Further Development of a Chronic *Ampelisca Abdita* Bioassay as an Indicator of Sediment Toxicity

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ABSTRACT

This report provides data on one component of a larger study funded by the RMP to develop an *A. abdita* sediment toxicity test using growth rate and chronic survival as endpoints. Two series of experiments were conducted: Experiment 1—Comparative sensitivity of a growth vs. mortality endpoint for *A. abdita*. The general approach was to spike sediments with cadmium or crude oil and to conduct parallel standard 10-day mortality tests and a 17-day growth tests, both using *A. abdita*. The objective was to establish if the growth endpoint was a more sensitive measure of toxicity (i.e., effects demonstrable at a lower toxicant concentration) than 10-day mortality. Experiment 2—Comparative sensitivity of *A. abdita* to *E. estuarius* and *R. abronius*. The general approach was to spike sediments with cadmium, DDT, or crude oil and to conduct parallel tests with all three species under identical conditions. The objective was to establish if *A. abdita* was more or less sensitive to the toxicants than the other species.

The data from these experiments allowed consideration of whether a growth endpoint provided greater sensitivity to toxicants than a mortality endpoint. Neither growth nor mortality endpoints provided a clear advantage in two of the five trials involving the three toxicants examined. In two other cases, growth was the more sensitive endpoint; i.e., toxicant concentrations necessary to depress growth rates were less than those causing mortality. Finally, in one case growth was a less sensitive endpoint, but interpretation of these data were problematic.

If toxicant sensitivity is defined on the basis of LC_{50} values in comparing the relative toxicant sensitivities of the three species, then these experiments yielded the following ranking:

Cadmium sensitivity:	*R. abronius* > *A. abdita* >> *E. estuarius*
DDT sensitivity:	*E. estuarius* > *A. abdita* > *R. abronius*
Crude oil sensitivity:	*R. abronius* = *A. abdita* = *E. estuarius*

R. abronius was the most sensitive species to cadmium but only slightly more so than *A. abdita*. One of the most striking observations in this study was the dramatic tolerance of *E. estuarius* to cadmium. A sediment concentration of 0.1% cadmium had no affect on acute mortality of *E. estuarius*, yet resulted in 81% mortality in *A. abdita* and 100% mortality in *R. abronius*. Thus, *E. estuarius* is a poor choice for toxicity testing if cadmium is among the potential toxicants, and relative sensitivity to other metals is unknown but would merit further evaluation. *E. estuarius* was the most sensitive of the three species to DDT, yet the differences in sensitivity among the three species were relatively small and LC_{50}s were within a factor of two. All three species had virtually identical sensitivity to weathered crude oil. Taken together, the data show that use of *A. abdita* for sediment toxicity testing does not result in any appreciable loss in sensitivity relative to *R. abronius* or *E. estuarius*. To the contrary, use of the species provides a substantial increase in sensitivity relative to *E. estuarius* in cases of cadmium toxicity and potentially other metals.

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INTRODUCTION

Sediment toxicity tests are a critical component in many programs to assess environmental quality. The San Francisco Estuary Regional Monitoring Program (RMP), for example, regularly monitors the toxicity of Bay sediments to the benthic amphipod *Eohaustorius estuarius* using acute mortality as the measurement endpoint. Recently there has been interest expressed in using growth rate of the amphipod *Ampelisca abdita* as another potential measure of sediment toxicity (Scott and Redmond, 1989; Redmond *et al.*, 1994). The use of an *A. abdita* growth toxicity test offers several attractive features. First, the species has been often used for a 10-day mortality test (DiToro *et al.*, 1990; ASTM,1993; EPA, 1994), but a chronic growth rate test could be a more sensitive indicator of pollution than acute mortality, and thus the use of a growth test may provide a greater degree of environmental protection. Secondly, standardized procedures for collection and laboratory maintenance of the species, at least on a short-term basis, are already established (ASTM, 1993; EPA, 1994). Thirdly, some information already exists on sensitivity to toxicants. Exposure to contaminated harbor sediments has caused a reduction in growth rate and reduced egg production by the smaller females (Scott and Redmond, 1989), demonstrating that an impaired individual growth rate can have negative consequences at the population level. This linkage between smaller females and reduced egg production has also been demonstrated specifically in San Francisco Bay populations (KLI, 1983).

A. abdita is widespread in San Francisco Bay, living in subtidal muds and muddy sands. In some areas its membranous tubes carpet the sediment surface, with animal densities exceeding 80,000 individuals m^{-2} (KLI, 1983). As a dominant organism in the Bay, it is a particularly attractive species for sediment toxicity testing because of the direct and immediate relevance of results to the Bay ecosystem. Moreover, if growth rate can be shown to be a sensitive indicator of sediment toxicity, then it may be possible to acquire similar data from size-frequency analysis of field populations. The use of the same endpoint for both laboratory toxicity tests and monitoring of field populations is an attractive unifying concept that has been largely unexplored.

This report provides data on one component of a larger study funded by the RMP to develop an *A. abdita* sediment toxicity test using growth rate and chronic survival as endpoints. Earlier results have already been presented (RMP, 1995) and include the following conclusions:

- *A. abdita* juveniles have been readily available in collections to date (May through September);
- Greater than 90% survival is consistently achieved in a wide variety of Bay sediments having very different grain size distributions and organic contents;
- Growth rates of about 1 mm/month are observed in the laboratory populations fed an algal diet;
- Bay sediments considered to be non-toxic based on *A. abdita* growth have also been shown to be relatively unimpacted based on chemical analyses and benthic community structure.

If *A. abdita* growth is to be used for sediment toxicity testing in San Francisco Bay, it is important that growth rate tests be of equal or greater sensitivity than acute mortality tests and that the species be no less sensitive to toxicants than other amphipod species that have been routinely used for toxicity testing (such as *Rhepoxynius abronius* and *Eohaustorius estuarius*). Since *E. estuarius* is currently used in the RMP, comparative sensitivity to this species is of particular concern. This report provides results on comparative toxicity tests, using sediments spiked with cadmium, DDT, or crude oil. Results are described within two experimental series:

Experiment 1—Comparative sensitivity of a growth vs. mortality endpoint for *A. abdita*. The general approach was to spike sediments with cadmium or crude oil and to conduct parallel standard 10-day mortality tests and a 17-day growth tests, both using *A. abdita*. The objective was to establish if the growth endpoint was a more sensitive measure of toxicity (i.e., effects demonstrable at a lower toxicant concentration) than 10-day mortality.

Experiment 2—Comparative sensitivity of *A. abdita* to *E. estuarius* and *R. abronius*. The general approach was to spike sediments with cadmium, DDT, or crude oil and to conduct parallel tests with all three species under identical conditions. The objective was to establish if *A. abdita* was more or less sensitive to the toxicants than the other species.

We are also in the midst of regular monitoring of a San Francisco Bay *A. abdita* population in order to establish: 1) if juveniles are available throughout the year for growth-based toxicity tests; and 2) if laboratory growth rates are independent of the time of collection provided constant exposure conditions (e.g., temperature, food supply) are maintained. These results are not included here, but will be provided in later RMP reports.

METHODS

Sediment preparation

Experiment 1 (*A. abdita* growth vs. mortality comparison) was conducted in June and July 1995. Test sediment was collected from the RMP Alameda site, the home sediment for the individuals used in the tests. The sediment was sieved through a 0.5 mm screen, thoroughly homogenized, and used to prepare one of the following treatments:

1. Cadmium—Cadmium chloride, dissolved in distilled water, was added to the sediment to achieve nominal concentrations of 22, 45, 90, and 180 mg cadmium · kg^{-1} dry weight.
2. Crude oil—Alaskan North Slope crude oil was placed in an open container and allowed to weather outdoors for one week. The weathered crude was then added to the sediment at nominal concentrations of 150, 460, 1,400, and 4,200 mg crude oil · kg^{-1} dry weight.
3. Home sediment control—Sediment from the Alameda collection site, without any added toxicants.

After addition of the toxicant spike the mixture was homogenized for one minute in a high-speed food blender. The sediment was then distributed to the test containers (one quart glass canning jars), and left undisturbed at 4°C for 12–13 days. Chemical analyses of the sediments are still in progress, therefore all concentrations reported herein are nominal values.

