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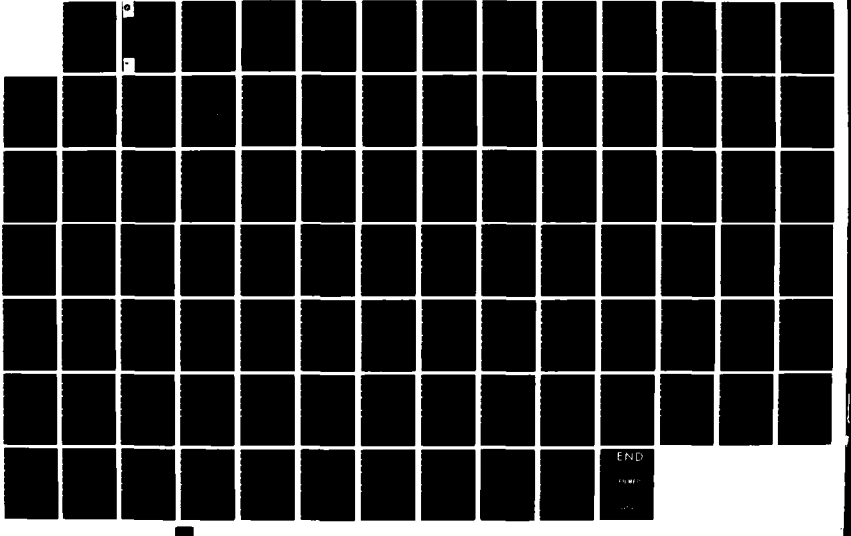

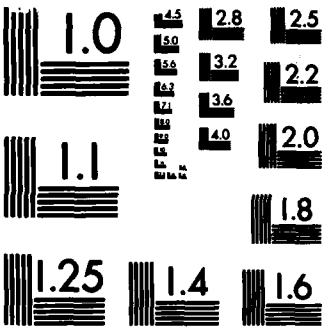

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US Environmental Protection Agency

AD-A160 507

FIELD VERIFICATION PROGRAM (AQUATIC DISPOSAL)

TECHNICAL REPORT D-85-8

APPLICATION OF LABORATORY POPULATION RESPONSES FOR EVALUATING THE EFFECTS OF DREDGED MATERIAL

by

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Environmental Research Laboratory
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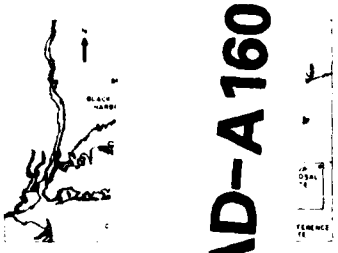

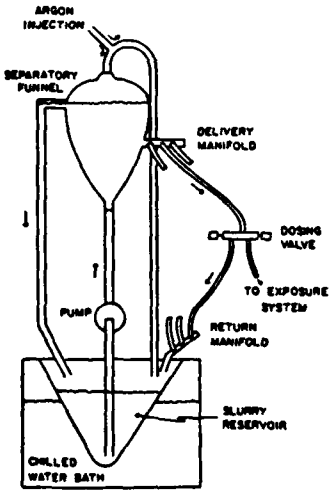

US Army Corps of Engineers

September 1985
Final Report

Prepared for DEPARTMENT OF THE ARMY
US Army Corps of Engineers
Washington, DC 20314-1000

and US Environmental Protection Agency
Washington, DC 20460

Monitored by Environmental Laboratory
US Army Engineer Waterways Experiment Station
PO Box 631, Vicksburg, Mississippi 39180-0631

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The D-series of reports includes publications of the Environmental Effects of Dredging Programs:

Dredging Operations Technical Support

Long-Term Effects of Dredging Operations

Interagency Field Verification of Methodologies for Evaluating Dredged Material Disposal Alternatives (Field Verification Program)

SUBJECT: Transmittal of Field Verification Program Technical Report Entitled "Application of Laboratory Population Responses for Evaluating the Effects of Dredged Material"

TO: All Report Recipients

1. This is one in a series of scientific reports documenting the findings of studies conducted under the Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives (referred to as the Field Verification Program or FVP). This program is a comprehensive evaluation of environmental effects of dredged material disposal under conditions of upland and aquatic disposal and wetland creation.

2. The FVP originated out of the mutual need of both the Corps of Engineers (Corps) and the Environmental Protection Agency (EPA) to continually improve the technical basis for carrying out their shared regulatory missions. The program is an expansion of studies proposed by EPA to the US Army Engineer Division, New England (NED), in support of its regulatory and dredging missions related to dredged material disposal into Long Island Sound. Discussions among the Corps' Waterways Experiment Station (WES), NED, and the EPA Environmental Research Laboratory (ERLN) in Narragansett, RI, made it clear that a dredging project at Black Rock Harbor in Bridgeport, CT, presented a unique opportunity for simultaneous evaluation of aquatic disposal, upland disposal, and wetland creation using the same dredged material. Evaluations were to be based on technology existing within the two agencies or developed during the six-year life of the program.

3. The program is generic in nature and will provide techniques and interpretive approaches applicable to evaluation of many dredging and disposal operations. Consequently, while the studies will provide detailed site-specific information on disposal of material dredged from Black Rock Harbor, they will also have great national significance for the Corps and EPA.

4. The FVP is designed to meet both Agencies' needs to document the effects of disposal under various conditions, provide verification of the predictive accuracy of evaluative techniques now in use, and provide a basis for determining the degree to which biological response is correlated with bioaccumulation of key contaminants in the species under study. The latter is an important aid in interpreting potential biological consequences of bioaccumulation. The program also meets EPA mission needs by providing an opportunity to document the application of a generic predictive hazard-assessment research strategy applicable to all wastes disposed in the aquatic environment. Therefore, the ERLN initiated exposure-assessment studies at the aquatic disposal site. The Corps-sponsored studies on environmental consequences of aquatic disposal will provide the effects assessment necessary to complement the EPA-sponsored exposure assessment, thereby allowing ERLN to develop and apply a hazard-assessment strategy. While not part of the Corps-funded FVP, the EPA exposure assessment studies will complement the Corps' work, and together the Corps and the EPA studies will satisfy the needs of both agencies.

SUBJECT: Transmittal of Field Verification Program Technical Report Entitled "Application of Laboratory Population Responses for Evaluating the Effects of Dredged Material"

5. In recognition of the potential national significance, the Office, Chief of Engineers, approved and funded the studies in January 1982. The work is managed through the Environmental Laboratory's Environmental Effects of Dredging Programs at WES. Studies of the effects of upland disposal and wetland creation are being conducted by WES and studies of aquatic disposal are being carried out by the ERLN, applying techniques worked out at the laboratory for evaluating sublethal effects of contaminants on aquatic organisms. These studies are funded by the Corps while salary, support facilities, etc., are provided by EPA. The EPA funding to support the exposure-assessment studies followed in 1983; the exposure-assessment studies are managed and conducted by ERLN.

6. The Corps and EPA are pleased at the opportunity to conduct cooperative research and believe that the value in practical implementation and improvement of environmental regulations of dredged material disposal will be considerable. The studies conducted under this program are scientific in nature and will be published in the scientific literature as appropriate and in a series of Corps technical reports. The EPA will publish findings of the exposure-assessment studies in the scientific literature and in EPA report series. The FVP will provide the scientific basis upon which regulatory recommendations will be made and upon which changes in regulatory implementation, and perhaps regulations themselves, will be based. However, the documents produced by the program do not in themselves constitute regulatory guidance from either agency. Regulatory guidance will be provided under separate authority after appropriate technical and administrative assessment of the overall findings of the entire program.

James Choromokos, Jr., Ph.D., P.E.
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Agency

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER Technical Report D-85-8	2. GOVT ACCESSION NO. AD-A160 507	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) APPLICATION OF LABORATORY POPULATION RESPONSES FOR EVALUATING THE EFFECTS OF DREDGED MATERIAL		5. TYPE OF REPORT & PERIOD COVERED Final report 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) John. H. Gentile, K. John Scott, Suzanne Lussier, Michele Redmond		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS US Environmental Protection Agency Environmental Research Laboratory Narragansett, Rhode Island 02882		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS Field Verification Program (Aquatic Disposal)
11. CONTROLLING OFFICE NAME AND ADDRESS DEPARTMENT OF THE ARMY, US Army Corps of Engineers, Washington, DC 20314-1000 and US Environmental Protection Agency, Washington, DC 20460		12. REPORT DATE September 1985 13. NUMBER OF PAGES 86
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) US Army Engineer Waterways Experiment Station Environmental Laboratory PO Box 631, Vicksburg, Mississippi 39180-0631		15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

Available from National Technical Information Service, 5285 Port Royal Road, Springfield, Virginia 22161.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Studies were conducted to determine the effect of Black Rock Harbor (BRH) dredged material on the survival, growth, reproduction, and population responses of the benthic amphipod, Ampelisca abdita, and the epibenthic shrimp, Mysidopsis bahia. Exposure system designs are described that permit continuous dosing of suspended solids at concentrations of 300 mg/l while proportionally mixing contaminated and reference sediments with reliability and precision.

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20. ABSTRACT (Continued).

Ninety-six-hour LC50 values were 290 mg BRH/1 for M. bahia and 82 mg BRH/1 for A. abdita, with reproducibility and precision being excellent. Chronic exposure indicated that survival was significantly decreased at 150 mg BRH/1 for M. bahia and at 12.5 mg BRH/1 for A. abdita. Growth was a sensitive indicator of stress for A. abdita whose effects were reflected in delays in reproduction in A. abdita but not for M. bahia. Reproduction was the most sensitive chronic response measured for both species. The number of ovigerous females of A. abdita were significantly reduced at 4.0-5.0 mg BRH/1, while the number of young produced in M. bahia was reduced at 32 mg BRH/1. The population parameters, intrinsic rate of growth, and multiplication rate per generation measured for M. bahia and A. abdita were significantly depressed at 42 and 4.7 mg BRH/1 sediments, respectively.

This investigation is the first phase in developing field-verified bioassessment evaluations for the Corps of Engineers and the US Environmental Protection Agency regulatory program for dredged material disposal. This report is not suitable for regulatory purposes; however, appropriate assessment methodologies that are field verified will be available at the conclusion of this program.

PREFACE

This report describes work performed by the U.S. Environmental Protection Agency (EPA), Environmental Research Laboratory, Narragansett, R.I. (ERLN), as part of the Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives Program (Field Verification Program - FVP). This program is sponsored by the Office, Chief of Engineers (OCE), and assigned to the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Mississippi. The OCE Technical Monitors for FVP were Drs. John R. Hall and William L. Klesch. The objective of this interagency program is to field verify existing predictive techniques for evaluating the environmental consequences of dredged material disposal under aquatic, wetland, and upland conditions. The aquatic portion of the FVP study is being conducted by ERLN, with the wetland and upland portion being conducted by WES.

The principal ERLN investigators for this aquatic study and authors of this report were Drs. John H. Gentile and K. John Scott and Ms. Suzanne Lussier and Ms. Michele Redmond. Technical support was provided by Mr. John Sewall and Ms. Ann Kuhn. A special note of recognition is extended to Dr. Clifford Katz for his assistance in designing the appropriate life-cycle graphs, defining the data requirements, and performing the linear matrix analysis. The authors wish to thank Dr. Hal Caswell for providing the theoretical and conceptual framework for using life-cycle graphs with the species used in this study. Appreciation is also extended to Ms. Martha Marcy for the use of her computer program for life-table analysis; Dr. James Heltshe for statistical design; Mr. Jeffery Rosen and Ms. Pam Sherman for data management; and Ms. Catherine Leavene for manuscript preparation.

The EPA Technical Director for the FVP was Dr. John H. Gentile; the Technical Coordinator was Mr. Walter Galloway; and the Project Manager was Mr. Allan Beck.

The study was conducted under the direct WES management of Drs. Thomas M. Dillon and Richard Peddicord and under the general management of Dr. C. Richard Lee, Chief, Contaminant Mobility and Criteria Group; Mr. Donald L. Robey, Chief, Ecosystem Research and Simulation Division; and Dr. John Harrison, Chief, Environmental Laboratory. The FVP coordinator was Mr. Robert L. Lazor, and the EEDP Managers were Mr. Charles C. Calhoun, Jr., and Dr. Robert M. Engler.

COL Tilford C. Creel, CE, and COL Robert C. Lee, CE, were Commanders and Directors of WES during the conduct of the study. COL Allen F. Grum, USA, was Director of WES during the preparation and publication of this report. Mr. Fred R. Brown and Dr. Robert W. Whalin were Technical Directors.

This report should be cited as follows:

> Gentile, J.H., et al. 1985. "Application of Laboratory Population Responses for Evaluating the Effects of Dredged Material," Technical Report D-85-8, prepared by US Environmental Protection Agency, Narragansett, R.I., for the US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

APPLICATION OF LABORATORY POPULATION RESPONSES FOR EVALUATING THE EFFECTS OF DREDGED MATERIAL

PART I: INTRODUCTION

1. The regulation of potential pollutants in aquatic environments is generally based upon toxicological information involving the quantification of a biological response with a pollutant concentration for some finite period of exposure. Traditionally, decisions have been made utilizing acute toxicity data where the exposure period is 96 hrs and the measured biological response is lethality (Sprague 1976). It is well recognized that this type of information while useful is insufficient to identify acceptable nontoxic concentrations that are protective of chronic effects on an organism's growth and reproduction (Mount 1968; Sprague 1971, 1976). This limitation has been addressed by the development of chronic toxicity tests designed to assess pollutant effects on growth, survival, and reproduction over long periods of exposure, often an entire life cycle. Even though chronic toxicity tests measure effects on survival, growth, or reproduction over longer time intervals (Rand 1980; Hansen and Garton 1982), until these endpoints are coupled in a predictive way to populations they are still tests at the individual species level of biological organization.

2. The National Research Council's report on "Testing for the Effects of Chemicals on Ecosystems" (1981) recommends that appropriate and relevant decisions regarding the release of potentially toxic chemicals into the environment be based upon a hierarchy of biological tests with the population being particularly crucial to such an assessment. Relating

short-term toxicant effects to population dynamics poses two problems: one due to time scales and one due to the diversity of toxicant effects. Since population changes take place on a time scale of generations, the direct approach of simply applying a toxicant to a population and observing the resulting dynamics is feasible only for the most rapidly growing organisms. Even when such an approach is possible, it reveals very little about the mechanisms generating the population response. The only way around this difficulty is to infer the population consequences from data on the responses of individual organisms to the toxicant. Since population growth is obviously in some sense the result of the survival, growth, maturation, development, and reproductive rates of individuals, this inferential approach has some promise. Using it, however, requires a determination of which individual characteristics are relevant, and how their population consequences are to be inferred. By adapting demographic techniques originally developed for the study of human populations, population ecologists can calculate a variety of population statistics from data collected on individual organisms (Hutchinson 1978).

3. Classical demography originated in the seventeenth century with the introduction of the life table as a means of integrating age-specific mortality and fecundity. Mathematically, separate measures of survival and fecundity may be linked together and used to estimate the intrinsic growth rate of a population.

This type of analysis has been frequently used in studies of the responses of both natural and laboratory populations to different environmental conditions (Birch 1948; Deevey 1947; Frank 1960; Hutchinson 1978), as well as in studies of human demography (Keyfitz and Flieber 1968). The use of life-tables for assessing the effects of chronic concentrations of pollutants, however, is limited to only a few studies (Marshall 1978; Hummon 1974; Winner and Farrell 1976; Winner et al. 1977; Daniels and Allan 1981; Gentile et al. 1982; Gentile et al. 1983).

