chambers were 1-L glass beakers with a 2-cm hole drilled into them at the 800-ml mark. This hole was covered by a 0.250-mm screen to prevent amphipods from escaping. Six hundred mil-
liliters of sand-filtered 20°C Narragansett Bay (NB) seawater were added to each chamber using a turbulence reducer (plastic disk supported by a long plastic handle to diffuse the flow of seawater). Throughout the study, the physical conditions maintained for the NB seawater were salinity = 30%, pH = 7.8, and dissolved oxygen = 6.0 mg/L. Sediments in exposure chambers were allowed to equilibrate (flushed with flow-through sand-filtered 20°C NB seawater, ~15 ml/min/replicate) over a 7-d period prior to adding test organisms. Previous tests showed that this amount of time allowed excess cadmium to partition into and exit the flowing, overlying water.

Following the equilibration period, chambers were removed from the equilibration table and the overlying water was poured off. One replicate per treatment (replicate E) was put aside for day 0 chemical analysis of AVS and SEM. Diffusion samplers (peepers, filled with deaerated seawater) were added to the remaining four replicates [13]. Peepers are used to measure concentrations of contaminants residing within the interstitial sediment matrix. These peepers were constructed from polyethylene vials (21-mm height, 20-mm diameter, 5-ml capacity) with a molded hinged cap. A 1.6-cm hole was drilled into the cap [14]. Once filled with the deaerated seawater, a 1-µm polycarbonate membrane was kept in place by the cap. Using a plastic spoon, a furrow was made in the sediment and a single peeper was placed into the furrow and covered over with sediment. Sand-filtered NB seawater (20°C) was again added to each chamber using a turbulence reducer.

Chambers were then placed into the test table provided with gentle aeration and flow-through, sand-filtered 20°C NB seawater (~15 ml/min/replicate) and allowed to equilibrate for approximately 1 h before adding test organisms. The exposure chambers for the 10-d acute bioassay did not receive any feeding. Physical data (pH, salinity, dissolved oxygen) was collected in every chamber once during the 10-d exposure period. Daily observations included emerged organisms, temperature, and flow rates.

**Exposure design for 70-d chronic population bioassay**

Sediment sample preparation for the 70-d chronic population bioassay was identical to that described above for the 10-d acute bioassay (e.g. rehomogenization, 200 ml sediment/chamber, 7-d equilibration, peepers deployment, addition of organisms). The 70-d chronic population bioassay also used seven replicate test chambers per treatment (labeled treatments A–G). Following the equilibration period, chambers were removed from the equilibration table and the overlying water was poured off. One replicate per treatment (replicate G) was put aside for day 0 chemical analysis of AVS and SEM. Chambers were placed into the test table provided with gentle aeration and flow-through, sand-filtered 20°C NB seawater (~15 ml/min/replicate) and allowed to equilibrate for approximately 1 h before adding test organisms (see below). These steps were repeated seven times (every 10 d) during the 70-d chronic population bioassay.

**Collection of neonates for initiation of 70-d chronic population bioassay**

Neonates (1–3-d-old individuals) were collected from the culture bins by sieving them free of the sediment and retaining them on a 0.3-mm sieve. Fifty neonates were randomly enumerated into transfer cups, recounted, and gently rinsed into each test chamber (six biological replicates [A–F] per treatment). One hour after adding the neonates, chambers were checked for tubes and moribund individuals, which were replaced. Gentle aeration and flow-through 20°C filtered seawater to the test chambers was reestablished. Amphipod test chambers were fed daily (100 ml for the first 14 d, 150 ml for the remainder of the test) of 50:50 laboratory-cultured phytoplankton Isochrysis:Tetraselmus. The flow-through seawater supply was turned off during feeding (1630–0800 h) while continuous gentle aeration was supplied. The test chambers were maintained on a 16:8-h light:dark cycle. Daily maintenance included cleaning by siphoning excess algae from the sediment surface and observations of temperature, salinity, flow rates, and the presence of dead or emerged animals. Physical data (pH, dissolved oxygen, and salinity) were measured in each replicate once during each 10-d exposure.