Experiment 2 (multiple species comparisons) was conducted March through May 1996. Test sediment was collected from an intertidal area along the channel into Bodega Bay, California, and the same sediment was used for tests with *A. abdita, R. abronius,* and *E. estuarius*. The sediment was sieved through a 0.5 mm screen, thoroughly homogenized, and then frozen. Two weeks prior to initiation of a toxicity test, the required amount of sediment was thawed and used to prepare one of the following treatments:

1. Cadmium—Cadmium chloride, dissolved in test water, was added to the sediment to achieve nominal concentrations of 12, 37, 110, 330, and 1,000 mg cadmium · kg^{-1} dry weight.
2. DDT—DDT (1,1-bis(p-chlorophenyl)-2,2,2-trichloroethane; Sigma Chemical, St. Louis, MO) was dissolved in acetone and added to the sediment to achieve nominal concentrations of 120, 370, 1,100, 3,300, and 10,000 µg DDT · kg^{-1} dry weight. The volume of acetone was held constant for all treatments, producing a nominal concentration of 1,050 mg kg^{-1} dry weight, although much of this would have been lost from the sediments by dissolution and volatilization prior to addition of the test animals.
3. Crude oil—Alaskan North Slope crude oil was placed in an open container and allowed to weather outdoors for one week. The weathered crude was then added to the sediment at nominal concentrations of 60, 180, 530, 1,600, and 5,000 mg crude oil · kg^{-1} dry weight.
4. Bodega Control—Bodega Bay sediment without any added toxicants or carrier solvents.

5. Solvent control—Acetone was added to the sediment to achieve a nominal concentration of 1,050 mg kg^{-1} dry weight, identical to that attained in all DDT-spiked sediments due to the use of an acetone carrier.
6. Home sediment controls—Sediments from the collection sites for each of the three amphipod species, without any added toxicants or carrier solvents.

The sediment samples were spiked in batches of 0.65 kg wet weight along with 200 ml test water to create a slurry. After addition of the toxicant spike, the mixture was homogenized for one minute in a high-speed food blender. The sediment was then distributed to the test containers (one quart glass canning jars), and left undisturbed at 4˚C for 9–12 days. Chemical analyses of the sediment samples are still in progress, therefore all concentrations reported herein are nominal values.

Test organism collection

A. abdita were collected in central San Francisco Bay, either from the RMP Alameda station (Experiment 1) or east of the Tiburon Peninsula (Experiment 2). Sediment was collected using a 0.025 m^2 Ponar grab, and the material in the grab sieved on stacked 2.0, 1.0, and 0.5 mm screens. The material on the 2.0 screen (mostly tubes from *A. abdita* and other macrofauna) was discarded. *A. abdita* retained on the 1.0 screen were discarded. Since it was our intent to measure growth, we did not use the larger, fully-grown individuals that would be retained by the 1.0 sieve. In addition, males die shortly after mating (ASTM, 1993), and elimination of this group from the test would likely increase our overall measures of survival. The amphipods and other material retained on the 0.5 mm screen were gently lowered into a pan of seawater, and the amphipods trapped by the surface tension were skimmed off with a dip net. They were placed in plastic dishes with seawater, and kept cool until arrival at the lab later that same day. In Experiment 1 the animals were held in seawater overnight, and the exposures began the following day. Home site temperatures and salinities were close to the conditions used in the exposures (home = 15–19˚C, 20–24‰ during the two collection events; test = 19˚C, 20‰). In Experiment 2, the amphipods were transferred to sediment-filled plastic trays and allowed to rebuild tubes. The animals were initially held at conditions of the collecting site (13˚C, 23 ‰), but gradually shifted to test conditions (17˚C, 25 ‰). Experiment 2 amphipods were held in the laboratory for 5–10 days prior to use in the toxicity tests, and fed the diatom *Phaeodactylum tricornutum* daily during this period.

Eohaustorius estuarius were collected from the beach adjacent to the EPA laboratory in Newport, Oregon. Sediment was collected by shovel at low tide, and sieved through a 1.0 mm screen to retain the amphipods. They were then placed in plastic containers, and flown to the laboratory at the University of California later the same day. The animals were initially held at collecting site conditions (8˚C, 13 ‰), and were raised to test conditions in increments of no more than 3˚C or 5 ‰ per day. The laboratory holding period ranged from 5 to 8 days.

Rhepoxynius abronius were supplied by Brezina and Associates (Dillon Beach, CA), and obtained from subtidal areas near Deception Pass, Washington. They were shipped to the laboratory by overnight mail, and initially held at 11˚C and 32 ‰. The animals were acclimated to test conditions by gradually changing temperature and salinity in increments of no more than 3˚C or 5 ‰ per day. The laboratory holding period ranged from 6 to 7 days.

Toxicant exposures

The quart canning jars containing the test sediments were retrieved from the 4°C holding area, and the overlying water removed. The sediment (approximately 3–4 cm deep) was left undisturbed, and 500 ml of test water was slowly added to the jars. The test water was either home site water (Experiment 1) or AE glass fiber filtered Pacific Ocean water obtained from the Bodega Marine Laboratory, with distilled water added to reduce salinity (Experiment 2). Jars were allowed to sit undisturbed for 24 hours before adding amphipods. Aeration was provided throughout the experiments through a pipette in each jar with the tip placed a few centimeters above the sediment-water interface.

Establishing appropriate testing conditions in Experiment 2 was complicated by the fact that standard amphipod testing protocols (ASTM, 1993; EPA, 1994) recommend unique temperature and salinity conditions for each of the three species tested. However, it was necessary in these comparative experiments that test conditions be kept identical for all species since the conditions could affect bioavailability and toxicity (e.g., temperature-dependent toxicity, salinity effects on cadmium toxicity). Therefore, the test conditions selected (17°C, 25 ‰) represent a compromise expected to be acceptable for all three species (Table 1).

Table 1. Experiment 2 test conditions in comparison to those recommended for each species in standard protocols (ASTM, 1993[a], EPA, 1994[b]).

Parameter	Test Conditions	*A. Abdita*	*E. Estuarius*	*R. Abronius*
Temperature (°C)	17	20[a,b] (tested at 8–25)[a]	15[a,b] (tolerates 5–21)[a]	15[a,b] (survives 0–20)[a]
Salinity (‰)	25	28[b] (tested at 20–35)[a]	20[b] 2–28[a]	28[a,b] (<25 not advised)[a]

Twenty amphipods were added to each test container, with five replicate containers per treatment. The test was conducted under 24-hour light since preliminary data (ASTM, 1993; pers. observ.) suggested this was more effective in insuring that ampeliscid amphipods did not emerge from their tubes and become trapped in the surface tension at the air-water interface. While continuous light may not have been necessary for all species, it was used because of the potential for phototoxicity in the crude oil treatments and the need for uniform conditions for all species tested in Experiment 2. Water quality conditions (temperature, salinity, pH, dissolved oxygen) were monitored in 5 randomly selected containers every 2–3 days. Seawater was not replaced except for a small amount of distilled water added to compensate for evaporation.

Experiment 1 compared *A. abdita* mortality after 10-day exposure (standard protocol) to *A. abdita* mortality and growth after 17 days. Animals in the 10-day exposures were not fed; animals held for 17 days growth tests were supplied 15–20 ml of *Phaeodactylum tricornutum* culture each day per container (approximately 10^7 cells ml^{-1} in culture, thus providing about $9x10^6$ cells $amphipod^{-1}$ day^{-1}). In Experiment 2 we were comparing *E. estuarius* and *R. abronius* mortality after 10 days to *A. abdita* mortality and growth after 17 days. While feeding is not part of standard protocols for 10-day mortality tests, it was necessary for the *A. abdita* growth test. Since we were interested in comparing the relative sensitivity of the various species to the toxicant spikes, and the addition of algae could alter contaminant bioavailability (e.g., through changes in sediment organic content), it was necessary to supply 15–20 ml algal culture to all species each day.

After completion of the exposures, the sediment was sieved on a 1 mm *(E. estuarius, R. abronius*) or 355–500 μm (*A. abdita*) screen to recover the amphipods. Surviving amphipods were either counted immediately (*E. estuarius, R. abronius*) or were preserved in 10% buffered formalin (*A. abdita*) for later enumeration and measuring. Body length in *A. abdita* was measured along the

dorsum from the insertion point of the first antennae to the base of the telson, using a drawing tube to trace the dorsal outline and a map-measuring device to obtain a length from the drawing.

A positive control treatment was established for all species using cadmium chloride as a toxicant. This test was done as a 96-hour, water-only exposure without aeration.

Statistical analyses

Percentage survival data was transformed by arcsine square root, and tested for normality by Shapiro-Wilks Test and for heterogeneity of variance by Bartlett's Test. Data found to meet the assumptions for parametric tests were analyzed by Dunnett's multiple comparison test. Data which did not meet the necessary assumptions were tested non-parametrically using Steel's Test. In Experiment 1 all treatment effects were evaluated relative to the unspiked home sediment control. In Experiment 2 all treatment comparisons were relative to the Bodega control sediment except in the case of the DDT treatments with *A. abdita*. In this case, comparisons were made relative to the solvent control since survival was slightly depressed relative to the Bodega control. LC_{50} values were calculated by the trimmed Spearman Karber method. All the above statistics were done using Toxcalc 5.0 (Tidepool Scientific Software).