4. The unique feature of demographic theory is that it solves the problems of time scale and diversity of effects. It is no longer necessary to follow the dynamics of the population for multiple generations. The estimation of a cohort life table requires only a single generation of observation, and there are techniques to speed up this process in cases where individuals can be aged and marked (Caughley 1977). The Euler equation for r also solves the problem of the diversity of life history effects by specifying exactly how survival and fecundity information must be combined to obtain an index of population growth.

5. There are three primary objectives in the aquatic portion of the EPA/CE Field Verification Program (FVP). The first objective is to demonstrate the applicability of the intrinsic rate of population growth as a measure of effects of dredged material and to determine the degree of variability and reproducibility inherent in the procedure. We are proposing to apply this technique to Mysidopsis bahia, an epibenthic crustacean, and Ampelisca abdita, an infaunal crustacean. This phase of the study, Laboratory Documentation, will involve exposing these organisms throughout their entire life cycle to suspended particulate and solid

phases of Black Rock Harbor (BRH) dredged material and is the subject of this report. The second objective is to field verify the response observed in the laboratory and thus to determine the accuracy of the laboratory prediction. Consequently, this portion of the study is referred to as the Field Verification Phase. The third objective is to determine the degree of correlation of tissue residues resulting from the bioaccumulation of contaminants from dredged material and ecologically significant alterations in organism viability as observed in both the laboratory and the field. The second and third objectives will be combined in a final report as appropriate for the FVP due in September 1985.

PART II: METHODS AND MATERIALS

General Methods

Sediment collection, processing, and storage

6. Two sediment types were utilized to conduct the solid and suspended particulate phase tests of these studies. The reference sediment (REF) was collected from the South reference site in Long Island Sound (40°7.95'N and 72°52.7'W) by a Smith-MacIntyre grab (0.1 m^2), press sieved through a 2-mm sieve, and stored at 4°C until used (Figure 1).

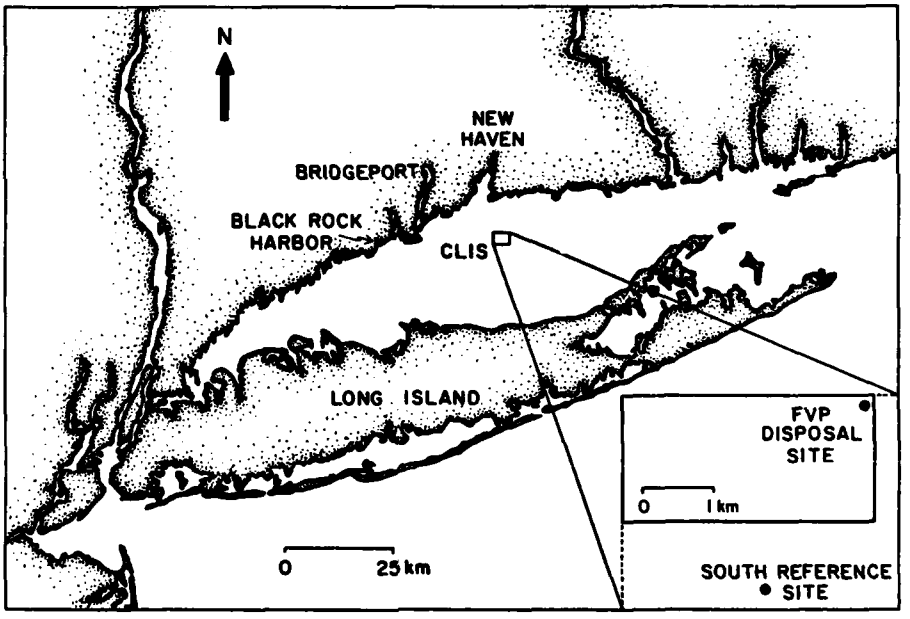


Figure 1. Central Long Island Sound disposal site and South reference site

Prior to dredging, contaminated sediment was collected from Black Rock Harbor (BRH) (41°9'N and 73°13'W) with a gravity box corer (0.1 m^2) to a depth of 1.21 m, thoroughly mixed, press sieved through a 2-mm sieve, and

refrigerated (4°C) until used (Figure 2). In all experiments, sediments were allowed to reach test temperature and mixed prior to use.

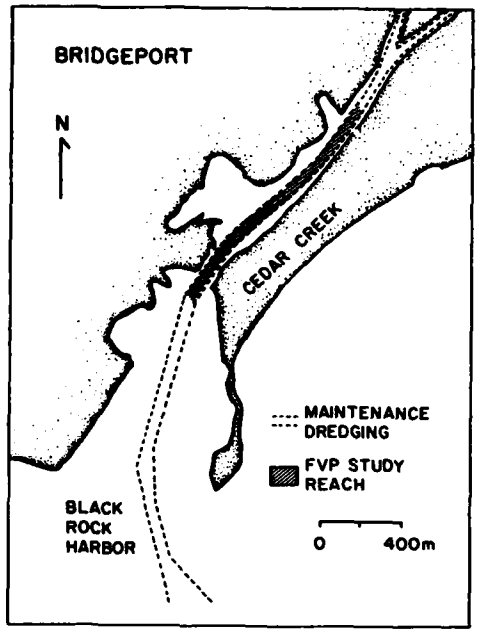


Figure 2. *Black Rock Harbor, Connecticut,* source of dredged material

Experimental design

7. To meet the objectives of this study, a series of initial experiments was conducted to characterize those aspects of exposure analogous to the field exposure conditions at the disposal site. The intent, however, was not to simulate environmental exposure conditions, but rather to include the necessary exposure components, which reflect a level of resuspension containing a mixture of contaminated and uncontaminated sediments.

8. Table 1 summarizes the experimental approach for the initial short-term testing. These studies were conducted to characterize the

Table 1

Experimental Approach for Short-Term Studies

Task Description	Suspended Solids Concentration	Reference Sediment Concentration	BRH Sediment Concentration	Solid Phase
Determine the effect of variable uncontaminated (reference) suspended solids concentrations on measured biological response	Variable over a range of 10-400 mg/l	Variable over a range of 10-400 mg/l	None	Reference sediment
Determine the effect of variable contaminated (BRH) suspended solids over a range of concentrations previously determined (above) to produce no effect on the measured biological response	Variable over a previously determined no-effect range of concentrations	None	Variable over no-effect range from previous experiment	Reference sediment or no-effect concentration of BRH and/or REF mixture
Determine the effect of a range of BRH sediment concentrations coupled with reference sediment to maintain a constant suspended solids concentration	Fixed concentration	Variable in proportion with BRH sediment	Variable in proportion with REF sediment	Reference sediment or same proportion as the suspended solids tests

potential contribution of two interrelated exposure variables, total suspended particulates and proportion of contaminants, on the measured biological responses. The first task was to determine the concentration of uncontaminated total suspended solids that each test species could tolerate. This was necessary to ensure that if biological effects were observed, they were caused by the contaminated dredged material and not by the particle density. Selecting a "no-effect" concentration from this study, a second test was conducted using only BRH-contaminated sediments to determine a contaminant dose-response relationship. The final test in this series was analogous to a field exposure which would have a fixed suspended particulate concentration, with variable amounts of BRH and reference sediments. The highest proportion of BRH sediment represents the center of the disposal mound with decreasing BRH sediment toward the edge where primarily reference sediment would predominate. These experiments form the basic design employed in the long-term chronic exposure from which population responses were determined for the epibenthic mysid shrimp, Mysidopsis bahia, and the infaunal amphipod, Ampelisca abdita.

Statistical analysis

9. Acute toxicity data were analyzed using probits, moving average, binomial, and graphical methods, as appropriate (Stephan 1977). Analysis of variance was used to analyze survival, growth, and reproductive data from all tests. Significant treatment differences were identified from Dunnett's and Tukey-Kramer's pairwise comparison tests (Snedecor and Cochran 1980). In addition, reproductive data for A. abdita were evaluated by analysis of covariance to account for differences in female size.

Sediment dosing system

10. Implementation of the experimental design required the construction of two identical sediment dosing systems to simultaneously provide either BRH or REF material as suspended sediment. The dosing systems (Figure 3) consisted of conical-shaped slurry reservoirs placed

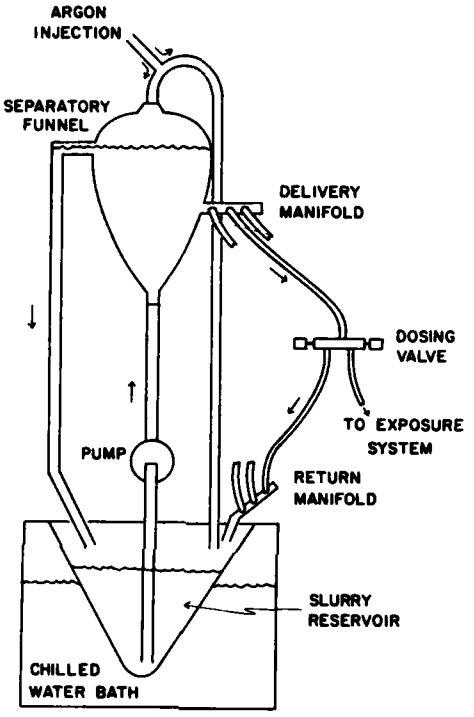


Figure 3. Sediment dosing system with chilled water bath and argon gas supply

in a chilled fiberglass chamber, a diaphragm pump, a 4-l separatory funnel, and several return loops that directed the particulate slurry through dosing valves. The slurry reservoirs (40 cm diam x 55 cm high) contained 40 l of slurry comprised of 37.7 l of filtered seawater and 2.3 l of either BRH or REF sediment. The fiberglass chamber (94 cm x 61 cm x 79 cm high) was maintained between 4° and 10°C using an externally chilled water source. (The slurry was chilled to minimize microbial degradation during the test.) Polypropylene pipes (3.8 cm diam) placed at the bottom

of the reservoir cones were connected to the diaphragm pumps (16 to 40 l/min capacity) that had Teflon® diaphragms. These pumps were used to circulate the slurry but minimize abrasion so that the physical properties and particle sizes of the material remained as unchanged as possible.

11. The separatory funnel was connected to the pump and returned to the reservoir by polypropylene pipes. The separatory funnel served two functions: (a) to ensure that a constant head pressure was provided by the overflow, and (b) to serve as a connection for the manifold located 4 cm below the constant head level. The manifold served to distribute the slurry by directing a portion of the flow from the funnel (through 6-mm inside diameter polypropylene tubes) through the Teflon® dosing valves (Figure 3) and back to the reservoir. At the dosing valves, the slurry was mixed with seawater to provide the desired concentrations for the toxicity tests. Argon gas was provided at the rate of 200 ml/min to the reservoir and separatory funnel to minimize oxidation of the sediment/seawater slurry. Narragansett Bay seawater filtered (to 15 u) through sand filters was used. The dosing valves were controlled by a microprocessor that was connected to a transmissometer (Figure 4) in the preliminary toxicity studies. The microprocessor can be programmed to deliver a pulse with a duration of 0.1 sec up to continuous pulse delivery and at intervals from once every second to once every hour.

Test Methods for M. bahia

Culture

12. Mysidopsis bahia is an epibenthic crustacean important in estuarine and marine food webs. The life cycle of this species lends itself to population studies for two reasons: the life cycle is short, being

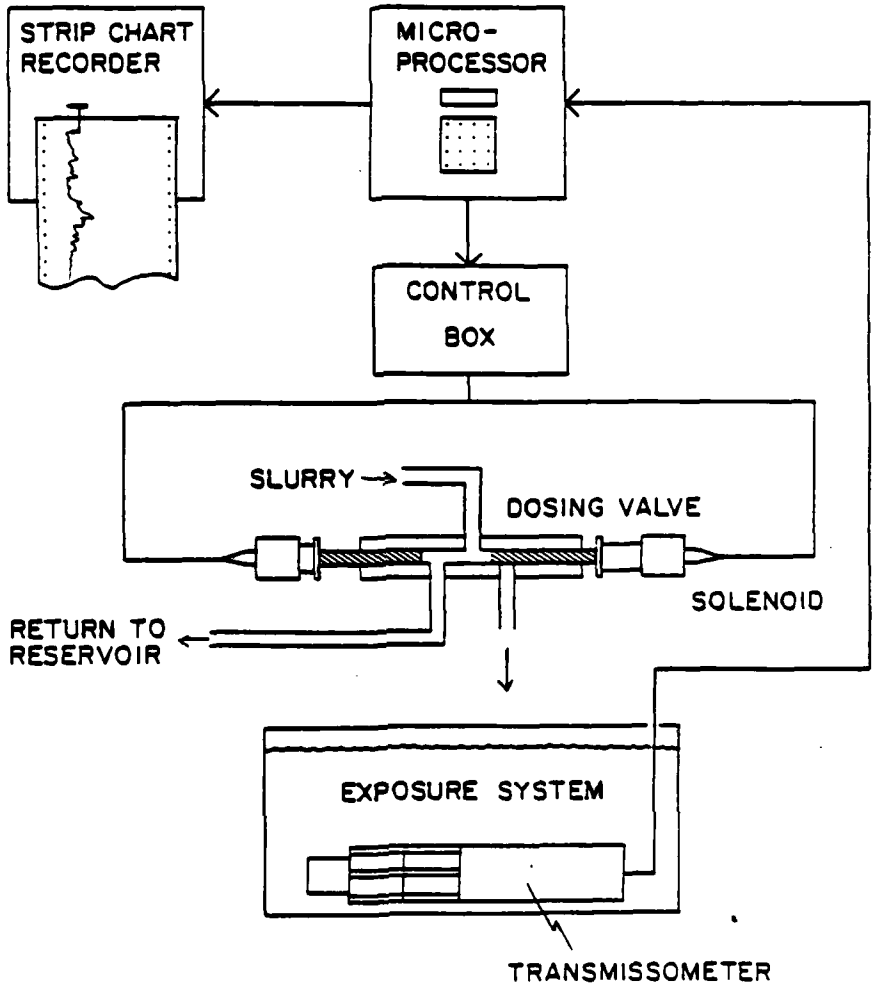


Figure 4. Suspended sediment feedback control loop and strip chart recorder

completed in 25 days at 25°C, and because the young are carried in a brood pouch, reproductive processes can be easily monitored and quantified.

13. Mysidopsis bahia were cultured in flow-through 76-l glass aquaria continuously supplied with filtered (15 u) natural seawater at a salinity of 28 ± 2 ppt and 25 ± 2°C. A photoperiod of 14L:10D was maintained by microprocessor to simulate dawn and dusk. Flow rates of 200 ml/min provided a 99 percent volume exchange every 24 hr. Sub-gravel

filters were used to provide aeration and a feeding current with a 25.4-mm-deep dolomite substrate.

14. Cultures were fed continuously, ad libitum, with 24-hr posthatch Artemia salina (reference strain, Sorgeloos 1981) at a rate of 7×10^4 nauplii/day for each 76-l culture.