**Collection of age-specific schedules of survival and fecundity every 10 d**

Every 10 d during the 70-d exposure period, test chambers were removed from the table and carefully emptied of overlying water over a 0.3-mm sieve, ensuring that no amphipods were poured off. One replicate from each treatment (replicate F) was put aside and destructively sampled (organisms were not sieved out or enumerated) for day 10 chemical analysis of AVS, SEM, and interstitial water. These F replicates included age-appropriate amphipods so that these sediment chemistry replicates would have the same organism effects (bioturbation, etc.) as the biological replicates. Peepers were carefully removed from each replicate (A–F) and rinsed free of sediment over the test vessel using a squirt bottle filled with filtered seawater. Peeper contents were removed by puncturing the membrane with an acid-stripped pipette tip and were transferred via pipette to sample containers (acid-stripped, 7-ml polycarbonate vial). These samples were acidified with 50 µl of concentrated nitric acid (pH < 1), archived, and later analyzed for interstitial water cadmium. Following removal of the peepers, amphipods were sieved free of sediment using a gentle spray of filtered 20°C seawater over a 0.3-mm sieve. Amphipods and tubes were transferred to picking bowls labeled by treatment and replicate. Samples were examined under a dissecting microscope so that the amphipods could be removed from the remaining organic matter and enumerated as adults, gravid adults, or juveniles. Any missing amphipods were assumed to have died and decomposed. All samples were recounted by another investigator as a measure of quality assurance. Surviving organisms were then returned to the appropriate treatment and replicate chamber of newly equilibrated sediment, which had been under flowing seawater for 7 d prior in the equilibration table. Offspring were enumerated and preserved (they were not reintroduced to the test chambers). Cultured organisms were used to continue the F replicate vessels (50/vessel), which were destructively sampled for chemistry every 10 d. After 70 d (the last 10-d interval), the remaining organisms were enumerated and then preserved in 10% buffered formalin so that adults could be later examined for sex determination.

**Chemical analyses**

Samples for AVS and SEM were taken at each day 0 (following the 7-d equilibration period). Acid volatile sulfide, SEM, and interstitial water (IW) were taken on each day 10 (following organism exposure) interval for both the 70-d chronic population and 10-d acute mortality bioassays. Prior to metals analysis, all IW samples were acidified with 1 µl...
nitric acid per milliliter of sample. High-concentration metals samples were analyzed on an Applied Research Laboratories inductively coupled plasma atomic emission spectrometer (Valencia, CA, USA) model 3410. Samples with concentrations at or below inductively coupled plasma atomic emission spectrometer detection limits (Cd < 10.0 μg/L) were analyzed by a Perkin-Elmer (Norwalk, CT, USA) simultaneous multiple element atomic absorption model 6000 graphite furnace spectrophotometer. The detection limits using this instrument were Cd > 0.005, Cu > 0.4, Ni > 1.0, Pb > 0.4, and Zn > 1.0 μg/L. Both instruments were calibrated using National Institute for Standards and Technology (Gaithersburg, MD, USA) traceable calibration standards and multipoint calibrations. Metals samples were analyzed utilizing blank and duplicate samples along with matrix spikes in order to assess the precision and accuracy of the analytical work. Analysis of duplicate samples were within 5% relative percent deviation while matrix spike additions were within 15% of calculated values. Acid volatile sulfide content of the sediments was determined by methods adapted from Cornwell and Morse [15].

Population model parameterization, exposure–response models, and model manipulations

Demographic data (survival and reproduction) collected at 10-d intervals during the 70-d chronic population bioassay were used to parameterize the A. abdita age-classified matrix population model. A 10-d time step was used for each of the seven age-classes so that the influence of early life-stage (10-d) acute mortality on the model population could be examined. These data provided age-specific schedules of survival (S) and fecundity (f) for the controls and each exposure concentration of cadmium. Survivorship was calculated by comparing the number of amphipods alive at the end of each 10-d interval with the number alive from the previous 10-d interval. Age-specific female fecundity was calculated as the number of female offspring produced per live adult for each time step, assuming a sex ratio of 1:1 of the offspring.

Population growth rate (λ) or the finite rate of increase was calculated for each treatment and the control using Risk Analysis and Management Alternatives Software/stage software [16]. This dominant eigenvalue or λ measures the finite rate of the population’s increase once it has reached stable distribution. The value of λ provides information regarding the status of the population, where λ > 1 indicates a growing population, λ < 1 indicates a declining population, and λ = 1 indicates no change in population size through time (i.e., zero population growth).

An exposure–response model (measured SEM Cd/AVS vs λ) was developed using the methods described by Bruce and Versteeg [17]. The nonlinear (NLIN) procedure [18] was used to fit a nonlinear regression model using the least squares method to estimate model parameters and point estimates [17]. The 10-d acute mortality and measured SEM Cd/AVS were also developed into an exposure–response model so that population growth rate could be regressed against 10-d acute mortality to establish the relationship between the two.