Treatments were analyzed for growth differences only if at least 40 of the 100 initial amphipods (5 replicates of 20 animals each) survived the exposure and provided body length data. For analysis of the size data in *A. abdita* the body length data from the five replicates for each treatment were first tested by Kruskal-Wallis one-way analysis of variance to determine if they could be considered drawn from the same population. If differences among the replicates were not significant ($p>0.05$), the replicates were composited for subsequent analyses. If the replicates showed significant differences, as in about one-third of the cases, then the aberrant replicate was identified by inspection of the data, discarded, and the remaining four replicates were then re-tested for significant differences and composited. If it was necessary to discard more than one replicate in order to obtain non-significance among remaining replicates, then the data from that treatment were not used and the high interreplicate variability is noted in results.

Once a homogeneous population was identified for each treatment (consisting of a four or five replicate composite), its size-frequency distribution was tested against that of the unspiked control using a one-tailed Kolmogorov-Smirnov Test with $p<0.05$ considered significant. If the size-frequency distribution of a toxicant treatment was significantly shifted towards smaller individuals than the unspiked control, this is interpreted herein as a reduction in growth rate. While this is certainly the case in treatments with high survival, in those treatments where mortality was substantial it is not possible to differentiate between growth reduction and selective mortality of larger individuals.

RESULTS

Sediment Characteristics

Only sediment from the RMP Alameda site was used in Experiment 1. It contained negligible amounts of sand greater than 500 μm, but tubes and other debris larger than 500 μm were removed by sieving prior to use of the sediment. The percentage of particles (by weight) in the remaining material was: medium sand (250–500 μm) 0.7%; fine sand (125–250 μm) 11.6%; very fine sand (63–125 μm) 16.5%; and silt and clay (<63 μm) 71.2%. The total organic carbon content of sediments at this site is 0.95% (RMP, 1995).

Particle size data for Experiment 2 are provided in Table 2. *E. estuarius* and *R. abronius* are typically found in sandy sediments. In these collections *E. estuarius* home site consisted of 97% fine and medium sand, with no measurable silt and clay. Qualitative assessment of the *R. abronius* home sediment indicated a similar particle size distribution. *A. abdita*, however, prefers muddy sediments and was collected from a San Francisco Bay site with 31% silt and clay.

In order to use the same test sediment for all species, it was necessary to obtain a muddy sand. The Bodega Bay sediment, containing 14% silt and clay, proved suitable for these experiments. The total organic carbon content of this sediment was 0.55%.

Table 2. Particle size distributions of Bodega Bay test sediment used for all Experiment 2 toxicant spikes and the home sediment from the amphipod collecting sites. No quantitative data are available from Deception Pass, home site of *R. abronius*. However, qualitative inspection indicated the sediment was similar to that of the *E. estuarius* site, containing well-sorted sand with negligible silt and clay particles. The small percentage of coarse sand in all sediments would have been removed by sieving prior to use of the sediments in toxicity tests.

	Bodega Bay (test sed.)	*A. abdita* home sed. (S.F. Bay, East of Tiburon)	*E. estuarius* home sed. (Yaquina Bay, OR)
coarse sand (>500 μm)	1.4	2.2	0.4
medium sand (250–500 μm)	30.6	5.2	63.5
fine sand (125–250 μm)	50.4	26.5	33.6
very fine sand (63–125 μm)	3.5	35.2	2.4
silt and clay (<63 μm)	14.0	31.0	0

Cadmium Positive Control

In a 96-hour, water only exposure, the *A. abdita* cadmium LC_{50} values and their 95% confidence intervals were:

- Experiment 1 at time of tests with cadmium-spiked sediments: 0.34 mg l^{-1} (0.29–0.40);
- Experiment 1 at time of tests with oil-spiked sediments: 0.89 mg l^{-1} (0.78–1.03);
- Experiment 2: 0.95 mg l^{-1} (0.71–1.26).

In all previous tests within our lab the mean LC_{50} has been 0.68 mg l^{-1}. Our values have been somewhat higher than values reported in the literature for the species (0.20–0.58 mg l^{-1}; Redmond *et al.,* 1994) indicating that our source population in San Francisco Bay is either inherently less sensitive to cadmium or that the animals were less stressed by other factors at the time of testing.

The 96-hour LC_{50} for *E. estuarius* was 5.03 mg l^{-1} (95% C.I. = 4.52 to 5.60). This value should be considered tentative since a shortage of individuals made it necessary to reduce the number of treatments (3 concentrations of 2 replicates each, rather than the typical 6 concentrations of 3 replicates each). Past reported values have been somewhat higher (9.33 mg l^{-1}; ASTM, 1993).

The 96-hour LC_{50} for *R. abronius* was 0.16 mg l^{-1} (95% C.I. = 0.05 to 0.24), a value that should also be viewed with caution because of high mortality in the water-only positive control (although not in sediment tests, as discussed below).

Experiment 1—Mortality

Experiment 1 was intended to determine if use of 17-day mortality and growth endpoints provided more sensitive indicators of cadmium and crude oil toxicity than the standard 10-day mortality test. Mortality results are provided in Table 3.

Good survival was obtained in the home sediment (Alameda control) in most of the Experiment 1 trials. After a 10-day exposure survival rates were 93 and 91% for cadmium and crude oil experiments, respectively. After a 17-day exposure the equivalent survival rates were 88 and 83%. The latter value is slightly lower than typical for our laboratory. Past work has shown survival in uncontaminated sediments to generally fall between 88 and 95%, even with exposure periods up to 1 month.

Cadmium significantly decreased survival (to 70–75%) at the lowest concentration used of 22 mg kg^{-1}. The 10-day exposures were noteworthy in that this survival rate remained essentially constant from 22 through 180 mg kg^{-1}, rather than following a typical dose-response relationship.

Table 3. Percent survival of *A. abdita* in Experiment 1 using Alameda home sediments, and cadmium or crude oil-spiked home sediments. Values shown are means and standard deviations. Those treatments significantly different from the home sediment control at $p<0.05$ are denoted with *.

Sediment	10-day survival	17-day survival
Cadmium		
Home (Alameda)	93 ± 12	88 ± 5
22 mg kg^{-1}	75 ± 6*	70 ± 85*
45 mg kg^{-1}	71 ± 6*	74 ± 14
90 mg kg^{-1}	59 ± 10*	45 ± 6*
180 mg kg^{-1}	75 ± 7*	15 ± 18*
Weathered crude oil		
Home (Alameda)	91 ± 9	83 ± 9
150 mg kg^{-1}	90 ± 12	94 ± 6
460 mg kg^{-1}	90 ± 11	85 ± 7
1400 mg kg^{-1}	79 ± 12	88 ± 10
4,200 mg kg^{-1}	25 ± 11*	33 ± 15*

A preliminary study in our lab using the same sediment also found ~70% survival to be the case up to 270 mg kg^{-1}, but with near total mortality at 810 mg kg^{-1}. The results from the 17-day exposures followed the more typical pattern of increasing mortality with increasing dosage, although the dose-response relationship was interrupted by the absence of significant mortality at 45 mg kg^{-1}. Cadmium LC_{50} values were >180 mg kg^{-1} for the 10-day test and 91 mg kg^{-1} (95% C.I. = 78–106) for the 17-day test.

In the weathered crude oil exposures, survival remained comparable to the control at concentrations of 150, 460, and 1,400 mg kg^{-1}. A dramatic reduction in survival to 25–33% occurred at 4,200 mg kg^{-1} in both the 10- and 17-day exposures. Oil LC_{50} values were 2,768 mg kg^{-1} (2,690–2,848) for 10 days and 3,334 mg kg^{-1} (3,259–3,409) for 17 days.

10- and 17-day experiments provided very similar results. With only one exception (45 mg kg^{-1} cadmium) the concentrations which produced significant 10-day mortality were the same ones that produced significant 17-day mortality. For the single exception, 17-day survival was marginally higher, not lower as might be expected. In non-toxic sediments, mean survival of amphipods in the 17-day tests was higher than after 10 days in two of the five cases.

Experiment 1—Growth

Growth in the cadmium tests of Experiment 1 was atypically rapid, probably because of the small size of the amphipods collected at that time. The amphipods used had an initial mean body length of 2.3 mm (s.d. ± 0.6). (Length at the time of hatching is approximately 1.5 mm.) Within the 17 days of the test, average length in control sediments had increased to 3.8 mm (± 0.7), or an increase in length of 65%. The oil exposures were conducted approximately 40 days later, but in the intervening period the animals in the source population had grown to a mean length of 3.2 mm (± 0.5). Over the 17 days of the oil exposure tests, animals in the control sediment grew to an average length of 3.9 mm (± 0.5), or 22%.

Relative to the control sediment, cadmium concentrations of 22 and 45 mg kg^{-1} had no effect on rate of growth. Animals held at 90 mg kg^{-1}, however, had a size-frequency distribution that was significantly shifted towards smaller animals after 17 days (Figure 1).