Exposure system design

15. The suspended sediment proportional diluter (Figure 5) was

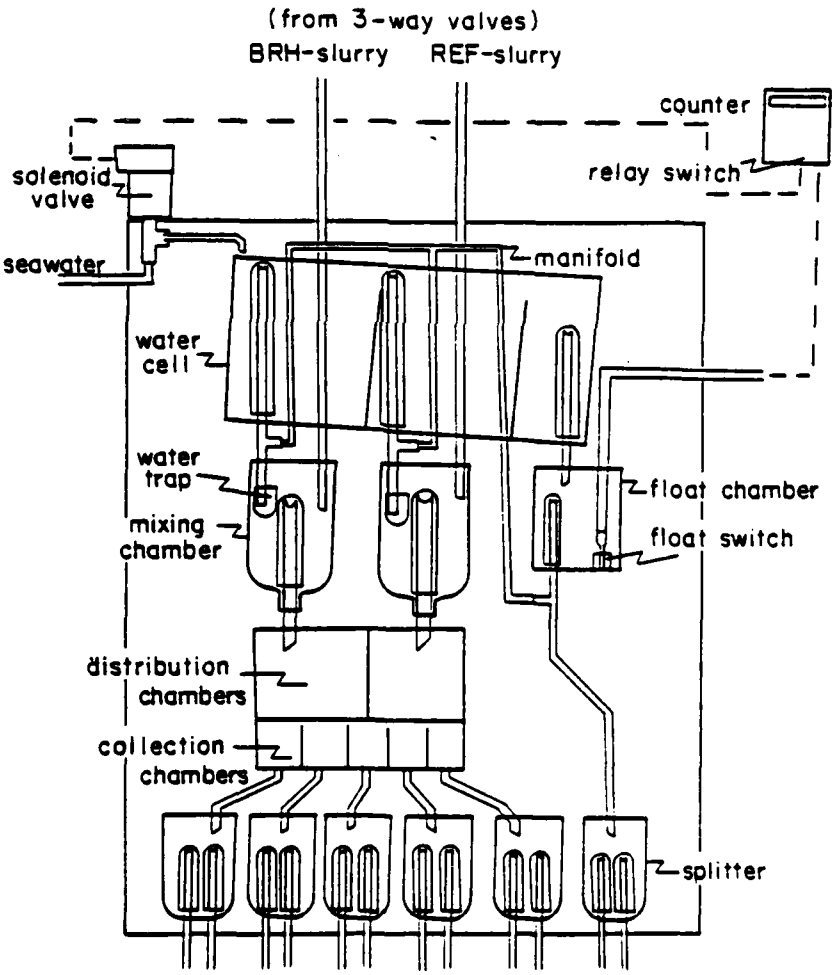


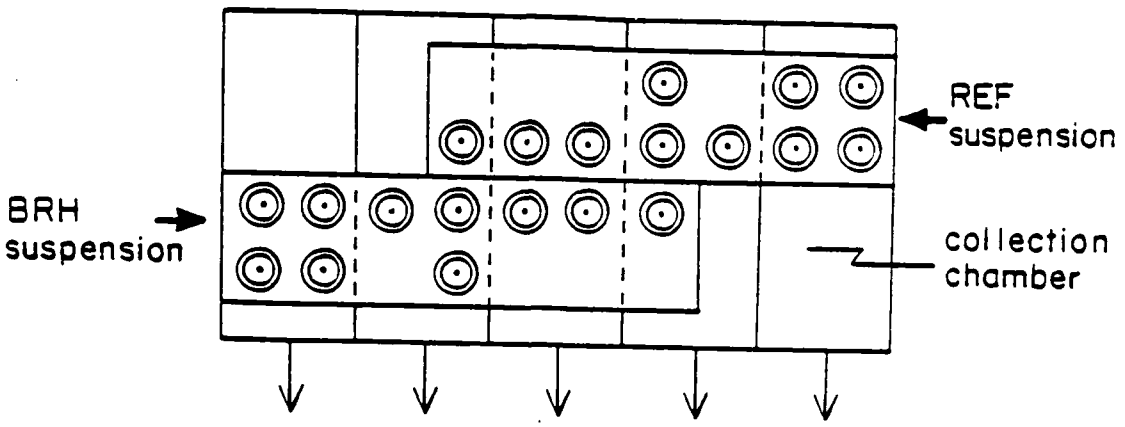
Figure 5. Suspended sediment proportional diluter for M. bahia

designed to mix small quantities of concentrated slurries of suspended marine sediments (10-20 g/l from the sediment dosing system with seawater to produce two dilute sediment suspensions in the mg/l range. It then combines slurries of different types (e.g., REF and BRH sediment suspensions) proportionally to maintain the same concentration of suspended particulates with different ratios of the two sediments. It can also function with one sediment diluted with seawater to produce a range of suspended solids concentrations. The diluter delivers two replicates for each of five treatments and a seawater control.

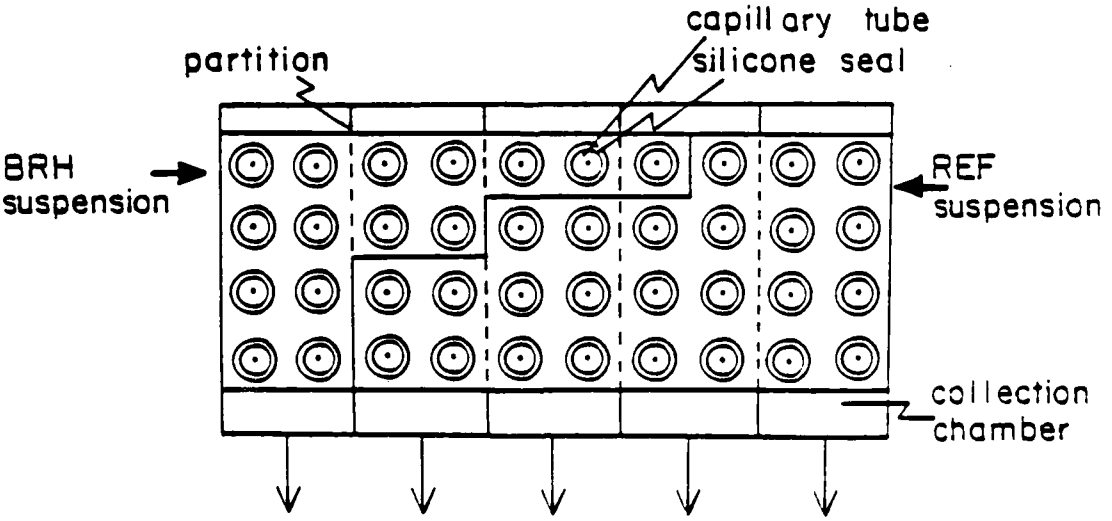
16. The distribution chamber (Figure 6) is partitioned into two cells and works by dividing the two volumes of suspension among a number of capillary tubes thereby delivering different volumes of each suspension to the five splitters depending upon the number of capillary tubes draining into them.

17. Each of the cells of the collection chamber drains its contents of a proportioned suspension into one of the five splitters (Figure 5). Each splitter contains two self-priming siphons, each of which takes half the volume of suspension and delivers these portions of suspension to the replicate animal exposure chambers.

18. In summary, the diluter system employed for quantitatively delivering suspended solids to the population tests consists of four tiered components. The first tier consists of the water cells which measure a predetermined volume of seawater and three-way valves which deliver microprocessor quantities of slurry from the sediment dosing system. The second tier consists of the mixing chambers which combine the slurry and the seawater to produce the desired concentrations of suspended sediments.



a. Short-term studies



b. Long-term studies

Figure 6. Proportional diluter distribution chamber configuration for M. bahia

The third tier includes the distribution and collection chambers where the REF and BRH slurries are proportionally mixed to produce the five treatment concentrations. The fourth and final tier includes the splitters where each treatment concentration is divided into two replicates and delivered to the exposure chambers.

Exposure chamber design

19. The animal exposure chambers (Figure 7) consist of wide-mouth

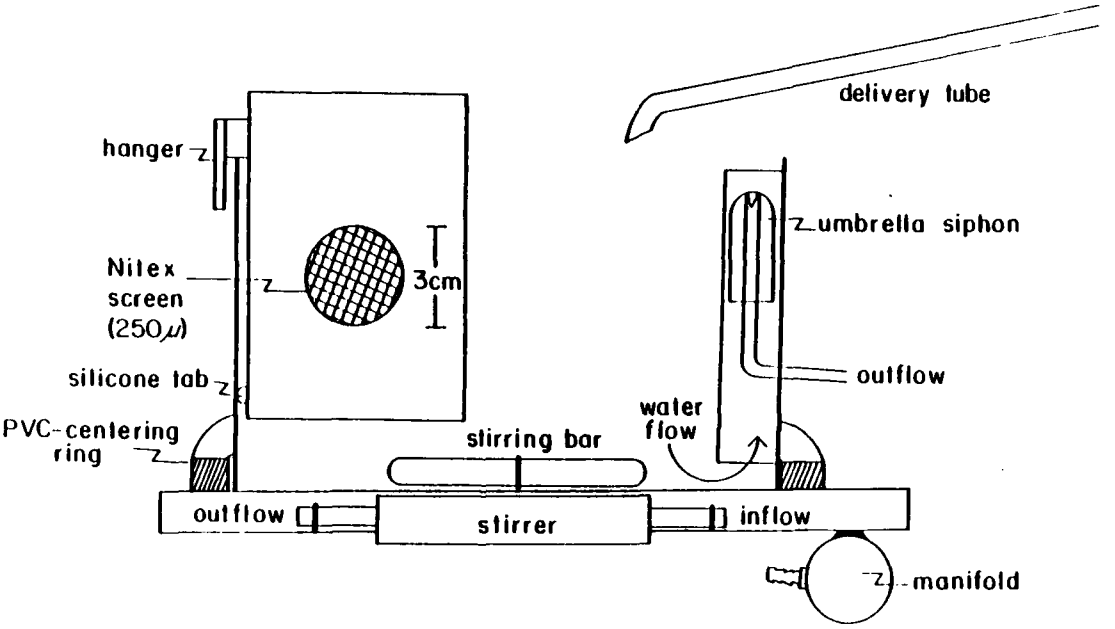


Figure 7. Exposure chamber design for M. bahia

glass jars, 8.5 cm high x 7.5 cm wide with two 3.2-cm holes drilled in opposite sides and centered 4.2 cm from the bottom. These holes are screened with 250-u Nitex® screen netting glued with clear silicone sealant to the inside to eliminate a ridge where test organisms could be trapped. Four glass cups are suspended in each of twelve crystallizing dishes (190 mm x 100 mm) by a small glass tab glued to the top rim. To maintain the vertical position of each jar, a small drop of silicone

sealant is placed at the bottom side of each cup just under the tab. Each treatment (six total) consists of two replicate crystallizing dishes; each replicate contains four observation cups for a total of eight per treatment.

20. Suspended sediment from the diluter flows through glass delivery tubes which empty into the center of each exposure chamber at the surface. Drainage from each chamber is accomplished by an enclosed umbrella siphon. When a chamber fills to about 1 cm from the top, the siphon is primed and drains about one third of the dish. This excursion of the water level ensures proper water circulation through the exposure cups. The enclosure around the umbrella siphon forces water outflow to be from the bottom of the water column. Inflow at the top and outflow from the bottom help eliminate the potential for vertical size partitioning of suspended particulates.

21. The twelve exposure chambers are held in position on a fiberglass grating in a temperature-controlled water bath by plastic rings 3.2 cm high by 20.3 cm diameter. These rings center the dishes over water-driven magnetic stirrers (embedded in the grating) which are used to spin 6.4-cm x 0.64-cm Teflon®-coated spin bars in the dishes to keep the sediment in suspension. The magnetic stirrers are driven by a manifold supplied with deionized water from the temperature control bath by Teel, epoxy-magnetic submersible pumps, Model #1P681A. This also serves to circulate the bath to ensure uniform temperature.

22. The position of the dishes in the bath is randomized. Bath temperature is maintained with Teflon® heat-exchanging coils under microprocessor control. Microprocessor controlled lighting is designed to simulate natural day/night cycles with the florescent lights growing

gradually brighter at dawn and dimmer at dusk. The construction materials that contact the test solutions or the animals are glass, silicone rubber (to cement and seal), Nitex® screen, and Teflon®.

System monitoring

23. Bioassays were conducted at a temperature of 25° ± 2°C, salinity of 28° ± 2 ppt, and illumination of 1000 lux on a 14L:10D cycle. Dissolved oxygen was measured daily with a YSI dissolved oxygen probe. Three times each week suspended particulate concentrations from the control and exposure chambers were analyzed by dry weight determination conducted according to Standard Methods (American Public Health Association (APHA) 1976) with the following modifications: the filters were washed with a 50-ml aliquot of deionized water before sample filtration, and then with three 10-ml rinses of deionized water immediately after sample filtration to remove salt.

Biological design

24. Tests were initiated with 24- to 30-hr postrelease juvenile M. bahia which were randomly distributed into three exposure cups of five animals each in two replicate exposure chambers randomized among the exposure concentrations. In the second chronic test, an individual pair of males and females was placed in each exposure cup. Each cup was fed 24-hr posthatch reference Artemia daily. Each cup was removed and monitored daily for mortality in the acute tests and both mortality and reproduction in the life cycle tests. Test organisms could be seen most easily over the sediment by shining an intense beam of light horizontally from the side of the test cup at the sediment/water interface.

25. For the solid phase portion of the test exposure, sediment at

room temperature from the reference site and from Black Rock Harbor was stirred and shaken vigorously before being mixed to obtain the desired percentage of BRH sediment for each treatment. The sediment was added to the exposure cups to a depth of 2 cm and allowed to equilibrate for 1 hr in the bioassay system in flowing test solution before animals were introduced.

Short-term tests

26. There are two variables in these tests which can be responsible for producing a biological response: suspended solids concentration and contaminant concentration. In order to ensure that the reported responses were the result of BRH contaminants, the sensitivity of M. bahia to a range of suspended solids concentrations had to be determined using a relatively uncontaminated reference sediment. This was followed by assessing effects of BRH contaminants within the range of suspended solids determined acceptable, and then combining REF- and BRH-contaminated sediments to provide a constant suspended solids concentration while varying the BRH contaminant concentration.

27. Preliminary 96-hr flow-through range-finding tests were conducted to determine the appropriate suspended particulate concentration for the definitive acute and chronic life cycle assays. The first test used only reference suspended particulates to determine the concentration of suspended particulate which would produce no effect on M. bahia juveniles. Previous tests (Rogerson et al. 1984) had determined that 25 mg/l of reference suspended particulates had no effect on the survival of juvenile M. bahia. Therefore, the treatments chosen for the first assay were 102, 76.5, 51, and 25.5 mg/l reference suspended particulates over a

solid phase of reference sediment with a seawater control (no sediment).

28. Subsequent preliminary tests used only BRH suspended material to determine the concentration which would produce an effect on M. bahia juveniles in a 96-hr exposure period. The treatments chosen were: 200, 150, 100, and 50 mg/l BRH suspended particulate over a solid phase of BRH sediment with a seawater control (no sediment). The experiment was repeated in order to obtain accurate and consistent dosing and mixing of the suspended sediment.

29. The definitive acute test used a 300 mg/l suspended particulate concentration which consisted of individual or combinations of reference and BRH material with a matching solid phase sediment in the following percentages: 100%BRH/0%REF, 75%BRH/25%REF, 50%BRH/50%REF, 25%BRH/75%REF, 0%BRH/100%REF, and a seawater control (no sediment).