The influence of early life-stage (10-d) acute mortality on the model population projections was examined by manipulating the survivorships with each age class in an iterative fashion. The base model, parameterized with the measured survivorship (P) and fecundity (f) values from the controls from the 70-d chronic population bioassay, was used as a basis for these manipulations. The manipulations included three different scenarios, including applying 10-d acute mortality (S10) as a percentage of the control base model, replacing all P values of the control base model with acute S10 values, and replacing only P(20,30) survivorship between days 20 and 30) values of the base model with acute S10 values. For these three model manipulations, only survivorship (P) values were replaced with acute S10 values while the fecundity (f) values remained the same as the control base model values since fecundity is not measurable in the 10-d acute mortality assay. Elasticity analysis was used to evaluate the effects of these three different model manipulation scenarios on population growth rate for A. abdita.

RESULTS

Sediment test concentrations

The overall mean measured concentrations of SEM/AVS in cadmium-spiked (normalized to acid volatile sulfide) sediment for both the acute and chronic bioassays were in general lower than the nominal concentrations by a factor of two (Table 1). The final reported concentrations of SEM and AVS values were calculated from the means of day 0 and day 10 sediment chemistry samples for each 10-d interval, which were then averaged over the 70-d time period (n = 7). The day 0 and day 10 values within each 10-d interval were consistently similar and did not change during the 10-d exposure periods. The SEM and AVS values for each treatment were relatively stable over the entire sampling period of 70 d, with coefficients of variation (CVs) ranging from 3 to 13% for SEM and 7 to 12% for AVS. The IW concentrations of cadmium, which were measured using peepers, were based on average day-10 values (n = 5 for each sampling event) for each concentration averaged over 70 d (n = 7 sampling events) (Table 1). The IW values were not as consistent as the SEM and AVS values and demonstrated a high degree of variability within treatments and between sampling periods. The original IW values had
Table 2. Summary of results from acute 10-d bioassay with *Ampelisca abdita* exposed to cadmium-spiked (normalized to acid volatile sulfide [AVS]) sediment. Measured cadmium concentrations for simultaneously extracted metal (SEM) and AVS are based on the average of the day 0 and day 10 samples and interstitial water (IW) averages are based on the day 10 values.

<table>
<thead>
<tr>
<th>Nominal concentration of cadmium (SEM/Cd/AVS)</th>
<th>Measured SEM/Cd/AVS</th>
<th>Average IW toxic units</th>
<th>% Survival (S10)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR control</td>
<td>0.14</td>
<td>0.01</td>
<td>0.97</td>
</tr>
<tr>
<td>0.6</td>
<td>0.39</td>
<td>0.09</td>
<td>0.98</td>
</tr>
<tr>
<td>1.2</td>
<td>0.52</td>
<td>0.10</td>
<td>0.88</td>
</tr>
<tr>
<td>1.6</td>
<td>0.65</td>
<td>0.10</td>
<td>0.87</td>
</tr>
<tr>
<td>2.0</td>
<td>0.70</td>
<td>0.23</td>
<td>0.97</td>
</tr>
<tr>
<td>4.0</td>
<td>1.19</td>
<td>1.822</td>
<td>0.12*</td>
</tr>
</tbody>
</table>

* Expressed as the molar ratio of cadmium to acid volatile sulfide.
* SEM/Cd/AVS = simultaneously extracted metal/acid volatile sulfides.
* S10 acute percentage survival from 10-d bioassay.
* NR = Narrow River (NR), Rhode Island, USA, reference control sediment.
* Denotes statistical difference from NR reference control using analysis of variance (p = 0.05).

Ten-day acute mortality bioassay

The results from the 10-d acute mortality bioassay with cadmium-spiked sediment are presented in Table 2. These 10-d acute mortality results are referred to as S10 values, which were used in subsequent regression analyses and empirical model manipulations. In general, these results indicate survival effects were not evident except at the highest concentration of cadmium-spiked sediment (1.19 SEM Cd/AVS) (Table 2). In accordance with the U.S. Environmental Protection Agency’s (U.S. EPA) Draft Equilibrium Partitioning Derived Sediment Guidelines (ESG), the acute water-only 50% lethal concentration (LC50) of 36.0 μg/L [13] was used to calculate the Cd IW toxic units since this is an acute 10-d exposure (Table 2).