A crude oil concentration of 1,400 mg kg^{-1} depressed the growth of *A. abdita* to the point that there was little if any change in body length over the 17-day exposure (Figure 2). Mean body length of both the initial population and the individuals exposed to 1,400 mg kg^{-1} for 17 days were identical (3.2 ± 0.5). It is possible that growth was impaired at lower concentrations of crude oil, but high interreplicate variability prevented statistical analysis. The size-frequency distributions of animals in both the 150 and 460 mg kg^{-1} treatments superficially appeared to contain smaller individuals than the control (Figure 2), but it was not possible to attain a homogeneous population for these treatments without elimination of two or more replicates. As discussed in the methods section, this situation prevents determination of growth effects.

Experiment 2—Mortality

Experiment 2 was intended to determine the relative toxicant sensitivity of *A. abdita, E. estuarius,* and *R. abronius*, when all three species were tested under identical conditions. The three amphipod species tested differ in their preferred sediment type, thus finding a single sediment suitable for all species was problematic. Nevertheless, the Bodega Bay sediment used for the Bodega control and all toxicant spikes appeared to be acceptable for all species. For *E. estuarius*, survival in Bodega control sediment was identical to that in the control (both with a mean survival of 93%; Table 4). For *R. abronius*, survival tended to be lower in the Bodega control (mean of 79% survival vs. 92% in home sediment), but because of interreplicate variability these means were not statistically different. The survival rate of *A. abdita* in Bodega control sediment was 89%, higher than the home sediment, and typical for uncontaminated sediments tested in our lab.

Solvent control survival rates were indistinguishable from the Bodega control for *E. estuarius* and *R. abronius*, demonstrating no acute toxicity of the acetone carrier solvent used for the DDT spikes. Solvent control survival was less than Bodega control only in the *A. abdita* tests, but the lower concentration DDT treatments had survival rates as high as the Bodega control, suggesting that the mortality in the solvent control was unrelated to the acetone.

Survival data upon exposure to the three toxicants are shown in Tables 4, and Figure 3. It should be recognized that the *E. estuarius* and *R. abronius* tests were done for 10 days as in standard protocols (ASTM, 1993; EPA, 1994), while the *A. abdita* tests represent a 17-day exposure since survival data were collected as part of a growth test. Previous work in our laboratory has shown that survival rates of *A. abdita* in uncontaminated sediments were comparable between 10 and 30 days, and in Experiment 1 (exposures to cadmium and crude oil) there was little difference between 10-and 17-day mortality rates. Thus, mortality comparisons between the three species should be valid in spite of the different exposure periods.

Concentrations of cadmium up to 330 mg kg^{-1} had no effect on mortality rates for any of the amphipod species. An increase in cadmium concentration to 1,000 mg kg^{-1} resulted in the complete mortality of *R. abronius* in all replicates, and an average survival rate of only 19% for *A. abdita. E. estuarius*, however, was unaffected by the highest cadmium concentration used of 1,000 mg kg^{-1} with a survival rate of 94%, a value comparable to the unspiked controls.

Despite the insensitivity of *E. estuarius* to cadmium, it was the first species to show decreased survival with increasing sediment concentrations of DDT. At a DDT concentration of 370 μg kg^{-1},

survival rates for the species dropped from 93% (Bodega control) to 77%. Survival rates of the other two species remained indistinguishable from the controls. At a concentration of 1,100 μg kg^{-1}, the

Table 4. Percent survival of the three species tested in their respective home sediments, Bodega control sediment (used for all spiked treatments), the solvent control (acetone carrier used in DDT spikes), and the various spiked treatments. Values shown are means and standard deviations. Those treatments significantly different from the Bodega control (or solvent control in the case of DDT exposures for *A. abdita*) at $p<0.05$ are denoted with *.

Sediment or Treatment	*A. abdita*	*E. estuarius*	*R. abronius*
Home sediment	75 ± 13*	93 ± 8	92 ± 9
Bodega control	89 ± 5	93 ± 3	79 ± 16
Solvent control	79 ± 4*	97 ± 4	74 ± 17
Cadmium			
12 mg kg^{-1}	83 ± 19	86 ± 4	74 ± 17
37 mg kg^{-1}	89 ± 14	92 ± 4	84 ± 17
110 mg kg^{-1}	95 ± 6	95 ± 5	84 ± 2
330 mg kg^{-1}	83 ± 6	93 ± 8	67 ± 16
1000 mg kg^{-1}	19 ± 19*	94 ± 7	0 ± 0*
DDT			
120 μg kg^{-1}	79 ± 13	95 ± 6	78 ± 4
370 μg kg^{-1}	88 ± 8	77 ± 7*	80 ± 8
1,100 μg kg^{-1}	14 ± 7*	9 ± 2*	35 ± 12*
3,300 μg kg^{-1}	0 ± 0*	0 ± 0*	0 ± 0*
10,000 μg kg^{-1}	0 ± 0*	0 ± 0*	0 ± 0*
Weathered crude oil			
60 mg kg^{-1}	83 ± 15	91 ± 7	85 ± 10
180 mg kg^{-1}	84 ± 10	88 ± 6	73 ± 12
530 mg kg^{-1}	44 ± 17*	42 ± 10*	31 ± 10*
1,000 mg kg^{-1}	5 ± 6*	23 ± 12*	14 ± 23*
5,000 mg kg^{-1}	1 ± 2*	1 ± 2*	5 ± 11*

survival rates of all species dropped precipitously (means of 9 to 35% survival), and no individual of any species survived exposure to DDT concentrations of 3,300 μg kg^{-1} or more.

The toxicity of weathered crude oil was very consistent among the three species, with no measurable affect of 180 mg kg^{-1}, and about 40% survival at a concentration of 530 mg kg^{-1} (*A. abdita* = 44%; *E. estuarius* = 42%; *R. abronius* = 31%). Survival continued to decline in all species with increasing crude oil concentrations, with only a few individuals surviving exposure to 5,000 mg kg^{-1}.

Differences in sensitivity among the three species are illustrated in Table 5 on the basis of LC_{50} values. The cadmium LC_{50} for *E. estuarius* is shown only as >1,000 mg kg^{-1}, since the highest concentration used in this study had no affect on mortality rate of the species.

Table 5. LC_{50} values and 95% confidence intervals for the three amphipod species exposed to cadmium, DDT and weathered crude oil in Experiment 2.

Toxicant	*A. abdita* LC_{50}	*E. estuarius* LC_{50}	*R. abronius* LC_{50}
Cadmium	643	>1,000	479
(mg kg^{-1})	(632–654)		(426–538)
DDT	769	554	1036
(µg kg^{-1})	(708–835)	(510–601)	(882–1217)
Crude oil	528	630	505
(mg kg^{-1})	(444–627)	(540–735)	(429–594)

Experiment 2—Growth

A. abdita individuals that survived exposures to the controls or spiked sediments were measured and used to determine a growth rate endpoint in addition to the mortality endpoint discussed above. Individuals used in the growth assays had an initial mean body length of 3.2 mm (s.d.=0.6). After 17 days in the Bodega control sediment their length had increased to an average of 4.2 mm (s.d.=0.6), or a gain of 31%. Body sizes of animals in the acetone control were indistinguishable from the Bodega control ($p>0.05$, all size comparisons by Kolmogorov-Smirnov test). Body size was greater in home sediment (mean = 4.6 mm ± 0.6) than in Bodega control even though the survival rate was reduced, perhaps suggesting differential mortality of smaller individuals.

In the cadmium treatments the size-frequency distribution after exposure to the 37 mg kg^{-1} treatment was significantly shifted towards smaller individuals relative to the Bodega control (Figure 4). The same was true of the 110 mg kg^{-1} cadmium treatment, but the difference was marginally non-significant ($0.1>p>0.05$). At a concentration of 330 mg kg^{-1} the difference was significant once again ($p<0.05$).

DDT had no adverse affect on growth rate at the two lowest concentrations, 120 and 370 µg kg^{-1} (Figure 5). At the next highest concentration of 1,100 µg kg^{-1} there was an 86% mortality, leaving too few individuals to reliably estimate growth rates.

Weathered crude oil did not adversely affect growth rates at concentrations of 180 mg kg^{-1} or less. Significant effects ($p<0.05$) were apparent at 530 mg kg^{-1} (Figure 6).