Long-term tests

30. Replicate life cycle tests were conducted to assess the effects of suspended particulate exposures of percentage combinations of BRH and REF sediments on the survival, growth, and reproduction of M. bahia. To determine which response parameters were the best indicators of stress, the developmental stages related to reproductive functions were examined in detail. Specifically, time to sexual maturity, appearance of embryos in brood sacs, and time to first brood release were determined for all treatments. Two approaches were used to quantify productivity. First, the number of juveniles released per female was determined for each treatment. From this the fecundity and reproductive variability between females could be determined. A second more integrative approach was to determine the total productivity for each treatment. To estimate total

productivity, it was necessary to normalize differences in the number of females available per exposure concentration. The number of available female reproductive days (AFRD) was calculated by multiplying the number of sexually mature females by the number of days each survived during the reproductive period. Productivity was estimated by calculating the following ratio: number of young produced per AFRD.

31. Life tables were used to calculate age-specific survivorship for controls and exposure concentrations (Birch 1948). Starting with an initial number of newborn females, the percentage of this initial population alive at every age was calculated by sequentially subtracting the percentage of deaths of each age. The fraction surviving at age x gives the probability that an average newborn will survive to that age (which is designated l_x).

32. Age-specific fecundity m_x is the number of female offspring produced by a female of age x during a designated age period. Specifically, the types of data collected included the number of juveniles released per day and the number of sexually mature females.

33. Age-specific survivorship l_x and fecundity m_x were used to calculate, by successive approximation, the intrinsic rate of population growth r from the Euler equation:

$$l_x m_x e^{-rx} = 1$$

Where

l_x = the probability of a female surviving to age x
m_x = the number of female offspring per female of age x produced during the interval x to x + 1
e = the natural logarithm
r = the intrinsic rate of population growth
x = the age class

Test Methods for A. abdita

Collection

34. Ampelisca abdita is a tube-dwelling amphipod which constructs a soft, upright, membranous tube 3 to 4 cm long in surface sediments. It occurs from Maine to Louisiana from the intertidal zone to depths of 60 m. Ampelisca is a particle feeder, ingesting either surface-deposited particles or particles in suspension. These amphipods brood their young after mating and fertilization occurs in the water column. The egg-carrying females return to the sediment and young are released into the surrounding sediment. They are reproductively active at 5 mm and grow to a maximum length of 7 to 9 mm. Available information indicates that each female will reproduce only once.

35. Ampelisca were collected from tidal flats in Narrow River, a small estuary flowing into Narragansett Bay, R.I. The sediment containing the amphipods was immediately transported to the laboratory. The sediment was sieved through a 0.5-mm screen and the Ampelisca collected by flotation from the air/water interface. Since collection temperatures were close to the experimental temperatures, no acclimation or holding was necessary for the first long-term chronic test. For the second long-term test, amphipods were collected from Narrow River and acclimated at 1°C/day up to 20°C. During acclimation, these animals were fed, ad libidum, laboratory-cultured Phaeodactylum tricornutum. After the animals were sieved from the sediment, they were sorted by size and randomly placed in 100-ml plastic beakers for subsequent distribution to the appropriate exposure chamber.

Exposure system design

36. The composite dosing system supplied suspensions of REF and BRH sediments as previously described (Figure 3). The appropriate amount of material was delivered to the amphipod dosing system via a three-way valve which was controlled by a microprocessor. For the acute tests, each slurry was delivered to a mixing chamber (glass 4-l reagent bottle) where it was initially diluted with seawater at a preset flow rate (right side of Figure 8). The diluted suspension then passed from the bottom of

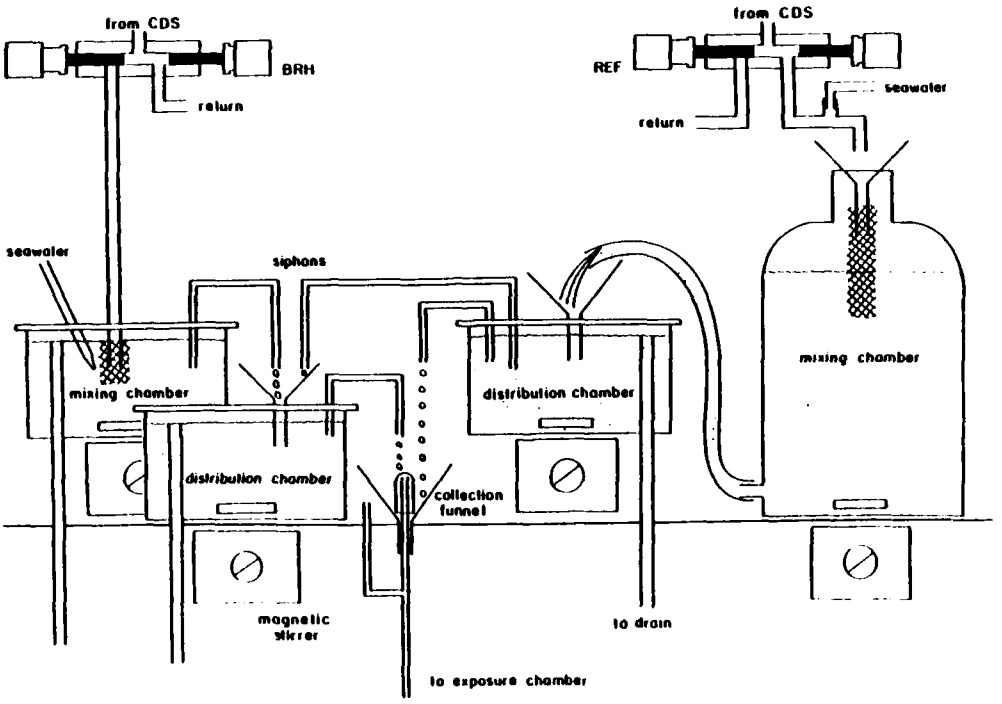


Figure 8. Suspended sediment proportional diluter system used for short- and long-term studies with A. abdita

the bottle to a distribution chamber (17 cm diam x 9 cm high) fitted with a standpipe to maintain a constant water level. The suspension then flowed through a siphon at a flow rate controlled by head pressure to a collection funnel which then distributed the material to the exposure

system. Each collection funnel had an umbrella siphon which acted as a flow accelerator to rapidly mix materials collected in the funnels. For the short-term acute tests, the mixing and distribution chambers with siphons were set up in duplicate and, using either REF or BRH sediment, one mixing chamber setup was dosed with the test sediment and the other was fed filtered seawater only.

37. To achieve a test concentration of 100 mg/l, where the mixing chamber concentration was 200 mg/l, the sediment mixing chamber siphon and the seawater mixing chamber siphon would be set at equal flow rates, e.g., 40 ml/min of each. Likewise, to achieve 50 mg/l of the test sediment, the seawater-siphon flow rate would be three times the suspension flow rate, e.g., 60 ml/min seawater and 20 ml/min sediment suspension. For the chronic tests, a constant suspended particulate density was desired, where BRH sediments were diluted with REF sediment instead of with seawater. To achieve this, REF sediment was dosed to one mixing chamber and BRH was dosed to the other so that there was an equal particle concentration in each distribution chamber. As for the seawater dilutions, equal siphon flow rates yield a 50-percent BRH concentration, a 3 to 1 REF to BRH yields a 25-percent BRH concentration, and so on.

38. The limiting factor of this dosing design is the minimum flow rate, 10 ml/min, that can readily be attained from the siphons. If each exposure chamber is to receive 20 ml/min, the total flow to the collection funnel is 40 ml/min; or for a 25-percent BRH concentration, 30 ml/min of REF sediment and 10 ml/min of BRH sediment. At 25-percent BRH, toxicity levels were high, so a modified design was implemented (left side of Figure 8) where the BRH concentration in the distribution chamber was set

to 20 percent by feeding that chamber 100 ml/min of REF and 25 ml/min of BRH at equal particle densities. The BRH concentrations of 10 and 5 percent were subsequently achieved as described above.

39. The collection funnels fed the exposure chambers through a polypropylene tube which was fitted with a polypropylene tee with 5-mm outside diameter glass elbows to split the flow to two exposure systems.

Exposure chamber design

40. The exposure chambers for this study were of two types, an acute test chamber and a chronic test chamber. The acute chamber consists of a capped glass jar with screened holes in the sides (Figure 9). Reference

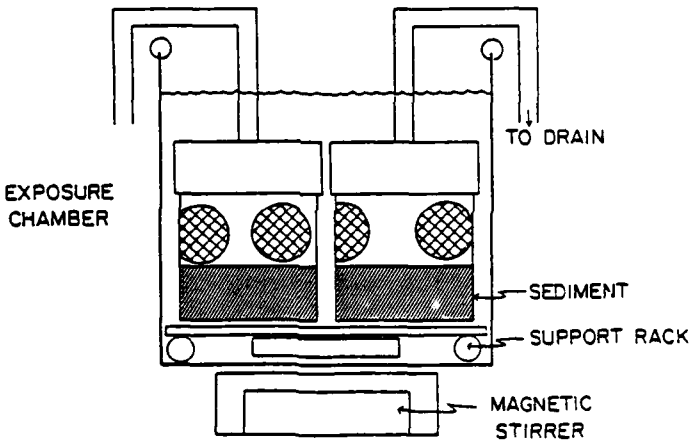


Figure 9. Acute exposure chamber for short-term tests with A. abdita

sediment was filled to the bottom of the holes. The test suspension flowed from the "funnel-tubing-tee" delivery system to a gallon jar containing two exposure chambers. The suspension passed through the screens and out of the cap via a glass elbow to a water trap and then to drain. The sediment was kept in suspension by a magnetic stirrer.

41. The chronic test chamber was a gallon jar filled with 0.75 l of REF sediment (5 cm) (Figure 10). The test suspension was fed to the gallon jar from the tee and was maintained in suspension by aeration. The suspension was removed from the jar by a siphon which collected material from just above the sediment surface. The effluent entered a water trap with a screened standpipe which permitted monitoring of mating activity.

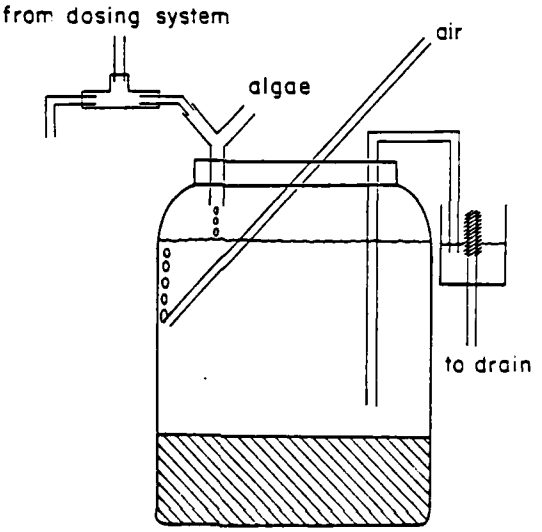


Figure 10. Chronic exposure chamber for long-term tests with A. abdita

42. The flow rate to each gallon jar was 40 ml/min for the acute tests and was reduced to 20 ml/min for the chronic tests in order to maximize the concentration of food supply. The diatom Phaeodactylum tricornutum was cultured (Guillard and Ryther 1962) and delivered to each gallon jar at 1 ml/min using a Harvard peristaltic pump.

System monitoring

43. The total suspended solids concentration in the exposure system was monitored using total dry weight as milligrams per liter. One-hundred-milliliter samples were taken biweekly from each gallon jar using a free-standing siphon to minimize disturbance.

Biological design

44. As the Ampelisca were being sorted for distribution to each test chamber, an extra container with the requisite number of Ampelisca was set aside for initial size measurements. In both the short-term test and the preliminary chronic test, juvenile (immature) amphipods were used. In the two chronic tests, the dosing and exposure system design were identical; however, the life stages that were used at the start of the test were different. In the first test 100 subadult amphipods were used in four replicates of each of three treatments, while in the second test 15 egg-bearing females were used in four replicates of each of three treatments. In the second chronic test, the number of eggs of each of 30 females were enumerated from an initial sample. The first chronic test lasted 45 days with an interim harvesting of two replicates from each treatment at 28 days. The second test ran for 58 days with an interim harvesting of two replicates at 32 days.

45. During the acute tests, each container was checked daily and the number dead were enumerated and removed. In the chronic tests, the overflow water trap was checked daily and the amphipods were enumerated and sexed when possible. At the end of each test, all containers were sieved and the amphipods were enumerated. Any animals not accounted for (either removed earlier or recovered on sieving) were considered dead.

46. In addition, animals from the two chronic tests were sexed and measured by use of a computerized digitizer and camera lucida device. The data collected at the interim sampling period in both chronic tests included: survival, mean size, and proportion of amphipods that were mature. In addition, the number of eggs from each ovigerous female were enumerated

and sized. At the termination of the test, these data were collected along with the number of young amphipods produced per chamber. In Chronic Test 2, any sex class having greater than 60 amphipods was subsampled for size measurements using a Folsom plankton splitter which randomly splits the sample in half. A minimum sample size of 30 was selected by examining the size variability that was found in Chronic Test 1 and by determining the sample number necessary to observe a treatment effect at the 5-percent probability level.

Short-term tests

47. A 96-hr range-finding test was run for REF and BRH sediments individually. The nominal concentrations for each run were 200, 100, 50, 25 mg/l suspended sediment, and a seawater control. The solid phase treatment for all tests described in this report was REF sediment. For each treatment, there was a comparison of the acute and chronic test chambers; therefore, each treatment had one chronic chamber with 50 Ampelisca and one gallon jar with two acute chambers, with 25 Ampelisca/ chamber.

Long-term tests

48. A preliminary chronic test was run for 18 days at a constant particle load of 50 mg/l, a seawater control, and concentrations of BRH suspended sediment of 75, 50, 25, and 0 percent. There were two chronic replicates per chronic treatment, each replicate containing 100 Ampelisca. The nominal particle load for the two definitive chronic tests was 50 mg/l and, due to high mortalities in the preliminary chronic test, the BRH concentrations were reduced to 10 and 5 percent with REF sediment. There were four containers/treatment; two replicates were sieved after 28 and 32 days

and the other two replicates were sieved at 45 and 58 days, respectively, which coincided with the production of young in the controls.

PART III: RESULTS AND DISCUSSION

Mysidopsis bahia

Preliminary tests

49. Prelimninary tests were conducted to determine the effect of contaminated (BRH) and uncontaminated (REF) suspended solids concentrations on the survival of *M. bahia*. A 4-day exposure to REF suspended solids concentrations of 300 mg/l did not cause mortality. A similar exposure to BRH-contaminated suspended solids resulted in 73-percent mortality, and a 96-hr LC50 for BRH suspended solids of 245 mg/l. Since a concentration of 300 mg/l of REF suspended solids produced no observable acute effects, this concentration of total suspended solids was used in the design of the definitive short-term tests. In the latter tests, the total suspended solids concentration was held constant while the contaminant concentration was varied by altering the proportions of BRH and REF sediments in each treatment.

Short-term tests

50. The results of three definitive short-term tests are summarized in Table 2. The suspended solids diluter developed for these studies provided a consistent and reproducible exposure environment for all treatment combinations at a nominal suspended solids concentration of 300 mg/l. Analysis of variance detected no statistically significant differences ($P = 0.05$) in total suspended solids concentrations between treatments within an experiment, nor within specific treatments between experiments.