CVs ranging from 39 to 104%. The original data set was analyzed for extreme outlier values using Dixon’s criteria for testing extreme observations in a single sample [19]. After removal of outliers, the IW values still had CVs ranging from 39 to 46%.

Seventy-day chronic population bioassay and population model parameterization

The results from the 70-d chronic population bioassay demonstrate a definitive concentration-dependent response for survival (Fig. 1). Table 3 displays the percent cumulative survival for each treatment for each 10-d time step. Statistically significant (p < 0.05) differences for survival compared with the control were detected at average measured concentrations as low as 0.82 SEM Cd/AVS using analysis of variance (ANOVA) followed by Tukey’s honestly significant difference test [20] (Table 3). The reproductive endpoint demonstrated a general decrease in number of young produced per individual with increasing cadmium concentration (Table 3). This decrease in number of young produced was observed at even the lowest concentration (0.51 SEM Cd/AVS), where there was a 50% reduction compared with the control over the 70-d time period. These differences in number of young produced were not, however, statistically significant (p > 0.05) due to the large natural variance associated with reproduction. It is also interesting to note that increasing concentrations of cadmium (0.82 SEM Cd/AVS and higher concentrations) exhibited a delay in the onset of reproduction. This delay in reproduction can have a strong negative influence on a population’s viability in an ecological sense.

Table 3. Summary of 70-d chronic population bioassay with *Ampelisca abdita* exposed to cadmium-spiked (normalized to acid volatile sulfide) sediment

<table>
<thead>
<tr>
<th>Nominal concentration of cadmium (SEM/Cd/AVS)</th>
<th>Measured SEM/Cd/AVS</th>
<th>Day 10 S</th>
<th>Day 20 S</th>
<th>Day 30 S</th>
<th>Day 40 S</th>
<th>Day 50 S</th>
<th>Day 60 S</th>
<th>Day 70 S</th>
<th>Total no. of young</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR control</td>
<td>0.17</td>
<td>90</td>
<td>77</td>
<td>70</td>
<td>64</td>
<td>1</td>
<td>47</td>
<td>16</td>
<td>26</td>
</tr>
<tr>
<td>0.6</td>
<td>0.51</td>
<td>86</td>
<td>68</td>
<td>64</td>
<td>60</td>
<td>1</td>
<td>43</td>
<td>4</td>
<td>23</td>
</tr>
<tr>
<td>1.2</td>
<td>0.67</td>
<td>85</td>
<td>64</td>
<td>62</td>
<td>51</td>
<td>1</td>
<td>35</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>1.6</td>
<td>0.82</td>
<td>85</td>
<td>65</td>
<td>53</td>
<td>49</td>
<td>1</td>
<td>27*</td>
<td>7</td>
<td>8*</td>
</tr>
<tr>
<td>2.0</td>
<td>0.91</td>
<td>76</td>
<td>68</td>
<td>64</td>
<td>48</td>
<td>1</td>
<td>16*</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>4.0</td>
<td>1.70</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
</tr>
</tbody>
</table>

* Expressed as the molar ratio of cadmium to acid volatile sulfides (AVS).
* SEM/Cd/AVS = simultaneously extracted metals/acid volatile sulfides.
* % Cumulative survival = total number of adults alive/total number of amphipods at beginning of bioassay (n = 250 per treatment) × 100.
* No. young produced is the total number of five juveniles per treatment (n = 5 replicates).
* NR = Narrow River, Rhode Island, USA, reference control sediment.
* Denotes statistically significant difference from the control using analysis of variance and Tukey’s honestly significant difference (p = 0.05).
The age-specific schedules of survival ($P$) and fecundity ($f$) (number of female offspring produced per alive adults, assuming a sex ratio of 1:1 of the offspring) per treatment are presented in Table 4. These survival and fecundity schedules were measured every 10 d during the 70-d population bioassay and were used to parameterize each time step for the $A. abdita$ age-classified projection matrix model (Fig. 2) and produce the population growth rate ($\lambda$) estimates [16]. The resulting values for $\lambda$ are presented in Table 5 for the control and each treatment of cadmium-spiked sediment. These $\lambda$ values are regressed against the measured SEM Cd/AVS values in Figure 3.