DISCUSSION

Cadmium LC_{50} values were quite variable, both among the two sediments used in these experiments and in comparison to literature values. Our sediment cadmium 10-day LC_{50} of 479 mg kg^{-1} for *R. abronius* is far higher than the 10-day LC_{50} of 6.9 mg kg^{-1} reported for the species by Swartz *et al.* (1985) for Yaquina Bay sediment. Conversely, our sediment LC_{50}'s for *A. abdita* of >180 (Experiment 1, 10-day), 91 (Experiment 1, 17-day) and 643 mg kg^{-1} (Experiment 2) are far less than the values of 1,070 to 2,850 mg kg^{-1} using sediments from New England (DiToro *et al.,* 1990). These differences may reflect variations in sensitivity among populations of the test animals, or may reflect variation in cadmium bioavailability among the sediments. The work of DiToro *et al.* (1990) suggests bioavailability differences may be related to the concentrations of

acid volatile sulfides (AVS) in the sediments. These AVS analyses, however, are still in progress with our sediments, so consideration of this possibility is premature.

Crude oil toxicity to *A. abdita* also varied among sediments from a 17-day LC_{50} of 3,334 mg kg^{-1} in the Alameda sediment to 528 mg kg^{-1} in the Bodega Bay sediment. In part these differences may be related to differing organic contents of the sediments and its affect on bioavailability. Normalization of the LC_{50} values to organic carbon reduced the difference between the sediments from 6-fold to 4-fold (0.95% TOC at Alameda = LC_{50} of 350 mg g^{-1} o.c.; 0.55% TOC at Bodega Bay = LC_{50} of 96 mg g^{-1} o.c.).

When normalized to organic carbon, our LC_{50} measurements for DDT show remarkable consistency with other data reported in the literature. Based on an organic carbon content of 0.55% in the Bodega Bay sediment, our DDT 17-day LC_{50} values were 101 µg g^{-1} o.c. (*E. estuarius*), 140 µg g^{-1} o.c. (*A. abdita*), and 188 µg g^{-1} o.c. (*R. abronius*). 10-day LC_{50} values for *Hyalella azteca* tested in three sediments range from 272-473 µg g^{-1} o.c. (Nebeker *et al.,* 1989). In the Lauritzen Canal, few amphipods (except *Grandidierella japonica*) were found at DDT concentrations above 100 µg g^{-1} o.c. (Swartz *et al.,* 1994). On the Palos Verdes shelf, few or no amphipods were collected at DDT concentrations above about 200 µg g^{-1} o.c. (Swartz *et al.,* 1985; 1986; 1991; Ferraro *et al.,* 1991). All these data point to DDT toxicity at concentrations of one hundred to a few hundred µg g^{-1} o.c. In contrast, field-derived 10-day LC_{50} values (based on toxicity tests with contaminated sediments collected from the field and analyzed for DDT) were 2,500 µg g^{-1} o.c. for *E. estuarius* in the Lauritzen Canal and 1,040 µg g^{-1} o.c. for *R. abronius* on the Palos Verdes shelf (Swartz *et al.,* 1994). However, the absence of field populations of amphipods at these same locations in sediments with an order of magnitude less DDT indicates a much greater toxicity than is reflected in the 10-day LC_{50} measurements.

There have been few attempts to compare toxicant sensitivity of the various amphipod species used in sediment toxicity testing. In one of the few comparative studies, fluoranthene LC_{50} values differed by less than a factor of three among the amphipods *H. azteca* (15.4 mg kg^{-1}*), E. estuarius* (10.6 mg kg^{-1}), and *R. abronius* (5.1 mg kg^{-1}) (DeWitt *et al.,* 1989). In most cases, comparisons among studies is complicated by differences in test conditions, and often made uninterpretable because of potential bioavailability differences among sediments. In our experiments all conditions (e.g., temperature, salinity, light regime, feeding, water source, sediment) were held constant for tests with all three species. The tests differed only in the duration of exposure (17-day for *A. abdita* and 10-day for the other species), and Experiment 1 data have shown this difference to be of little consequence for cadmium and crude oil. Data are lacking for the 10-day versus 17-day mortality for DDT.

In comparing the relative toxicant sensitivities of the three species, it is apparent that no one species is consistently more or less sensitive than the others across all toxicants. If relative sensitivity is defined on the basis of LC_{50} values, then these experiments yield the following ranking:

Cadmium sensitivity:	*R. abronius* > *A. abdita* >> *E. estuarius*
DDT sensitivity:	*E. estuarius* > *A. abdita* > *R. abronius*
Crude oil sensitivity:	*R. abronius* = *A. abdita* = *E. estuarius*

R. abronius is the most sensitive species to cadmium toxicity, but it is only slightly more sensitive than *A. abdita* (LC_{50} values of 479 mg kg^{-1} for *R. abronius* and 643 mg kg^{-1} for *A. abdita*). One of the most striking observations in this study is the dramatic tolerance of *E. estuarius* to cadmium. A sediment concentration of 1,000 mg kg^{-1} (0.1% cadmium) had no affect on acute mortality of *E. estuarius*, yet resulted in 81% mortality in *A. abdita* and 100% mortality in *R. abronius*. This tolerance to cadmium was also apparent in the water-only 96-hour exposures in which the LC_{50} for *E. estuarius* was an order-of-magnitude higher than for the other species. These data indicate that *E. estuarius* is a poor choice for toxicity testing if cadmium is among the potential toxicants. Relative sensitivity to the other metals is unknown but would merit further evaluation.

E. estuarius is the most sensitive of the three species to DDT, yet the differences in sensitivity among the three species are relatively small. All DDT LC_{50} values varied by less than a factor of 2 (*E. estuarius* = 55 µg kg^{-1}; *A. abdita* = 77 µg kg^{-1}; *R. abronius* = 104 µg kg^{-1}). The three species are equally sensitive to crude oil, with LC_{50} values being essentially indistinguishable.

Taken together, the data show that the use of *A. abdita* for sediment toxicity testing does not result in any appreciable loss in sensitivity relative to *R. abronius* or *E. estuarius* for the conaminants tested. To the contrary, use of the species provides a substantial increase in sensitivity relative to *E. estuarius* in cases of cadmium toxicity. The tubiculos nature of *A. abdita* does not appear to compromise its usefulness for toxicity testing. Either the tube wall is not a barrier to diffusion of sediment-associated toxicants, or exposure via the near-bottom waters within a few mm of the sediment-water interface is comparable to that of the pore water exposure experienced by the two fossorial species.

The data from these experiments also permit consideration of whether a growth endpoint provides greater sensitivity to toxicants than a mortality endpoint (either 10- or 17-day exposure). Neither growth nor mortality endpoints provided a clear advantage in two of the five trials involving the three toxicants examined (Table 6; DDT, Experiment 2 and crude oil, Experiment 2). In two other cases, growth was the more sensitive endpoint; i.e., the toxicant concentrations necessary to depress growth rates were less than those necessary to cause mortality (cadmium, Experiment 2 and crude oil, Experiment 1). Finally, in one case (cadmium, Experiment 1), growth was a less sensitive endpoint. Interpretation of results in this one case are problematic. First, there is no apparent mechanism for mortality to be a more sensitive endpoint of cadmium toxicity in one sediment (Alameda sediment of Experiment 1), but growth to be a more sensitive endpoint in another sediment (Bodega Bay sediment of Experiment 2). It is plausible that the most sensitive endpoint would depend upon the toxicant (e.g., one which would impair growth and another whose toxic effects are through other growth-independent mechanisms), but it does not seem resonable that the most sensitive endpoint would be sediment dependent. Secondly, a cadmium concentration of 22 mg kg^{-1} was sufficient to increase mortality in both the 10- and 17-day exposures, yet there was no increased mortality after 17 days at 45 mg kg^{-1}. Thus, the lack of a consistent dose-response relationship makes interpretation of the cadmium mortality data difficult at the lower concentrations.

While a growth endpoint was a more sensitive measure than acute toxicity for some contaminants and sediments, it was not demonstrated to be consistently so in all cases. One reason for this may have been the 3x intervals used in sediment spiking (e.g., 12, 37, 110, 330, and 1,000 mg kg^{-1} cadmium). While these intervals would have been sufficient to detect large differences in sensitivity between the endpoints based on NOEC/LOEC values, the demonstration of more subtle differences would require more closely spaced intervals.

Table 6. Minimum effective concentration of the toxicants used in the experiments as based on each of the three endpoints. Values shown are NOEC and LOEC, with the minimum concentration necessary to elicit a response expected to fall between these two points. In cases where one endpoint provides a more sensitive measure than the others, the effective concentration is in bold type. Concentrations are: Cd = mg kg^{-1}; DDT = µg kg^{-1}; and weathered crude oil = mg kg^{-1}.

Toxicant	10-day survival	17-day survival	17-day growth
Cadmium (Exp. 1)	**0–22**	45–90[a]	45–90
Cadmium (Exp. 2)		330–1,000	**110–330[b]**
DDT (Exp. 2)		370–1,100	>370[c]
Crude oil (Exp. 1)	1,400–4,200	1,400–4,200	**<1,400[d]**
Crude oil (Exp. 2)		180–530	180–530

[a]Mortality was significantly greater than the control at 22 and 90 mg kg^{-1}, but not at 45 mg kg^{-1}. Therefore, the more conservative interpretation is taken here in presenting 45 and 90 mg kg^{-1} as the NOEC and LOEC, respectively.