51. Dissolved oxygen concentrations were within an acceptable range (4.9 to 7.0 mg/l) for all experiments and treatments. In Experiments 1 and 2, dissolved oxygen decreased with increasing percentages of BRH sediment.

Table 2

Results of Definitive Short-Term 96-hr Acute Toxicity Tests with Juvenile M. bahia

Treatment	Suspended Solids mg/l	Dissolved Oxygen mg/l	Temperature °C	Salinity mg/kg	Percent Mortality	LC50 mgBRH/l
			Experiment 1			
100%REF/0%BRH	266.8 ± 52.1	6.3 ± 0.2	25	30.0 ± 0	0.0	>358
75%REF/25%BRH	310.2 ± 49.6	5.7 ± 0.4			10.0	
50%REF/50%BRH	337.4 ± 36.5	5.4 ± 0.5			16.7	
25%REF/75%BRH	358.0 ± 92.6	4.9 ± 0.6			10.0	
0%REF/100%BRH	358.0 ± 26.7	4.0 ± 0.5			33.3	
			Experiment 2			
100%REF/0%BRH	297.3 ± 30.2	6.4 ± 0.8	25	30.0 ± 0	2.0	290
75%REF/25%BRH	260.2 ± 39.6	6.4 ± 0.8			5.0	
50%REF/50%BRH	309.7 ± 51.7	5.7 ± 0.6			2.0	
25%REF/75%BRH	366.8 ± 161.7	5.3 ± 0.6			31.0	
0%REF/100%BRH	311.5 ± 30.2	5.2 ± 0.4			81.0	
			Experiment 3			
100%REF/0%BRH	193.5 ± 64.5	6.7 ± 0.1	25	30.0 ± 0	0.0	410
75%REF/25%BRH	348.6 ± 106.5	6.6 ± 0.1			6.0	
50%REF/50%BRH	202.4 ± 23.5	6.7 ± 0.1			6.0	
25%REF/75%BRH	209.4 ± 29.5	6.7 ± 0.1			18.0	
0%REF/100%BRH	396.4 ± 126.1	6.6 ± 0.1			48.0	

52. Test temperatures and salinities of 25°C and 30 mg/kg were constant throughout all experiments.

53. The acute mortality patterns were similar in each experiment (Table 2). The ninety-six hour mortalities at approximately 300 mg/l total solids and 100% BRH are 33, 81, and 48 percent. This variability in acute mortalities is similar to ranges reported in intercalibration studies with M. bahia (Schimmel 1981; McKenney 1982). The estimated 96-hr LC50's for three definitive tests are >358, 290, and 410 mg BRH/l respectively. These results illustrate good reproducibility for the acute toxicity test method with M. bahia using BRH dredged material.

54. The acute mortality data can be utilized to examine the possible interactions between BRH contaminant-induced responses and the synergistic and antagonistic interactions resulting from the presence of reference sediment. In the preliminary test, where only BRH sediment was used, 16-percent mortality occurred at a concentration of 150 mg/l. In the three definitive tests, the 50%REF/50%BRH treatment at 300 mg/l total solids provides an analogous 150-mg BRH/l exposure, but in this case there is an equivalent amount of reference sediment present. The mortalities in the definitive tests were 16.7, 2.0, and 6.0 percent, respectively. Although these values are at the lower end of the dose-response curve and, consequently, subject to greater inherent variability, they do not indicate a strong interaction between the sediment types and acute mortality.

55. As previously stated, the principal objectives of the Laboratory Documentation of this program are to determine the applicability of the short-term acute tests with M. bahia for measuring the effects with dredged material, determine the sensitivity of the method, and assess the

reproducibility of the test method using BRH sediments. The results discussed above demonstrate that this method works well with dredged material. To assess the method's reproducibility and sensitivity, the following statistical analyses were conducted.

56. The first hypothesis examined was to determine if there were differences in the acute mortality patterns in the three experiments as a function of the total suspended solids exposure concentrations. Analysis of variance results indicate that there were no significant differences ($P = 0.05$) between mortality and suspended solids concentration within and between the experiments. The second hypothesis was to determine if mortality patterns were related to the BRH sediment treatment combinations. Statistically significant differences ($P<0.05$) in mortality patterns were found with treatments. In order to address the question of experimental reproducibility, a two-way analysis of variance of experiments and treatment combinations was conducted. The results of this analysis indicated that there were no significant differences in the mortality patterns between the experiments. Since significant differences were detected between treatments, we were able to address the issue of sensitivity. Treatment differences were analyzed by Tukey-Kramer's pairwise comparison test using the 100%REF/0%BRH as the control. The results of this comparison are summarized in Table 3. These results indicate that there were statistically significant differences ($P<0.05$) in mortality when the BRH sediment concentration reached 25%REF/75%BRH which is equivalent to 225 mg/l BRH sediment.

57. In summary, the short-term test with juvenile M. bahia performed satisfactorily when applied to dredged material. The reproducibility of this method was acceptable as judged from the range of estimated 96-hr LC50

values and from statistical analyses of mortality data (Table 2).

Table 3

Analysis of Treatment Differences for
Short-Term Acute Tests with Juvenile M. bahia

Treatment	N	Mortality*	Grouping**
100%REF/0%BRH	6	0.0375	A
75%REF/25%BRH	6	0.2379	A
50%REF/50%BRH	6	0.2327	A
25%REF/75%BRH	6	0.4607	B
0%REF/100%BRH	6	0.8333	C

*Arcsine transformation of mortality data.
**Same letters are not significantly different.

Long-term tests

58. Replicate long-term tests were conducted to determine the effect of BRH sediment on the survival, growth, and reproduction of M. bahia exposed throughout an entire life cycle. Data on survival and reproduction were then used to calculate the intrinsic rate of population growth and other population parameters.

59. The chronic dosing and exposure system proved to be reliable when operated at a nominal total suspended solids concentration of up to 300 mg/l for 30 days. The precision of the dosing system was within 15 percent at 300 mg/l total solids in the first chronic experiment and 22 percent at 200 mg/l total solids in the second chronic experiment. The mean dissolved oxygen concentration ranged from 6.3 to 7.0 mg/l and 6.3 to 6.8 mg/l for Experiments 1 and 2, respectively. Dissolved oxygen decreased slightly with increasing concentrations of BRH sediments. Both long-term tests were conducted at 25°C and 30 $\pm$ 0.5 mg/kg salinity.

60. The growth and reproductive results of the long-term chronic tests are summarized in Table 4. The growth of M. bahia, as measured by dry weight, was similar in all the long-term chronic experiments. Analysis of variance indicated that growth did not differ significantly (P = 0.05) between experiments nor were there significant differences resulting from exposure to BRH sediments in any of the experiments.

Table 4

Growth and Reproductive Results for Chronic Tests with M. bahia

BRH-sediment mg/l	Growth mg dry wt.	Sexual maturity days	Initial reproduction days	Young per AFRD	AFRD EC50 mg/l
		Experiment 1			
Zero*	0.61 ± 0.08	13	19	0.12	125
65	0.63 ± 0.16	13	20	0.17	
155	0.56 ± 0.19	19	23	0.03	
275	-----	19	--	----	
311	-----	--	--	----	
		Experiment 2			
Zero*	0.69 ± 0.09	12	19	0.20	47
87	0.54 ± 0.08	15	22	0.01	
156	0.52 ± 0.12	20	24	----	
396	-----	--	--	----	
		Experiment 3			
Zero*	0.57 ± 0.02	11	16	0.31	32
43	0.56 ± 0.02	12	18	0.10**	
95	0.49 ± 0.02	16	22	0.01**	

* REF Sediment Control
** Significantly different from REF sediment control (P = 0.05)

61. The three measures of reproductive function quantified in the long-term chronic tests were the times to sexual maturity and initial reproduction, and the number of young produced per available reproductive

day (AFRD) (Table 4). The times to reach sexual maturity in the REF controls were 13, 12, and 11 days, respectively, for Experiments 1, 2, and 3. In all experiments, the time to reach sexual maturity increased with increasing concentrations of BRH sediments. If we assume that the range in times to sexual maturity reported for the controls is indicative natural variability, then the 6-day delay at 155 mg/l in Experiment 1, the 8-day delay at 156 mg/l in Experiment 2, and the 5-day delay at 95 mg/l in Experiment 3 represent significant deviations from the control variability. No estimates of sexual maturation times were reported for the 311- and 396-mg/l treatments because of complete female mortality.

62. The times to reach initial reproduction in the REF were 19, 19, and 16 days, respectively, for Experiments 1, 2, and 3. The time to initial reproduction increased with increasing concentrations of BRH sediment for those concentrations where reproduction occurred. Major deviations from the controls occurred at 155, 156, and 95 mg/l BRH sediment in Experiments 1, 2, and 3, respectively. The brood duration for the REF sediment ranged from 5-7 days. At the 155-mg/l treatment in Experiment 1, the brood duration was 4 days resulting in only 3 young produced, while in the 156-mg/l treatment in Experiment 2, the brood duration was also 4 days and resulted in only 1 young being produced.

63. Production, estimated from the number of young per AFRD, in the REF sediment was 0.12, 0.20, and 0.31 for Experiments 1, 2, and 3, respectively. There was a distinct decrease in production with increasing BRH sediment concentration in all experiments and particularly at the 155- and 156-mg/l treatments in Experiments 1 and 2 where only 3 and 1 young were reproduced, respectively. The concentrations of BRH sediment that

produced a 50% decrease in the AFRD (EC50) were determined by graphical interpolation. The treatment AFRD values were converted to percent of the REF (100%) and graphed on the BRH sediment concentrations. The BRH sediment concentration at the intercept of the AFRD slope and the 50% response axis is the estimated EC50 concentration. Using this procedure, the EC50 values for Experiments 1, 2, and 3 are 125, 47, and 32 mg/l BRH sediment, respectively.

64. The reproducibility of the chronic responses was generally acceptable. There were no significant (P = 0.05) effects on growth between treatments nor between experiments. The times to sexual maturity and intitial reproduction, while differing slightly in absolute value between experiments, consistently decreased with increasing concentrations of BRH sediment. Treatment differences in these parameters were consistent and reproducible occurring at 155, 100, and 95 mg/l BRH sediment.

65. Comparisons of production (young/AFRD) from estimated EC50 concentrations provides an estimate of the reproducibility of this response parameter. The high to low ratio, 3.9, for the range of EC50's (125-32) concurs with interlaboratory calibration data for chronic testing with M. bahia (McKenney 1982). These studies reported high to low ratios of 5.7 and 3.6 for chronic test results conducted by six laboratories with the heavy metal, silver, and the pesticide endosulfan, respectively.

66. It is difficult to assess the reproducibility of productivity data, due to the high degree of variability inherent in this parameter. Initial analyses of variance of the number of female young per available reproductive days for Experiments 1 and 2 indicated there were no significant (P = 0.05) differences between treatments even though the actual total

production varied by an order of magnitude between the REF and the 155- to 156-mg/l exposure concentrations, respectively. The inability to detect statistical differences was directly attributable to the following sources of variability: the number of reproducing females, the number of replicates in the exposure design, and the size of the broods. The decision to modify the experimental design for Experiment 3 can be criticized for jeopardizing the true replicability needed to assess test method reproducibility within Laboratory Documentation. However, the authors believed that it was more important to improve the statistical limits of detectability for the reproductive portion of this test method than collect data that would be difficult to interpret because of limitations of the test method.

67. The design modification consisted of increasing the number of replicates from six to thirty, and reducing the number of exposure concentrations. The maximum nominal exposure concentration chosen for Experiment 3 was 100 mg/l BRH sediment based upon the 40-percent mortality and almost complete cessation of reproduction at 158 mg/l BRH sediment in Experiments 1 and 2. Statistical analysis of data from Experiment 3 revealed significant ($P < 0.05$) treatment differences between the REF control at both the 95-mg/l and 43-mg/l concentrations of BRH sediment. Thus, the improved experimental design enabled the statistical discrimination of reproductive changes in Experiment 3 that were undetected in Experiments 1 and 2.

68. In summary, the reproducibility of the chronic reproductive data developed within the Laboratory Documentation phase of this study with M. bahia is consistent with the expected variability of the test method. This is very encouraging in view of the complex contaminant profile of the dredged material and affirms the applicability of the

long-term chronic test method with M. bahia for assessing the effects of dredged material contaminants.

Population analyses

69. Population response parameters were calculated from life tables (Tables A1, A2, A3) that utilized age-specific survival and reproduction data from the whole life cycle long-term tests. The three response parameters examined are intrinsic rate of growth, r , the net reproductive value or multiplication rate per generation, and the mean generation time. The patterns of these parameters measured for M. bahia exposed to BRH sediments in three separate experiments are summarized in Table 5.

70. The differences in the absolute values between the three experiments for the intrinsic rate of growth, r , measured for the REF sediment are the result of differences in the growth rates of the populations in the two experiments. Values for r that are positive represent increasing population size, while values for r that are at or close to zero represent populations whose births and deaths are balanced, resulting in maintenance of the population. Strongly negative values for r are indicative of populations whose death rates greatly exceed the birth rates and ultimately would lead to extinction.

71. Negative values in Experiment 1 and 2 of -0.015 and -0.018, respectively, are low and are indicative of populations which are slowly moving toward extinction. This is in contrast to the positive value of +0.070 determined for the REF sediment in Experiment 3. In previous studies, r-values for laboratory populations of M. bahia were greater than +0.030 (Gentile et al. 1983). Because of the negative r - values in the first two experiments, the authors changed the experimental design for

Table 5

Population Responses for
Life Cycle Tests with M. bahia

BRH-sediment mg/l	Intrinsic rate of growth r	Multiplication rate per generation	Generation time days	EC50 for r mg/l
		Experiment 1		
Zero	-0.015	0.72	22.15	110
65	-0.002	0.96	23.40	
155	-0.099	0.13	21.00	
275	---	--	---	
311	---	--	---	
		Experiment 2		
Zero	-0.018	0.722	18.31	42
87	-0.107	0.062	26.00	
156	---	---	---	
396	---	---	---	
		Experiment 3		
Zero	+0.070	4.52	21.59	47
43	+0.038	2.16	20.69	
95	-0.078	0.17	22.36	

Experiment 3 by increasing the replication and decreasing the number of treatments.

72. Although the r - values were slightly negative in Experiments 1 and 2, there was still a definable decrease (negative increase) in the r-value as a function of BRH sediment concentration. A similar pattern was clearly discernable in the r - values for Experiment 3. Substantial decreases in r were observed at 155, 87, and 95 mg/l in Experiments 1, 2, and 3, respectively. At these concentrations of BRH sediments, the respective populations were moving toward extinction much more rapidly than the REF sediment population. EC50 values for r in Experiments 1, 2, and 3 of 110,

42, and 47 mg/l BRH sediments, respectively, were estimated using the graphical interpolation method previously described for the reproductive parameters.

73. The second population response parameter, multiplication rate per generation, showed a similar pattern of response to that described for the intrinsic rate of population growth. Specifically, values for the multiplication rate per generation decreased with increasing concentrations of BRH sediments. The absolute values in Experiments 1 and 2 were less than 1.0, indicative of a population that was not replacing itself, and the value of 4.52 for Experiment 3 denotes a four-fold increase in population size for each generation. There were no significant changes in the mean generation time for those treatments that reproduced successfully in any of the experiments, indicating that this parameter was not a sensitive index of stress.