**Relationship between 70-d population growth rate and 10-d acute mortality**

The 10-d acute mortality and 70-d population growth rate results are presented together with measured SEM Cd/AVS in Figure 3. A nonlinear model was first developed using only the 10-d acute mortality data. This model was then used to predict population growth rate estimates using the actual measured concentrations from the 70-d chronic population bioassay. The residual values from these predicted population growth rate estimates and the observed measured population growth rates were evaluated and a nonlinear model combining the two bioassay results (10-d acute and 70-d chronic population) was considered appropriate. The nonlinear regression describing the relationship between 10-d acute mortality and 70-d population growth rate resulted in $r^2 = 0.96$ and is presented in Figure 3. The regression model in Figure 3 also displays the proposed range of acceptable acute mortality ($\leq 20\%$) that correlates with higher population growth rate $\lambda$ estimates (e.g., $\lambda = 0.80$ and greater).

**Empirical model manipulations**

The relationship between 10-d acute mortality ($S_{10}$) and population growth rate was explored by empirically manipulating the survivorships for each age class in the control base model using the $S_{10}$ values. The control base model is constructed with the survivorship ($P$) and fecundity ($f$) values for each time step ($P_{(20,30)}$) for the control treatment (Table 6). The empirical manipulations for the model included applying $S_{10}$ values as a percentage of the control base model, replacing control base model survival values ($P$) with $S_{10}$ values, and replacing only the $P_{(20,30)}$ time-step control base model values with $S_{10}$ values. The $P_{(20,30)}$ time step is the representative age class for the 10-d standard bioassay. The amphipods routinely exposed in the 10-d standard bioassay are collected on sieves ranging from 0.71 to 1.0 mm. This size range correspond to the $P_{(20,30)}$ age class and is therefore an important time-step for these comparisons (Fig. 4). The calculations used for model manipulation number 1 (applying $S_{10}$ as a percentage of the control base model) and the control base model values used for each manipulation are shown in Table 6.

The population growth rate results from the three different model parameterization scenarios are presented in Table 5. A comparison of these model manipulations with the original 70-d population growth rate model is presented in Figure 5. The RAMAS/stage software was used to perform the elasticity analysis for the 70-d $A. abdita$ model, which is presented in Figure 6. Elasticity analyses were also performed on every treatment level for all three manipulation scenarios. No changes in elasticity compared with the 70-d control base model were detected in any of the treatments or manipulations.

**DISCUSSION**

**Ten-day acute mortality bioassay**

The results of the 10-d acute mortality bioassay agree in general with the U.S. EPA’s draft ESG, which is a recommendation of the concentration of a substance that may be present in a sediment while still protecting benthic organisms from the effects of that substance. These guidelines are applicable to a variety of freshwater and marine sediments because they are based on the biologically available concentration of the substance in those sediments. The ESG recommends normalization of sediment concentrations by utilizing the mea-
evaluated by the cadmium water-only LC50 of 36.0 mg/L for Ampelisca abdita acute mortality for 70-d population growth rate (\(l\)) measured average of 65,590 concentration had the equivalent of 1,822 toxic units based on a mortality (100%) in this 10-d acute exposure. The highest concentration (Tables 1 and 2) would have been expected to produce complete mortality (100%) in this 10-d acute exposure. The highest concentration had the equivalent of 1,822 toxic units based on a molar concentration of SEM, the sediment is predicted to be nontoxic with respect to cadmium. The acute bioassay done in this study performed as expected and did not demonstrate significant toxicity until the SEM/AVS threshold of 1.0 had been exceeded (Table 2).

The IW toxic units for this study also support the premise of the ESG, which predicts toxic effects will be observed when IW toxic units are greater than one. However, based on the ESG approach, the extremely high level of cadmium measured in the interstitial water of the highest concentration (Tables 1 and 2) would have been expected to produce complete mortality (100%) in this 10-d acute exposure. The highest concentration had the equivalent of 1,822 toxic units based on a measured average of 65,590 \(\mu\)g/L of cadmium (Table 1) divided by the cadmium water-only LC50 of 36.0 \(\mu\)g/L for A. abdita [13]. Therefore, it is surprising that 12% of the amphipods in this concentration were able to survive these conditions based on IW toxic units (Table 2).

Seventy-day chronic population bioassay

The chronic results for survival and reproduction also generally agree with the U.S. EPA's ESG approach. However, results from this chronic cadmium exposure demonstrate significant adverse effects (both survival and reproductive, Table 3) at molar ratios of SEM/AVS below 1.0, where toxic effects would not be expected according to the ESG.