[b]The size frequency distribution contained significantly smaller individuals relative to the control at 37 and 330 mg kg^{-1}. At 110 mg kg^{-1}, however, the effect was marginally non-significant ($0.05<p<0.1$). Therefore, the more conservative interpretation is taken here in presenting 110 and 330 mg kg^{-1} as the NOEC and LOEC, respectively.

[c]The LOEC is unknown since at DDT concentrations greater than 370 µg kg^{-1} the mortality rate was too great to provide sufficient individuals for growth determination.

[d]At oil concentrations of 150 and 460 mg kg^{-1} growth superficially appeared less than the control, but high interreplicate variability prohibited demonstration of statistical significance. Growth impairment was clearly evident at 1400 mg kg^{-1}, and that value is shown above, but the actual effective concentration may be much lower.

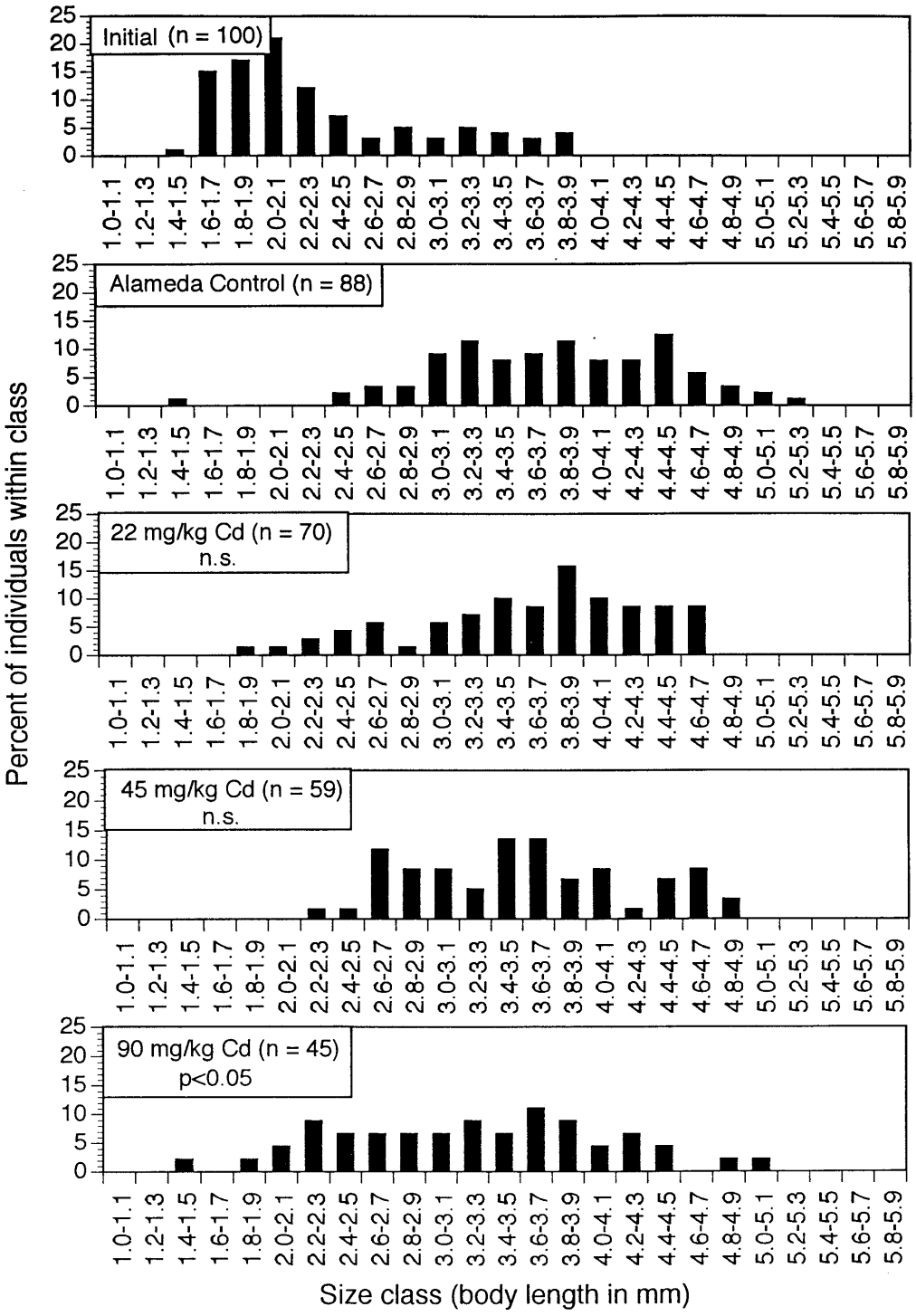

Figure 1. Size-frequency distribution of *A. abdita* from the cadmium treatments of Experiment 1. The initial distribution at the beginning of the test is shown, as well as the distribution after 17 days in the Alameda (home) control and three cadmium-spiked sediments. No size data are shown for the 180 mg kg^{-1} treatment because of a survival rate of less than 40%. The significance levels are derived from a Kolmogorov-Smirnov Test against the Alameda control.

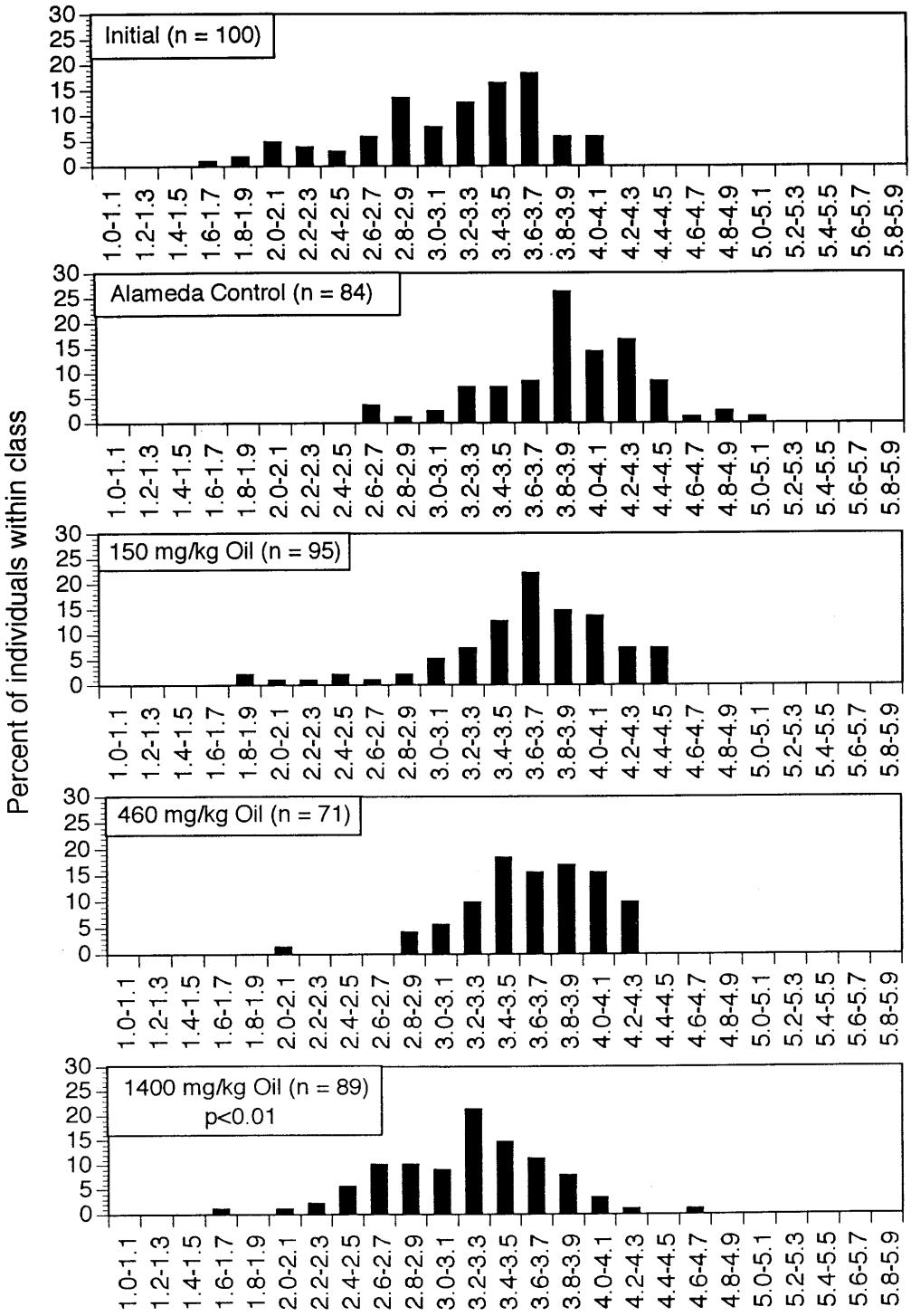

Figure 2. Size-frequency distribution of *A. abdita* from the crude oil treatments of Experiment 1. The initial distribution at the beginning of the test is shown, as well as the distribution after 17 days in the Alameda (home) control and three oil-spiked sediments. No size data are shown for the 4,200 mg kg^{-1} treatment because of a survival rate of less than 40%. The significance levels are derived from a Kolmogorov-Smirnov Test against the Alameda control. The 150 and 460 mg kg^{-1} treatments were not tested statistically because of high interreplicate variability.