74. Comparison of the range of EC50 values (42-110) for the intrinsic rate of population growth, r, indicates a high to low ratio of 2.6 for the three experiments that is well within the expected range of variability for a chronic life cycle test with M. bahia. In contrast, the negative values for r in Experiments 1 and 2 indicate that these populations were not growing satisfactorily, which necessitated a change in the experimental design for Experiment 3. The latter design changes resolved both the problems with the intrinsic growth rate and the problems associated with reproductive variability, thus improving the power of statistical analysis. The results of these studies with M. bahia indicate that: (a) the use of life cycle chronic tests are applicable for use in evaluating the impact of dredged material contaminants, (b) this

species is among the most sensitive to the contaminants in BRH sediments of the species tested to date, and (c) the reproducibility of the reproductive and population response parameters is within acceptable levels.

Ampelisca abdita

Exposure system monitoring

75. Table 6 shows monitoring data for the 96-hr range-finding tests. All the measured concentrations are close to the nominal, although in some cases standard deviations were as high as 50 percent of the mean, especially at the high concentrations. In these range-finding tests, particle concentration was affected by the design of the exposure system. The exposure system using the acute chambers had lower total solids concentrations than did the chronic test chambers. The presence of two exposure chambers in each acute exposure system (Figure 9) resulted in obstructed circulation and increased sedimentation. There was, however, no particle settling in the acute exposure chambers themselves. At the high concentrations (100 and 200 mg/l), there was 3 to 5 mm of sedimentation in the chronic exposure chambers; consequently, the acute exposure chambers appear to represent a truer water column exposure.

76. The monitoring data for the preliminary chronic test (18 days) is shown in Table 7. Replication was good, although there was higher variability at the REF and 25% BRH level than in the other treatments. This variation was caused by intially high levels of REF sediment at the beginning of the test.

77. Table 8 shows the particle concentrations for Chronic Test 1. Mean values ranged from 31.8 to 43.8 mg/l and those concentrations are the replicate values for the 28-day exposure of 10% BRH. The amount of BRH

Table 6

Ampelisca abdita 96-hr Mortalities and Suspended Particulate Concentrations ($\bar{x} \pm SD$) for REF and BRH Sediments, comparing two exposure chambers, acute (A) and chronic (C)*

Nominal Concentration mg/l	Exposure Chamber	REF Meas. Conc. mg/l $\bar{x} \pm SD$	REF Mortality %	BRH Meas. Conc. mg/l $\bar{x} \pm SD$	BRH Mortality %
0	A	--	2	--	2
Seawater control	C	--	6	--	4
25	A	29.0 ± 18.4	2	32.4 ± 4.8	24
	C	22.5 ± 1.8	2	33.9 ± 10.8	20
50	A	41.8 ± 18.3	6	47.0 ± 0.9	18
	C	60.2 ± 31.0	10	55.0 ± 9.2	20
100	A	93.1 ± 46.5	8	74.9 ± 12.2	42
	C	120.2 ± 25.2	0	92.0 ± 44.9	50
200	A	199.2 ± 54.4	4	186.8 ± 9.6	92
	C	233.6 ± 46.7	4	239.2 ± 30.1	88

*N for dry weight determinations = 2, N for % mortality = 50. Sizes (mm) of A. abdita ($\bar{x} \pm SD$) N = 25 : REF = 3.54 ± 0.65, BRH = 3.39 ± 0.47.

sediment in each treatment is also shown and ranges from 1.8 to 1.9 mg/l for the 5-percent BRH exposures and from 3.2 to 4.4 mg/l for the 10 percent exposures. In Chronic Test 2, the 3-way valves were adjusted to provide a slightly higher suspended particulate concentration at about 5 mg/l above the concentration in Test 1. Total suspended load ranged from 39.7 (control) to 50.1 mg/l (10 percent BRH) over all treatments and the resultant BRH sediment concentrations were 2.0 to 2.3 mg/l at 5 percent BRH and 4.3 to 5.0 mg/l at 10 percent BRH (Table 9). The exposure concentrations were less variable in Test 2; only replicate 4 at 5-percent BRH had a coefficient of variation greater than 30 percent. The variability of all the 58-day exposure concentrations was higher than the 32-day concentration

Table 7

Dry Weight and Ampelisca Mortality for an 18-day Exposure to BRH in the Suspended Phase Preliminary Chronic Test*

Treatment	Replicate	Dry Weight mg/l $\bar{x} \pm$ SD	N	BRH Sediment mg/l	Mortality %
Seawater	1	4.6 ± 3.2	3	0	13
control	2	4.0 ± 3.8	3	0	5
100% REF	1	56.3 ± 27.5	6	0	14
0% BRH	2	52.5 ± 35.9	6	0	9
25% BRH	1	50.1 ± 22.2	6	12.5	90
	2	51.2 ± 23.3	6	12.8	98
50% BRH	1	50.5 ± 12.0	6	25.3	100
	2	55.8 ± 8.9	6	27.9	100
75% BRH	1	55.9 ± 6.4	6	41.9	100
	2	53.8 ± 7.0	6	40.4	100

*The number of amphipods per replicate is 100. Initial mean size and SD = 3.11 ± 0.48 mm (N=91).

variability because of 3-way valve malfunctions which occurred during the last week of the test. Because the exposure design for the long-term chronic tests was different than that for the short-term tests (Figure 1), the suspended solids concentrations in the two distribution chambers and the BRH mixing chamber were checked weekly in Test 1 and twice per week in Test 2 (Table 10).

78. Temperature was measured daily and salinity was checked every other day. Temperature ranged from 19.5°to 20°C in Chronic Test 1 and from 19.5°to 21°C in Chronic Test 2. Salinity ranged from 28 to 32 ppt in both tests.

Table 8

Suspended Particulate Dry Weight, Standard Deviation, and Ampelisca Mortality for 28- and 45-day Exposure to BRH Suspended Phase in Chronic Test 1*

Treatment	Exposure Duration days	Replicate	Dry Weight mg/l	N	BRH Sediment mg/l	Mortality % Overflow	Mortality % Total
REF Control	28	1	43.0 ± 15.0	8	0	0	1
		2	40.4 ± 11.4	8	0	1	5
	45	3	36.3 ± 10.3	11	0	2	18
		4	41.0 ± 13.1	11	0	12	25
5% BRH	28	1	37.3 ± 8.4	8	1.9	0	3
		2	35.9 ± 12.4	8	1.8	0	12
	45	3	36.5 ± 9.8	11	1.8	3	17
		4	38.8 ± 12.7	11	1.9	34	61
					1.9 ± 0.1		
10% BRH	28	1	31.8 ± 7.8	8	3.2	0	4
		2	43.8 ± 13.2	8	4.4	0	9
	45	3	42.6 ± 14.8	10	4.3	13	35
		4	34.5 ± 9.5	11	3.5	2	21
					3.9 ± 0.6		

*Number of amphipods per replicate is 100. Overflow mortalities are those found in the overflow water trap.

Table 9

Suspended Particulate Dry Weight and Standard Deviation for Chronic Test 2 at Exposure Durations of 32 and 58 Days

Treatment	Exposure Duration days	Replicate	Dry Weight mg/l	N	BRH Sediment mg/l
REF control	32	1	42.5 ± 6.2	9	0.0
		2	40.7 ± 8.5	9	0.0
	58	3	39.7 ± 9.6	17	0.0
		4	42.9 ± 11.1	17	0.0
5% BRH	32	1	40.8 ± 5.0	9	2.0
		2	40.4 ± 7.0	9	2.0
	58	3	45.5 ± 12.3	17	2.3
		4	45.1 ± 19.9	17	2.3
					2.2 ± 0.2
10% BRH	32	1	46.5 ± 6.8	9	4.7
		2	42.5 ± 7.4	9	4.3
	58	3	50.1 ± 13.7	17	5.0
		4	49.1 ± 13.1	17	4.9
					4.7 ± 0.3

Table 10

Suspended Particulate Concentrations in the Dosing System

	Chronic Test Number	
	Experiment 1 mg/l ± SD(N)	Experiment 2 mg/l ± SD(N)
REF Distribution Chamber	84.6 ± 19.8(8)	93.3 ± 35.0(16)
BRH Mixing Chamber	91.3 ± 24.7(9)	78.3 ± 26.4(17)

Short-term tests: mortality

79. The REF and BRH 96-hr range-finding tests were designed to determine threshold mortalities for each sediment when diluted with seawater. The chronic and acute test chambers were each used in these short-term tests for comparative purposes to ensure that chamber design would not cause mortalities in the chronic tests.

80. The REF suspended phase, at nominal total solids concentrations of 200 mg/l and below, caused no significant mortalities (Tables B1 and B2). The BRH suspended phase did cause significant mortalities (Tables 6, B3, and B4). Mortality patterns in the two chamber types were comparable. The 96-hr LC50 value for the acute exposure chambers was 84.2 mg/l with 95-percent confidence limits of 72.8 to 97.4 mg/l. For the chronic exposure chambers, the LC50 value was 90.9 mg/l with 95-percent confidence limits of 76.2 to 110.7 mg/l. These LC50 values are not different from each other as shown by complete overlap of the 95-percent confidence intervals. The replication is good, especially considering that two different chamber designs were used, and that the suspended particulate concentrations were not exactly the same. Ampelisca's acute response to BRH sediments in the solid phase, which has been reported elsewhere (Rogerson et al., 1984), was also consistent among several series of tests.

81. Based on these mortality patterns, a preliminary chronic test was designed to have a constant total particle concentration of approximately one half the LC50 values and a BRH contaminant gradient was achieved by proportionally diluting the BRH sediment with REF sediment. The BRH suspended particle proportions were 75, 50, 25, and 0 percent. As Table 7

shows, significant mortalities were obtained at all BRH exposures.

82. The mortalities in these short-term tests are not surprising since Ampelisca is a particle feeder (Mills 1967) and the target organs affected by BRH sediment are the digestive tract and hepatopancreas. The absolute mortalities found in the range-finding tests and the 18-day preliminary chronic test are not strictly comparable, however. The diatom P. tricornutum was supplied to the amphipods in the 18-day test and not in the 96-hr tests. It is not known if the presence of food stimulates feeding, thereby increasing exposure to contaminated particles, or if the food supply ameliorates toxicity by providing a nutritional food source in the presence of a potentially toxic material.

Long-term tests

83. As there were significant mortalities at 25% BRH (12.5 mg/l BRH sediment) in the 18-day test, the exposure concentrations for the long-term test were decreased. The experimental design for the long-term tests included three treatments: 10- and 5-percent proportions of BRH sediment and a reference control at approximately 50 mg/l total suspended solids. Two additional replicates were added to each treatment in order to evaluate growth and survival at two time intervals, 25 and 45 days in Test 1 and 32 and 58 days in Test 2.

Mortality

84. Chronic mortalities for the 28-day exposure in Experiment 1 were not significant (Table 8) at 10% BRH and approximately 40 mg/l total suspended solids (3.8 mg/l BRH). In Chronic Test 2, survival was estimated by comparing the number of Ampelisca harvested on day 32 with the estimated initial number of young, assuming 100-percent survival of all eggs of the

ovigerous females that were introduced into the containers at day 0. These females had a mean egg number of 17.7 eggs (SD = 6.73) to yield 266 initial young. As shown in Table 11, survival in the control and 5-percent exposures was better than at 10-percent BRH. However, the 95-percent confidence interval around the mean egg number/female is 15.2 to 20.2 eggs which would yield between 227 and 303 initial young; only replicate 2 at 10-percent BRH falls significantly outside this range (Table 11). The mortality threshold appears to occur at 4.5 mg/l BRH sediment based upon the 32-day data.

Table 11

Estimated A. abdita Mortality in Chronic Test 2*

Treatment	Replicate	No. Harvested	Mortality %
		32 Days	
REF	1	240	9.8
	2	245	7.9
5% BRH (2.0 mg/l)	1	278	0
	2	307	0
10% BRH (4.5 mg/l)	1	227	14.7
	2	190	28.6
		56 Days	
REF	3	159	40.2
	4	127	52.3
5% BRH (2.3 mg/l)	3	73	72.6
	4	118	55.6
10% BRH (5.0 mg/l)	3	178	33.1
	4	206	22.6

*Assumming 100% hatching success of eggs from 15 egg-bearing females with with a mean egg number/female (± SD) of 17.7 ± 6.73 eggs to yield 266 initial young.

Based upon the results of the preliminary chronic (18-day) and the two definitive (28- and 32-day) chronic tests, the chronic mortality threshold for BRH suspended particulates is between 4 and 12.5 mg/l for A. abdita exposed for 18 days or longer. By converting the proportion of BRH sediment to milligrams per liter of BRH sediment, the mortalities from the 18- and 28-day exposure in Chronic Test 1 were analyzed to estimate an LC50 value. The 32-day data in Chronic Test 2 was not used because the mortality data are only estimates. Although the 28-day exposure is longer, the mortalities for an 18-day exposure are at least no greater than those for 28 days. This chronic LC50, calculated using the binominal test, is 7.03 mg/l with 95-percent confidence limits from 4 to 12.5 mg/l. The 96-hr LC50 for the chronic exposure chambers was 90.9 mg/l, yielding an acute mortality: chronic mortality ratio of 12.9.

85. The mortalities for the 45-day exposure in Chronic Test 1 increased, ranging from 18 to 51 percent over all treatments (Table 8). Of the total mortality in this experiment, 66 of 167, or 40 percent, were found in the overflow cups (Figure 3) that were checked daily. Of these 66 Ampelisca, 50 were mature males, and over half of these were found in the 5-percent BRH replicate 4. The cause of these mortalities does not appear to be BRH sediment since the mortalities were mostly adult males which frequently experience natural senility and die after mating (Mills 1967). In Test 2, the 58-day estimated mortality pattern was reversed (Table 11) with the greatest survival occurring in the 10-percent BRH exposure.

86. As will be shown below, natural mortality occurs across all treatments and is indicative of the stage of development of the population.

As such, only the 28-day, Test 1, mortality data were used to calculate the acute:chronic ratio. The goal of the exposure system design, to expose Ampelisca to nonlethal concentrations of BRH suspended particulates, was achieved in both chronic tests. The natural life span of Ampelisca at 20°C is 4 to 6 weeks, a period that is encompassed by the second two replicates of each test.

Population structure

87. Ampelisca abdita individuals can be easily sexed and classified into stages of sexual maturity according to morphological characteristics. Morphologically, adult males (M) are the most distinctive with a carinate urosome and elongated second antennae (Bousfield 1973). Males are specially adapted for swimming, which facilitates mating in the water column. They do not molt again and presumably die after mating. Whether they mate with more than one female is unknown.

88. Female A. abdita are more difficult to sex, but they can be divided into five progressively distinct groups: FDV, FE, FOV, FS, and OTH. The earliest stage, after sexual differentiation, is here termed developing female (FDV). This stage animal is distinguished by the presence of a brood plate on the interior of each of the first five coxal appendages. As the female grows, the brood plate increases in size and eggs begin to develop in the oviduct which is dorsal to the digestive tract, and which can be seen through the exoskeleton. This stage is termed developing egg female (FE). The females go through a series of molts until one of the final molts, at which time the brood plates develop long setae and the eggs are deposited through a gonopore into a fully developed brood pouch. This stage is termed ovigerous female (FOV). Presumably mating occurs at

this time. Since all eggs in a brood pouch are in the same stage of development, all eggs in the oviduct are assumed to be deposited at once. After releasing their young, the females do not immediately die but remain in the population for some undetermined time. They do not have eggs in the oviduct or in the brood pouch but do retain the setose oostegites. They are termed spent females (FS).