A perhaps better method for evaluating these chronic population results is to calculate a chronic value with the method used in the development of U.S. EPA water quality criteria [22]. The chronic value is defined as the geometric mean of the no-observed-effect concentration and the lowest-observed-effect concentration. This statistic is used to indicate the potential of chemical stressors to elicit biological effects in natural systems. In this study, the chronic value based on the chronic population bioassay results using the SEM Cd/AVS values would result in a chronic value of 0.74 (the geometric mean of the no-observed-effect concentration, 0.67 SEM Cd/AVS, and the lowest-observed-effect concentration, 0.82 SEM Cd/AVS).

These survival and reproductive effects at concentrations lower than expected (e.g., chronic value = 0.74 SEM Cd/AVS) are most likely the result of the time-dependent bioavailability

<table>
<thead>
<tr>
<th>Nominal SEM/AVS</th>
<th>Measured SEM/AVS</th>
<th>70-d population growth rate ((l))</th>
<th>% Survival</th>
<th>(S10) of control base model</th>
<th>(S10) replace all (P) values of base model</th>
<th>(S10) replace (P_{20,30}) of base model</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR control</td>
<td>0.17</td>
<td>0.90</td>
<td>0.97</td>
<td>0.88</td>
<td>1.06</td>
<td>0.91</td>
</tr>
<tr>
<td>0.6</td>
<td>0.51</td>
<td>0.79</td>
<td>0.98</td>
<td>0.89</td>
<td>1.06</td>
<td>0.91</td>
</tr>
<tr>
<td>1.2</td>
<td>0.67</td>
<td>0.83</td>
<td>0.88</td>
<td>0.81</td>
<td>0.97</td>
<td>0.90</td>
</tr>
<tr>
<td>1.6</td>
<td>0.82</td>
<td>0.72</td>
<td>0.87</td>
<td>0.80</td>
<td>0.96</td>
<td>0.90</td>
</tr>
<tr>
<td>2.0</td>
<td>0.91</td>
<td>0.71</td>
<td>0.97</td>
<td>0.88</td>
<td>1.06</td>
<td>0.91</td>
</tr>
<tr>
<td>4.0</td>
<td>1.70</td>
<td>0.00</td>
<td>0.12</td>
<td>0.15</td>
<td>0.18</td>
<td>0.67</td>
</tr>
</tbody>
</table>

\(a\) Expressed as the molar ratio of cadmium to acid volatile sulfide (AVS).

\(b\) Average Simultaneously extracted metal (SEM)/AVS for 70-d chronic bioassay.

\(c\) \(S10\) acute percentage survival from 10-d bioassay.

\(d\) Using control 70-d survival (\(P\)) and fecundity (\(f\)) values as base model and applying \(S10\) as a percentage of the base model.

\(e\) Replace all \(P\) values of base model with \(S10\).

\(f\) Narrow River (NR), Rhode Island, USA, reference control sediment.

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Fig. 3. Relationship between 70-d population growth rate (\(l\)) and 10-d acute mortality for Ampelisca abdita exposed to cadmium-spiked (normalized to acid volatile sulfide).

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Table 6. Summary of calculations for model manipulation number applying 10-d acute mortality (\(S10\)) as a percentage of the control base model (\(P\) [survivorship] and \(f\) [fecundity] values from the 70-d chronic population bioassay control)

<table>
<thead>
<tr>
<th>Age class</th>
<th>(P)</th>
<th>(f)</th>
<th>(S10)</th>
<th>(S10)</th>
<th>(S10)</th>
<th>(S10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2-1.0</td>
<td>0.90</td>
<td>0.87</td>
<td>0.88</td>
<td>0.79</td>
<td>0.78</td>
<td>0.87</td>
</tr>
<tr>
<td>1.0-2.0</td>
<td>0.86</td>
<td>0.83</td>
<td>0.84</td>
<td>0.76</td>
<td>0.75</td>
<td>0.83</td>
</tr>
<tr>
<td>2.0-3.0</td>
<td>0.92</td>
<td>0.89</td>
<td>0.90</td>
<td>0.81</td>
<td>0.80</td>
<td>0.89</td>
</tr>
<tr>
<td>3.0-4.0</td>
<td>0.91</td>
<td>0.006</td>
<td>0.88</td>
<td>0.89</td>
<td>0.80</td>
<td>0.79</td>
</tr>
<tr>
<td>4.0-5.0</td>
<td>0.73</td>
<td>0.143</td>
<td>0.71</td>
<td>0.72</td>
<td>0.64</td>
<td>0.71</td>
</tr>
<tr>
<td>5.0-6.0</td>
<td>0.55</td>
<td>1.629</td>
<td>0.53</td>
<td>0.48</td>
<td>0.48</td>
<td>0.53</td>
</tr>
<tr>
<td>6.0-7.0</td>
<td>0.74</td>
<td>0.000</td>
<td>0.72</td>
<td>0.73</td>
<td>0.65</td>
<td>0.72</td>
</tr>
</tbody>
</table>