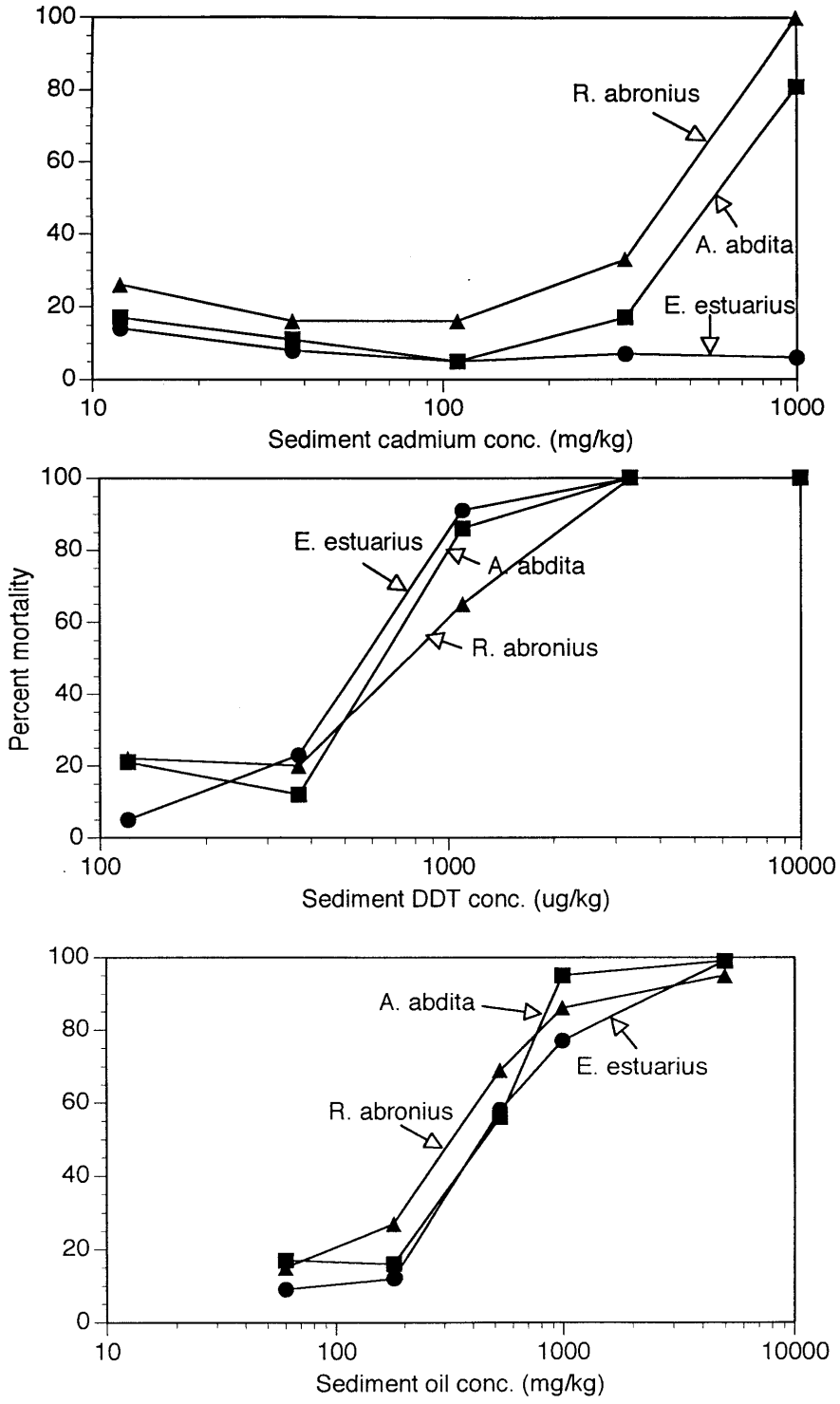

Figure 3. Mortality of the three amphipod species with increasing concentrations of cadmium, DDT, and crude oil. The values shown are means. Error bars are not provided for the sake of graphical clarity, but the data are available in Table 4.

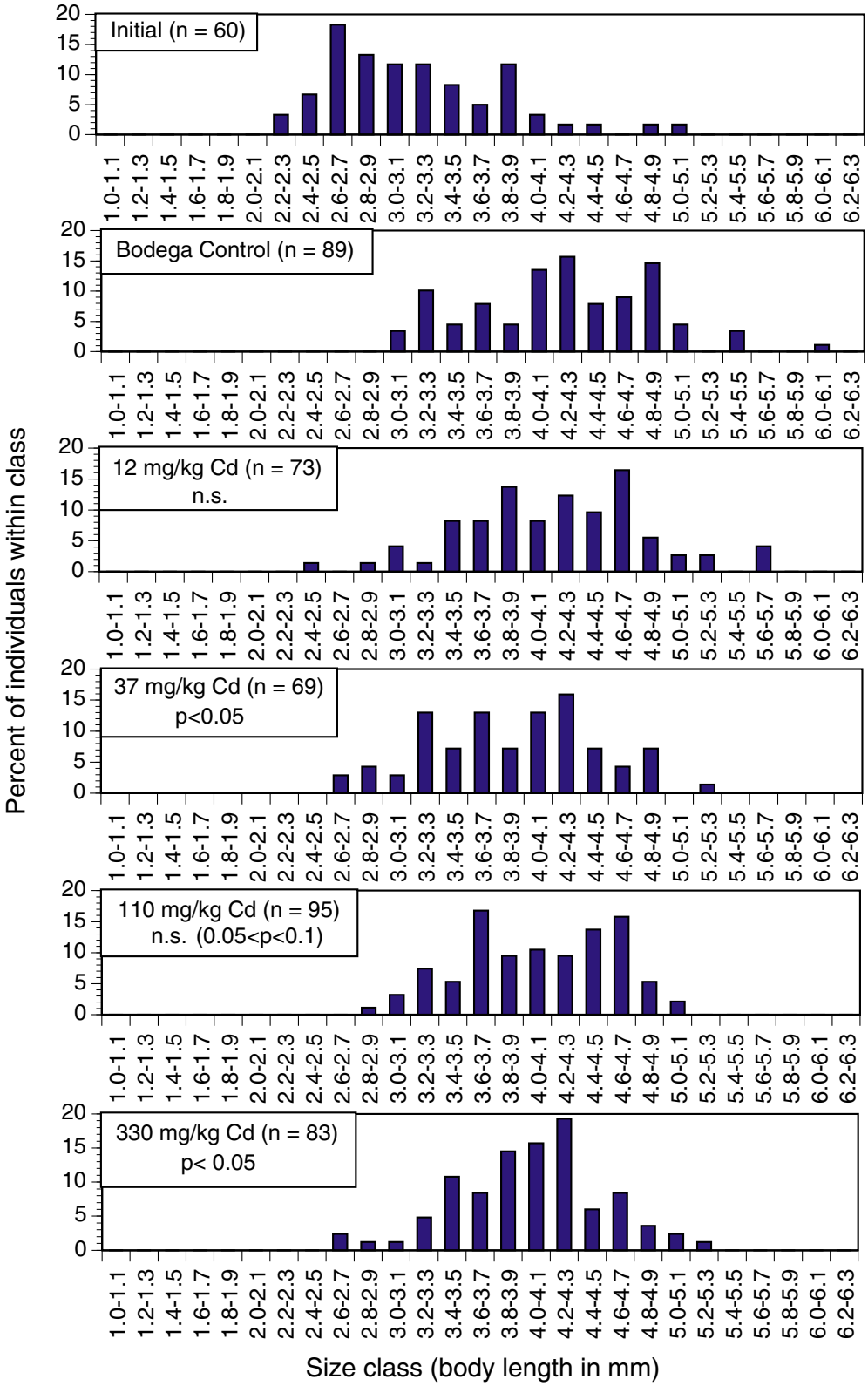

Figure 4. Size-frequency distribution of *A. abdita* from the cadmium treatments of Experiment 2. The initial distribution at the beginning of the test is shown, as well as the distribution after 17 days in the Bodega Bay control and four cadmium-spiked sediments. No size data are shown for the 1,000 mg kg^{-1} treatment because of a survival rate of less than 40%. The significance levels are derived from a Kolmogorov-Smirnov Test against the Bodega control.