89. There is one other group, termed undifferentiated (OTH). These include juveniles, undifferentiated females, and undifferentiated males. In most cases where this group was encountered, the animals were larger than 4.0 mm. These animals are probably males since females can be distinguished at a smaller size, when the brood plates first develop.

90. In Chronic Test 1, after the 28- and 45-day exposures, two replicates from each treatment were sieved to 0.5 mm and the amphipods were sex classified and measured. Development of the female population was progressively retarded as the BRH concentration increased (Table 12) at 28 days, as evidenced by the greater numbers of ovigerous females (FOV) in the REF and their total absence in the 10% BRH treatment. Conversely, the earliest stage of development (FDV) was the predominant developmental stage at the highest BRH concentration. The same results were evident for the adult males (M) and the undifferentiated (OTH) groups, where there were more males in the control treatment than in the BRH exposed groups. These data suggest that exposure to BRH sediment caused a delay in maturation and development over the 28-day exposure period. After a 45-day exposure, there does not appear to be any difference among the treatments, although in the 10-percent treatment there was a greater proportion of the undifferentiated (OTH) group, again indicating

Table 12

Number of A. abdita in Each Sex Category for 28- and 45-Day Exposures to BRH Suspended Particulates in Chronic Test 1*

Treatment	Replicate	FOV	FE	FDV	M	OTH
		28 Days				
REF	1	18	18	6	25	32
	2	9	26	7	16	37
	mean	13.5	22.0	6.5	20.5	34.5
5% BRH (1.9 mg/l)	1	4	35	2	18	38
	2	1	7	22	1	57
	mean	2.5	21.0	12.0	9.5	47.5
10% BRH (3.8 mg/l)	1	0	12	33	0	48
	2	0	3	40	3	48
	mean	0	7.5	36.5	1.5	48.0
		45 Days				
REF	3	22	18	2	29	4
	4	17	23	2	29	2
	mean	19.5	20.5	2.0	29.0	3.0
5% BRH (1.9 mg/l)	3	27	19	2	30	2
	4	14	10	0	13	0
	mean	20.5	19.5	1.0	21.5	1.0
10% BRH (3.9 mg/l)	3	13	7	13	6	23
	4	21	11	2	35	10
	mean	17.0	9.0	7.5	20.5	16.5

*Initial N for each replicate was 100 Ampelisca. See text for designation of classes.

that development of the males is also impacted. Chi-square tests of the mean proportions of each group showed significant treatment effects at $P < 0.05$.

91. Similar results were obtained in the second chronic test, but since the test was initiated with ovigerous females, the major effects occurred at the 58-day sampling rather than at 32 days (Tables 13, 14).

Table 13

Number of A. abdita in Each Sex Category for 32- and 58-Day Exposures to BRH Suspended Particulates in Chronic Test 2*

Treatment	Replicate	FOV	FE	FDV	FS	M	OTH
			32 Days				
REF	1	22	37	22		35	124
	2	3	32	86		3	121
5% BRH (2.0 mg/l)	1	10	66	53		32	117
	2	18	31	82		28	148
10% BRH (4.5 mg/l)	1	2	36	60		1	128
	2	1	6	45		0	138
			58 Days				
REF	3	46	15	20	19	27	32
	4	39	25	22	8	15	18
5% BRH (2.3 mg/l)	3	30	9	7	15	5	7
	4	53	17	5	17	13	13
10% BRH (5.0 mg/l)	3	23	25	29	3	31	67
	4	2	5	97	0	1	101

*See text for designation of classes.

At 32 days, the populations were similar, mainly because of the replicate variability. For example, the REF replicate 2 is similar to both 10% BRH replicates, in having low numbers of ovigerous females and adult males (Table 13). There is a trend, however, for more ovigerous females and adult males to be present in the control and 5% exposures. This trend becomes more dramatic at 58 days. The population exposed to 10% BRH had fewer ovigerous females and more developing females (FDV) and subadult males (OTH) than in the lower treatment conditions. Chi-square again showed significant treatment effects at $P < 0.05$. These results also suggest a delay in the maturation of the population in Chronic Test 2.

Table 14

Mean Percent of A. abdita in Each Sex Category for 32- and 58-Day Exposures to BRH Suspended Particulates in Chronic Test 2*

Treatment	FOV	FE	FDV	FS	M	OTH
			32 Days			
REF	5.2	14.2	22.3	--	7.8	50.5
5% BRH (2.0 mg/l)	4.8	16.6	23.1	--	10.3	45.3
10% BRH (4.5 mg/l)	0.7	10.1	25.2	--	0.2	63.8
			58 Days			
REF	29.7	14.0	14.7	9.4	14.7	17.5
5% BRH (2.3 mg/l)	43.5	13.6	6.3	16.8	9.4	10.5
10% BRH (5.0 mg/l)	6.5	7.8	32.8	0.8	8.3	43.8

*See text for designation of classes.

Growth

92. The mean sizes and variability estimates for each development stage and replicate for Chronic Tests 1 and 2 are shown in Tables A8 and A9, respectively. Analysis of variance tests were done to determine treatment effects for the 28- and 45-day samples in Test 1 and for the 32- and 58-day samples in Test 2. Although some of the stage-classified samples were split to reduce the sample size, it was felt that these smaller subsamples could be included in the ANOVA because they would reflect a conservative estimate of the statistical differences.

93. The initial mean size and standard deviation of Ampelisca for Test 1, measured on a sample of 100 animals from the original pool of test organisms, was 3.30 $\pm$ 0.51 mm. The survival (Table 8) and general growth of these animals (Table 15) was good for all treatments. In most of the treatment comparisons, the sizes of the 5% BRH-exposed Ampelisca were slightly larger than the control animals, but none of these differences

Table 15

Mean size (mm) of A. abdita Exposed to BRH Suspended Particulates in Experiments 1 and 2*

Treatment	FOV	FE	FDV	M	OTH
		Experiment 1			
		28 Days			
REF	6.71	7.15**	6.35	6.55	6.64
5% BRH (1.9 mg/l)	7.09	7.39	5.18	6.88	5.95
10% BRH (3.8 mg/l)	--	6.94	5.79	6.58	6.11
		45 Days			
REF	7.46	7.68**	6.43	6.70	7.37**
5% BRH (1.9 mg/l)	7.54	7.97	6.63	6.81	7.94
10% BRH (3.9 mg/l)	7.01	7.49	6.15	6.51	6.55
		Experiment 2			
		32 Days			
REF	6.14	6.37	5.29	5.50	5.74
5% BRH (2.0 mg/l)	6.34	6.39	5.22	5.59	5.47
10% BRH (4.5 mg/l)	6.75	6.04	4.67	5.25	4.42
		58 Days			
REF	6.89	7.58	7.18	6.40	7.33
5% BRH (2.3 mg/l)	6.25	6.99	6.88	6.24	7.23
10% BRH (5.0 mg.l)	6.12	6.69	5.80	5.83	6.15

*Sizes connected by the same line are not significantly different from each other at $P < 0.05$.
**Control and 10% BRH are not significantly different at $P < 0.05$.

were statistically significant. There were no significant treatment or time differences among male mean sizes, indicating that adult males were the same size regardless of the length of time that the growth took. Therefore, even though it took longer for the males to mature as a result of exposure to BRH sediment (Table 12), they were the same size at maturation across all treatments. When all females were pooled, there was a

treatment effect at 28 days between REF and 10% BRH with the reference females being significantly larger ($t = 8.167$, $df = 157$). As noted above, the females in the 5% BRH treatment were not significantly different from those in REF at 28 days because of the large size variation between replicates (Table C1). This variation is a reflection of the developmental stage difference between the two replicates. Analysis of variance, with replicates pooled, does show that the developing females (FDV) are significantly larger in the reference ($P < 0.05$). The same is true for the undifferentiated group (OTH) indicating that the growth of males and females is slowed.

94. At the 45-day exposure, the differences in growth are not as great, although there is a consistent trend in all classes for the 10% BRH Ampelisca to be the smallest. There was a significant ($P < 0.05$) difference among the ovigerous females with the reference and 5% BRH treatments being consistently larger than the 10% BRH exposed amphipods.

95. In Experiment 2, the statistical differences in sizes among the classes are more prevalent than those found in the first test. As expected from the results in Test 1, at 32 days there were no treatment effects on ovigerous females or adult males. The ANOVA showed that the 10% BRH exposure produced significantly smaller nonovigerous females and subadult males, indicating an adverse impact on growth. By day 58, all development stages showed significant treatment effects on size. In all cases, the REF were larger than the 10% BRH exposure condition. Additionally, the REF ovigerous females (FOV) and females with eggs in the oviducts (FE) were significantly larger than those in the 5% BRH exposure (Table 15).

96. The size data, in conjunction with the data on population

structure, presented in the previous section, has shown that a major effect of BRH suspended particulates is the retardation of growth and subsequent maturation. The more dramatic effects on growth (Table C2) that were found in Experiment 2 are the probable result of a more complete exposure of Ampelisca throughout its life cycle to BRH sediments. The results of the longer exposure condition also suggest that the earlier part of the life cycle may be more sensitive. Notwithstanding these differences, the replication of the growth dose response to BRH suspended particulates is very good.

Fecundity and productivity

97. The number of eggs produced per female in A. abdita is a function of female size (Mills 1967). Treatment effects were assessed using analysis of covariance, with female size as the dependent variable. Once the quantitative relationship between egg number and female size is determined, the differences in egg number/female among treatments can be analyzed by statistically adjusting the fecundity estimates based on the regression relationship.

98. Analysis of covariance showed positive correlation of egg number with size ($F = 30.26$, $P < 0.001$) for the 45-day data in Experiment 1. There was a trend for more eggs to be produced/female in the REF and 5% BRH treatments than in the 10% BRH exposure. However, when egg number/female was adjusted for female size in the covariance analysis, there were no differences among treatments (Table 16). Identical results were found for the day 58 ovigerous females in Experiment 2 (Table 16). Again the egg number/female tended to be greater in the REF and 5% BRH animals than at 10% BRH, but the differences were not significant

Table 16

Mean Number of Eggs/Ovigerous Female ± Standard Error for A. abdita Exposed to BRH Suspended Particulates for 45 days in Experiment 1 and 58 days in Experiment 2

Treatment	N	Raw $\bar{x}$ ± SE	Size Adjusted $\bar{x}$ ± SE	Total Young Produced
		Experiment 1		
REF	39	19.8 ± 2.08	18.5 ± 1.85 (A)	Present
5% BRH (1.9 mg/l)	41	21.8 ± 2.06	19.4 ± 1.86 (A)	Present
10% BRH (3.9 mg/l)	34	14.4 ± 1.89	18.3 ± 2.12 (A)	Absent
		Experiment 2		
Initial	30	17.7 ± 1.23	17.0 ± 1.52 (A)	---
REF	68	13.6 ± 1.17	12.44 ± 1.04 (B)	1212
5% BRH (2.3 mg/l)	59	12.3 ± 1.21	13.54 ± 1.12 (B,A*)	318
10% BRH (5.0 mg/l)	16	9.2 ± 1.23	10.93 ± 2.11 (B)	0

*Raw data and egg number adjusted for female size. Means with the same letter are not significantly different at $P < 0.05$.

when fecundity was adjusted for female size. In Chronic Test 2, all of the experimental fecundities were significantly lower than the egg production found in the INITIAL sample which was the original pool of ovigerous females at the start of the experiment. Although it was not statistically tested, the Test 2 fecundities also appear lower than the fecundities at all treatments in Experiment 1.

99. A possible explanation for the lowered fecundity in Experiment 2 may be that the Experiment 1 animals were the F1 laboratory generation, whereas, the Experiment 2 Ampelisca were an F2 laboratory generation. To test this hypothesis, all control ovigerous females were submitted to the

covariance analysis, including the 28-day Experiment 1, and the INITIAL and the 32-day Experiment 2 amphipods. The analysis revealed that, in fact, the 58-day Experiment 2 fecundities (Table 16) were significantly lower ($P < 0.05$) than all other control groups, except for the 28-day Experiment 1 animals. Lowered fecundities of an F2 generation probably result from the lowered nutritional quality of the laboratory food source (Phaeodactylum), which does not simulate the diverse detrital component found in nature.

100. The number of young produced in Experiment 2 is also shown in Table 16. The REF produced more young (F3) than the 5% BRH treatment and no young were found in the 10% BRH exposure. The causes are twofold: fewer ovigerous females were present at the highest BRH concentration (Tables 13 and 14), and they tended to carry fewer eggs (Table 16). In Experiment 1, a larger sieve size (0.5 mm mesh) was used and the young (F2) were not quantified; however, they were present in the REF and 5% BRH and were absent from the 10% BRH exposure.

Population analyses

101. As is the case with M. bahia, population response parameters were calculated from life tables using age-specific survival and reproduction data (Tables A4, A5). To construct these Ampelisca life tables, the test period was broken into 14-day intervals and survival was estimated for each age interval by knowing the initial number of amphipods, those harvested at the interim sampling, either day 28 or 32, and those found at the final sampling, either day 45 or day 58 for Experiments 1 and 2, respectively. The reproduction data for Experiment 1 were estimated based on the mean number of eggs per ovigerous female since the total number of

young produced was not quantified. In Experiment 2, the number of eggs per ovigerous female were included and combined with the actual number of young produced for each treatment. The population responses in the two experiments are summarized in Table 17. The responses shown are intrinsic rate of growth, r , the multiplication rate per generation, and generation time.

Table 17

Population Responses for Life-Cycle Tests with A. abdita

Treatment	Intrinsic rate of growth r	Multiplication rate per generation	Generation time (days)
	Experiment 1		
REF	.045	5.65	38.5
5% BRH (1.9 mg/l)	.038	4.93	41.7
10% BRH (3.9 mg/l)	.021	2.46	43.0
	Experiment 2		
REF	.023	3.50	54.0
5% BRH (2.2 mg/l)	.009	1.60	51.6
10% BRH (4.7 mg/l)	-.023	0.28	54.9

102. Both the experiments show the same dose response in intrinsic rate of growth and multiplication rate per generation: each response decreases with increasing BRH concentration. These results indicate that, although the absolute values of these responses are different, exposure to BRH sediments elicits a similar dose response pattern. This is an important consideration from a toxicological viewpoint since these two experiments are not true replicates in that the population stage was

different at the beginning of the two tests. In Test 1, field-collected subadults were used to initiate the tests, and therefore the early part of the life cycle was not exposed to BRH sediments. This would account for the proportionately greater number of ovigerous females across all treatments. Conversely, in Test 2, all Ampelisca were exposed from hatching and brood release. The steeper response pattern for r that was found in Test 2 results from fewer ovigerous females developing and the subsequent decreased contribution of young to the population. The differences in actual BRH sediment concentrations do not appear great enough to cause the observed drop in r. The longer generation time in Experiment 2 probably reflects the longer duration of the experiment. In addition to the fewer number of ovigerous females, as noted in the previous section, the number of eggs per female is lower in Experiment 2. The total reduction in fecundity is a probable result of the fact that the Ampelisca used in this test were an F2 generation laboratory stock that were reared on Phaeodactylum.