\(a\) Expressed as the nominal molar ratio of cadmium to acid volatile sulfide (AVS) concentrations for 10-d acute mortality bioassay.
of cadmium measured in the chronic as opposed to acute exposure. As exposure time increased from day 40 to day 50, the effect concentration for survival decreased demonstrating time-dependent mortality (Table 3). There are most likely different modes of action occurring between mortality and reproductive effects in the chronic as opposed to acute exposure [8,23]. This chronic effect is also exhibited by the estimated population growth rates, which decline 20% from \( \lambda = 0.90 \) for the control treatment to \( \lambda = 0.72 \) at the 0.82-SEM Cd/AVS concentration (Table 5). Population growth rates at this level (\( \lambda = 0.72 \)) would eventually lead to extinction over a rapid period of time for this species, which has a relatively short life span (~90 d).

**Relationship between 70-d population growth rate and 10-d acute mortality**

The results from this study indicate that survival of less than 80% in the 10-d acute mortality test correlates with a decline in population growth rate, \( \lambda \), which may be used to predict abundances in the field (Fig. 3). The relationship between 10-d acute mortality and population growth rate estimates demonstrates the predictive power of the standard acute sediment bioassay results for extrapolating chronic population-level effects. It should be stressed that the population model used in this study has not been validated in the field and further evaluations are necessary to define the predictive nature of this model using acute mortality from sediment toxicity tests (e.g., comparison of synoptically collected sediment toxicity, sediment chemistry, benthic species composition, abundance, and community structure with model projections parameterized with survival results from the acute sediment toxicity tests).

Data collected by the U.S. EPA’s Environmental Monitoring and Assessment Program (EMAP) for the Virginian Province (1990–1993) were examined to evaluate the relationship between survival of \( A. abdita \) in sediment toxicity tests and the presence of ampeliscids in field samples [24]. Less than 80% survival relative to control survival in the sediment samples was used as a benchmark for classifying toxic sediments, and less than 60% survival was used to define severely toxic sediments. This evaluation of \( Ampelisca \) survival in sediment toxicity tests and benthic community response (abundance of ampeliscids) indicates that generally amphipod abundance was positively correlated with high survival (>80%) in sediment toxicity tests. Ampeliscid abundances were generally low (0–1,140/m²) when survival in the toxicity tests was less than 50%.

A review of degraded sediment quality in U.S. estuaries reveals that about 10% of the estuarine sediments in the United States are significantly toxic and could pose toxicological threats to valuable resources [9]. These data from two major national sediment survey programs (National Oceanic and Atmospheric Administration’s National Status and Trends program and U.S. EPA’s EMAP) indicate that there is a very strong positive correlation between sediment toxicity (amphipod survival) and benthic community structure. Preliminary evidence from analyses of these data demonstrate that, as acute toxicity increased (survival <80%), alterations in the benthic community structure also increased. In general, these alterations in benthic community structure resulted in a decrease of infaunal crustaceans and metrics of total abundance and total...
species richness as toxicity and chemical concentrations increased. McGee et al. [25] also found that survival in 10-d acute toxicity exposures with *Leptocheirus plumulosus* (an estuarine amphipod) was positively correlated with abundances of this species at the test sites in Baltimore Harbor (MD, USA), suggesting that acute toxicity test results with *L. plumulosus* may be predictive of population-level effects [25]. These studies also demonstrate a negative relationship with concentrations of sediment-associated contaminants and benthic community structure (low species richness and abundance; increase in polychaete and mollusk species paralleling with a decline in infaunal crustaceans).