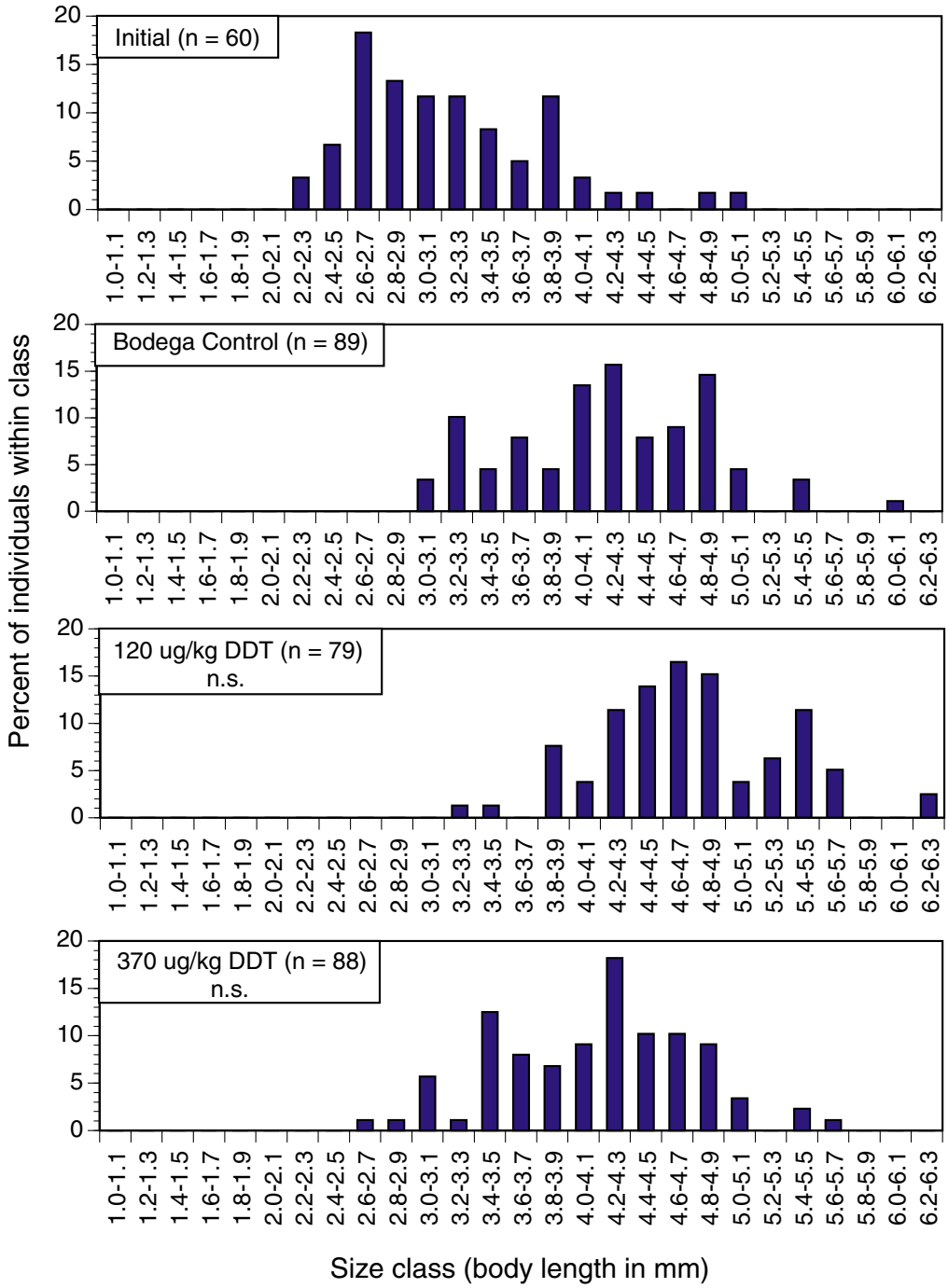

Figure 5. Size-frequency distribution of *A. abdita* from the DDT treatments of Experiment 2. The initial distribution at the beginning of the test is shown, as well as the distribution after 17 days in the Bodega Bay control and two DDT-spiked sediments. No size data are shown for the 1,100, 3,300 and 10,000 µg kg^{-1} treatments because of a survival rate of less than 40%. The significance levels are derived from a Kolmogorov-Smirnov Test against the Bodega control.

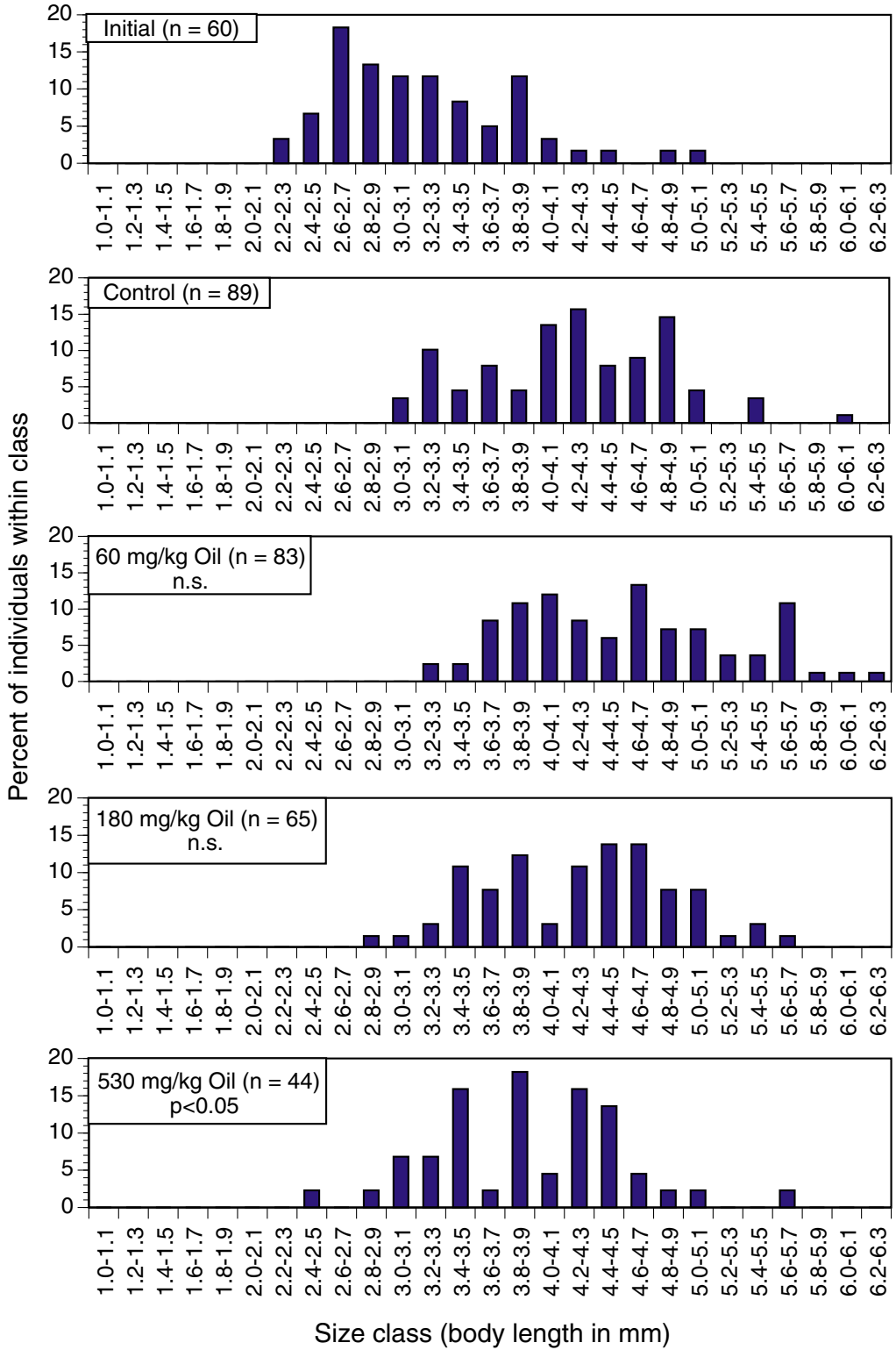

Figure 6. Size-frequency distribution of *A. abdita* from the crude oil treatments of Experiment 2. The initial distribution at the beginning of the test is shown, as well as the distribution after 17 days in the Bodega Bay control and three oil-spiked sediments. No size data are shown for the 1,600 mg kg^{-1} treatment because of a survival rate of less than 40%. The significance levels are derived from a Kolmogorov-Smirnov Test against the Bodega control.

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