**Empirical model manipulations**

These results suggest that the model manipulation number 1 (applying S10 as a percentage of the control base model) aligns most closely with the original 70-d population growth rate model (Fig. 5). This implies that applying the S10 as a percentage of the control base model would be the most conservative extrapolation method for using acute mortality data to predict chronic effects for *A. abdita*. The other manipulations tended toward overestimating the population growth rate compared with the original model. The scenario that appears to have grossly overestimated the population growth rate was the model that replaced all of the survivorship (P) values of the base model with the acute mortality S10 values. The other model manipulation that appears to be overestimating the population growth rate compared with the original model is, surprisingly, the number 3 manipulation, which replaces only the P20,30 time step of the control base model values with S10 values. The estimates of λ produced using this model manipulation differ from the original 70-d model in that the values for λ do not decline rapidly for the higher exposure concentrations (e.g., SEM Cd/AVS > 1.0 μM/g). This manipulation scenario would also result in an overestimation of population growth rate for concentrations where effects most likely be occurring.

These model parameterization scenarios highlight the importance of early life-stage survival in the *A. abdita* model. All of these model manipulation scenarios were performed by replacing and applying S10 (survivorship) values to the control base model, leaving fecundity as it is in the original 70-d population base model. An analytical tool that is being used increasingly [26–30] to calculate the proportional sensitivities and the relative contributions of life-history traits (e.g., survival and reproduction) to population growth rate, λ, is elasticity analysis. In all cases, the survivorship probabilities of the first five age classes were found to be most important in the analysis of relative contributions of small changes in demographic parameters to λ. Elasticities of survivorship of older classes became less important with age, while elasticities of fecundity were lower than the early life-stage survivorship (Fig. 6). The high elasticity of the early life-stage survivorship supports the use of this endpoint in the 10-d acute *A. abdita* mortality bioassay. The results of these analyses demonstrate the strong influence that early life-stage survival has on the population growth rate for this species.

**CONCLUSIONS**

The results from this study show that *A. abdita* sediment toxicity 10-d acute mortality can be used to predict population-level effects in a controlled laboratory experiment. The strong relationship of acute mortality and population growth rate indicate that, when acute mortality (as measured in the 10-d standard sediment test) starts to rise above 20%, the population growth rate estimates will begin to decline appreciably. These laboratory studies and their analyses highlight the value of an integrated approach using mathematical models to develop hypotheses that can be tested using experimental models. These results also suggest that relatively simple laboratory tests using easily measured endpoints can provide information that, when considered in context with species and stressor attributes, has direct relevance to the assessment of ecological risks by anthropogenic stressors.

This preliminary analysis agrees with earlier findings from the National Oceanic and Atmospheric Administration’s National Status and Trends program and the U.S. EPA’s EMAP studies [9–10, 24], which found ampeliscid field abundances positively correlated with high survival (>80% relative to the control survival) in the 10-d sediment toxicity tests. These results support the previously proposed range of acceptable acute mortality in the 10-d sediment test to be ≤20%. A confidence range concerning the uncertainty of this range of acceptable acute mortality will be established with field evaluation and validation of the current population model. A more thorough analysis using large databases such as EMAP and the National Status and Trends data will hopefully develop the relationship of numerous environmental factors (e.g., water depth, salinity, dissolved oxygen, sediment grain size) with benthic community structure regardless of contaminant loadings. When these relationships are better established and understood, then we can begin to explain the simultaneous effect contaminated sediments have on these benthic communities.

The results of the empirical model manipulations used in this study suggest that applying the acute mortality (S10) as a percentage of the control base model relates most closely to the original 70-d population growth rate estimates. Similarities between acute and population response curves are also consistent with elasticity analysis of the matrix model showing that juvenile survival is an important regulator of population growth rate for this species. Based on the demographic importance of juvenile survival, results of the standard acute *A. abdita* sediment tests will probably correlate closely with projections of population-level effects for other contaminants as well. This study showed that, even for those contaminants that produce reproductive impairments, information that cannot be captured by acute tests, predictions of population effects based on results of acute tests may be reasonably accurate. However, conclusions concerning the accuracy of population projections based on acute results may not be applicable to other species. For example, acute results may underestimate effects produced in those species with relatively higher reproductive output or longevity than *A. abdita*. Caution should be used when applying acute data in general to models for extrapolating population-level effects since many factors need to be considered, e.g., which life-history attribute is affected by chronic exposure (survival, growth, or reproduction) and the mode of action of the toxicant (nonpolar narcosis, polar narcosis, aspecific reactivity, specific reactivity, heavy metals).

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wish to thank Glen Thursby, John Kiddon, and Diane Nacci for critiquing earlier versions of this manuscript. The information in this document does not necessarily reflect the views and policies of the U.S. Environmental Protection Agency. Mention of trade names and commercial products does not constitute endorsement or recommendation for use.

REFERENCES