103. The differences in the experimental conditions of the two tests, i.e., the initial propulation stage, appear sufficient to explain the absolute differences in the intrinsic rate of growth and multiplication rate per generation between Test 1 and Test 2. More importantly, the magnitude of the differences is not great; e.g., for the controls, the tests differ by a factor of less than 2. The observed dose response of the population parameters is a good indication that population responses can be used to evaluate the long-term effect of dredged material in the suspended phase.

PART IV: CONCLUSIONS

104. The objectives of the Laboratory Documentation phase of the Field Verification Program are to demonstrate the applicability of using the chronic responses growth, reproduction, and intrinsic rate of population to determine the precision and reproducibility of the test methods and the response parameters measured.

105. The results of this study demonstrate the feasibility of conducting flow-through suspended solids whole life cycle toxicity tests with epibenthic and infaunal crustaceans for periods of 60 days. The suspended solids dosing systems developed for these studies were capable of proportionally mixing contaminated sediment (BRH) with reference sediment (REF) to produce a graded contaminant profile with consistency and precision.

106. Short-term tests with M. bahia resulted in 96-hr LC50's of 358, 290, and 410 mg/l BRH sediment while similar tests with A. abdita produced values of 84 and 91 mg/l. These results provide an estimate of the reproducibility of the short-term test methods developed for these species.

107. Long-term test methods were successfully developed to evaluate the effect of suspended solids on the growth, reproduction, and intrinsic rates of population growth, r , with M. bahia. Growth of M. bahia was not significantly affected by exposure to BRH sediments. Two measures of reproductive function, the times to sexual maturity and initial reproduction, while differing slightly in absolute values between experiments, consistently decreased with increasing concentrations of BRH sediments. Treatment differences in these parameters were consistent and reproducible occurring at 155, 156, and 95 mg/l BRH sediment. The third measure of reproductive function, the number of young per available reproductive day (AFRD), decreased with

increasing concentrations of BRH sediments in all experiments. The EC50 values for this parameter were 125, 47, and 42 mg/l for the three experiments, which is within the expected range of variability for the chronic test method with M. bahia. The measures of population response, intrinsic rate of population growth, r , and multiplication rate per generation, decreased with exposure to increasing concentrations of BRH sediments. The EC50 values for these parameters were 100, 42, and 47 mg/l for the three long-term experiments. These studies demonstrate the successful application of long-term chronic test methods using dredged material suspended solids exposure with M. bahia. Growth, reproductive, and population parameters measured using this method responded in a consistent and reproducible manner. The variability of these response parameters measured was within a factor of 3.0 for the three experiments.

108. The results of the long-term chronic tests with A. abdita indicate that this species is very sensitive to BRH sediment in the suspended phase. The BRH concentrations used for the chronic tests did not cause significant mortalities; however, effects on growth and reproduction were dramatic. In both replicates of the chronic tests, growth was inhibited at 4 to 5 mg/l BRH sediments (10% BRH) causing a delay in the maturation of adult females. Although there were test replicate differences in fecundity that were related to the generation of Ampelisca used, there were no treatment effects on the number of eggs produced per female. The effects of slower growth and delayed maturation were evident in the calculation of r , the intrinsic rate of population growth. In both tests, a consistent dose response was found for this parameter. The results of these studies demonstrate that the long-term chronic test method when applied to evaluating

the effects of dredged material suspended solids using the infaunal amphipod, A. abdita, gave a consistent and reproducible dose response for growth, population structure, and intrinsic rate of population growth.

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APPENDIX A

Life Tables for *M. bahia* and *A. abdita*

Table A1

Life Tables for M. bahia Exposed to BRH Sediments in Experiment 1

Age/Days	No. Females	l_x*	Female Young	m_x**
REF				
1- 5	18.	1.000	0.0	0.000
6-10	18.	1.000	0.0	0.000
11-15	18.	1.000	0.0	0.000
16-20	18.	1.000	3.0	0.167
21-25	18.	1.000	4.0	0.222
26-30	18.	1.000	6.0	0.333
31-35	0.	0.000	0.0	0.000
65 mg/l BRH Sediment				
1- 5	13.	1.000	0.0	0.000
6-10	13.	1.000	0.0	0.000
11-15	13.	1.000	0.0	0.000
16-20	13.	1.000	3.0	0.231
21-25	12.	0.923	0.5	0.042
26-30	11.	0.846	9.0	0.818
31-35	0.	0.000	0.0	0.000
155 mg/l BRH Sediment				
1- 5	12.	1.000	0.0	0.000
6-10	12.	1.000	0.0	0.000
11-15	12.	1.000	0.0	0.000
16-20	12.	1.000	0.0	0.000
21-25	12.	1.000	1.5	0.125
26-30	11.	0.917	0.0	0.000
31-35	0.	0.000	0.0	0.000

* l_x = the probability of a female surviving to age x
** m_x = female offspring per female per age interval

Table A2

Life Tables for M. bahia Exposed to BRH Sediments in Experiment 2

Age/Days	No. Females	l_x*	Female Young	m_x**
REF				
1- 5	18.	1.000	0.0	0.000
6-10	18.	1.000	0.0	0.000
11-15	18.	1.000	0.0	0.000
16-20	17.	0.944	8.0	0.471
21-25	13.	0.722	4.5	0.346
26-30	12.	0.667	0.0	0.000
31-35	8.	0.444	0.5	0.062
36-40	0.	0.000	0.0	0.000
87 mg/l BRH Sediment				
1- 5	16.	1.000	0.0	0.000
6-10	16.	1.000	0.0	0.000
11-15	16.	1.000	0.0	0.000
16-20	16.	1.000	0.0	0.000
21-25	14.	0.875	0.5	0.036
26-30	12.	0.750	0.0	0.000
31-35	11.	0.687	0.5	0.045
36-40	0.	0.000	0.0	0.000

* l_x = the probablility of a female surviving to age x
** m_x = female offspring per female per age interval

Table A3

Life Tables for M. bahia Exposed to BRH Sediments in Experiment 3

Age/Days	No. Females	l_x*	Female Young	m_x**
REF				
1- 5	28.	1.000	0.0	0.000
6-10	28.	1.000	0.0	0.000
11-15	28.	1.000	0.0	0.000
16-20	28.	1.000	51.5	1.839
21-25	24.	0.857	25.0	1.042
26-30	19.	0.679	33.5	1.763
31-35	10.	0.357	16.5	1.650
36-40	0.	0.000	0.0	0.000
43 mg/l BRH Sediment				
1- 5	34.	1.000	0.0	0.000
6-10	34.	1.000	0.0	0.000
11-15	34.	1.000	0.0	0.000
16-20	34.	1.000	33.5	0.985
21-25	34.	1.000	22.0	0.647
26-30	31.	0.912	7.0	0.226
31-35	24.	0.706	11.0	0.458
36-40	0.	0.000	0.0	0.000
95 mg/l BRH Sediment				
1- 5	32.	1.000	0.0	0.000
6-10	32.	1.000	0.0	0.000
11-15	32.	1.000	0.0	0.000
16-20	32.	1.000	0.0	0.000
21-25	32.	1.000	4.0	0.125
26-30	27.	0.844	1.5	0.056
31-35	24.	0.750	0.0	0.000
36-40	0	0.000	0.0	0.000

* l_x = the probability of a female surviving to age x

** m_x = female offspring per female per age interval

Table A4

Life Tables for A. abdita Exposed to BRH Sediments in Experiment 1

Age/Days	No. Females	l_x*	Female Young	m_x**
REF				
1-14	50	1.000	0.0	0.000
15-28	46	0.920	0.0	0.000
29-42	42	0.840	90.0	2.143
43-56	42	0.840	192.5	4.583
57-70	0	0.000	0.0	0.000
5% BRH (1.85 mg/l)				
1-14	50	1.000	0.0	0.000
15-28	45	0.900	0.0	0.000
29-42	40	0.800	23.0	0.575
43-56	40	0.800	223.5	5.588
57-70	0	0.000	0.0	0.000
10% BRH (3.85 mg/l)				
1-14	50	1.000	0.0	0.000
15-28	47	0.940	0.0	0.000
29-42	43	0.860	0.0	0.000
43-56	33	0.660	123.0	3.727
57-70	0	0.000	0.0	0.000

* l_x = the probability of a female surviving to age x
** m_x = female offspring per female per age interval

Table A5

Life Tables for A. abdita Exposed to BRH Sediments in Experiment 2

Age/Days	No. Females	l_x*	Female Young	m_x**
REF				
1-14	133	1.000	0.0	0.000
15-18	133	1.000	0.0	0.000
29-42	101	0.759	0.0	0.000
43-56	101	0.759	100.0	0.990
57-70	100	0.756	365.0	3.650
71-84	0	0.000	0.0	0.000
5% BRH (2.15 mg/l)				
1-14	133	1.000	0.0	0.000
15-28	133	1.000	0.0	0.000
29-42	133	1.000	0.0	0.000
43-56	103	0.774	82.5	0.801
57-70	76	0.571	130.0	1.711
71-84	0	0.000	0.0	0.000
10% BRH (4.73 mg/l)				
1-14	133	1.000	0.0	0.000
15-28	133	1.000	0.0	0.000
29-42	92	0.692	0.0	0.000
43-56	92	0.692	5.5	0.060
57-70	92	0.692	31.5	0.342
71-84	0	0.000	0.0	0.000

* l_x = the probability of a female surviving to age x
** m_x = female offspring per female per age interval

APPENDIX B

Acute Toxicity Data for *A. abdita*

TABLE B1
ACUTE TOXICITY DATA SHEET
COE/ERLN FVP

STUDY PLAN: 8 INVESTIGATOR: SCOTT/REDMOND

EXPERIMENT DESCRIPTION: SUSPENDED DATE OF TEST: 830701

TEST NUMBER: 1 ACUTE CHAMBER SPECIES: AMPELISCA SP

** EXPERIMENTAL CONDITIONS **

TEMPERATURE: 20.00 DEGREES CENTIGRADE RANGE: 19.90 - 22.00
SALINITY: 31.00 PARTS PER THOUSAND RANGE: -
EXPOSURE DURATION: 4 DAYS
PHOTOPERIOD: 14 HOURS
FLOW RATE: 80 ML8/MIN VOLUME ADDITIONS/DAY 30
NUMBER OF ANIMALS/REPLICATE: 25
NUMBER OF REPLICATES/TREATMENT: 2
ANIMAL'S LIFE STAGE: JUVENILE AGE: DAYS SIZE: MILLIMETERS
CONTROLS: SEAWATER/SOUTH REFERENCE SOLID
FOOD USED NONE

EXPOSURE CONCENTRATIONS (1)		OXYGEN	ANIMALS USED PER TREATMENT		NUMBER DEAD AT DAY				
NOMINAL	MEASURED(2)	MG/L	4 DAY	10	1	2	3	4	10
100MG REF/REF	93.09		50					4	
200MG REF/REF	199.19		50					2	
29MG REF/REF	29.00		50					1	
90MG REF/REF	41.79		50					3	
SEAWATER/REF			50					1	

96 HOUR LC50 NOT CALCULATED NO EFFECT CONCENTRATION 199.2MG/L REF/REF
ORGANISMS COLLECTED NARROW R. 830630, 3.54 +/- 0.69MM(N=25). SOLID PHASE REF SEDIMENT BATCH 3, BOTTLES 3,4. MG/L DETERMINED BY DRY WEIGHT MEASUREMENTS
(1) PERCENT FOR SOLID PHASE TESTS
(2) MILLIGRAMS/LITER FOR SUSPENDED PARTICULATE TESTS, DRY WEIGHTS OR COULTER COUNTS OR BOTH.

TABLE B2
ACUTE TOXICITY DATA SHEET
COE/ERLN FVP

STUDY PLAN: 8 INVESTIGATOR: SCOTT/REDMOND

EXPERIMENT DESCRIPTION: SUSPENDED DATE OF TEST: 830701

TEST NUMBER: 2 CHRONIC CHAMBER SPECIES: AMPELISCA SP

** EXPERIMENTAL CONDITIONS **

TEMPERATURE: 20.00 DEGREES CENTIGRADE RANGE: 19.50 - 22.00
SALINITY: 31.00 PARTS PER THOUSAND RANGE: -
EXPOSURE DURATION: 4 DAYS
PHOTOPERIOD: 14 HOURS
FLOW RATE: 80 MLS/MIN VOLUME ADDITIONS/DAY 30
NUMBER OF ANIMALS/REPLICATE: 50
NUMBER OF REPLICATES/TREATMENT: 1
ANIMAL'S LIFE STAGE: JUVENILE AGE: DAYS SIZE: MILLIMETERS
CONTROLS: SEAWATER/SOUTH REFERENCE SOLID
FOOD USED NONE

EXPOSURE CONCENTRATIONS (1)		OXYGEN	ANIMALS USED PER TREATMENT		NUMBER DEAD AT DAY				
NOMINAL	MEASURED(2)	MG/L	4	DAY 10	1	2	3	4	10
100MG REF/REF	120.19		50					0	
200MG REF/REF	233.60		50					2	
25MG REF/REF	22.50		50					1	
50MG REF/REF	60.20		50					5	
SEAWATER/REF			50					3	

96 HOUR LC50 NOT CALCULATED NO EFFECT CONCENTRATION 233.6MG/L REF/REF
ORGANISMS COLLECTED NARROW R. 830630, 3.54 +/- 0.69MM(N=29). SOLID PHASE REF SEDIMENT BATCH 3, BOTTLES 3,4. MG/L DETERMINED BY DRY WEIGHT MEASUREMENTS.
(1) PERCENT FOR SOLID PHASE TESTS
(2) MILLIGRAMS/LITER FOR SUSPENDED PARTICULATE TESTS, DRY WEIGHTS OR COULTER COUNTS OR BOTH.

TABLE B3
ACUTE TOXICITY DATA SHEET
COE/ERLN FVP

STUDY PLAN: 8 INVESTIGATOR: SCOTT/REDMOND

EXPERIMENT DESCRIPTION: SUSPENDED DATE OF TEST: 830711

TEST NUMBER: 1 ACUTE CHAMBER SPECIES: AMPELISCA SP

** EXPERIMENTAL CONDITIONS **

TEMPERATURE: 20.00 DEGREES CENTIGRADE RANGE: 20.00 - 20.50
SALINITY: 32.00 PARTS PER THOUSAND RANGE: -
EXPOSURE DURATION: 4 DAYS
PHOTOPERIOD: 14 HOURS
FLOW RATE: 40 MLS/MIN VOLUME ADDITIONS/DAY 15
NUMBER OF ANIMALS/REPLICATE: 25
NUMBER OF REPLICATES/TREATMENT: 2
ANIMAL'S LIFE STAGE: JUVENILE AGE: DAYS SIZE: MILLIMETERS
CONTROLS: SEAWATER/SOUTH REFERENCE SOLID
FOOD USED NONE

EXPOSURE CONCENTRATIONS (1)		OXYGEN	ANIMALS USED PER	NUMBER DEAD AT DAY				
NOMINAL	MEASURED(2)	MG/L	TREATMENT 4 DAY 10	1	2	3	4	10
100MG BRH/REF	74.90		50				21	
200MG BRH/REF	186.80		50				46	
25MG BRH/REF	32.40		50				12	
50MG BRH/REF	47.00		50				9	
SEAWATER/REF			50				1	

96 HOUR LC50 84.2(72.8-97.4)MG/L NO EFFECT CONCENTRATION LESS THAN 32.4 MG/L
ORGANISMS COLLECTED NARROW R. 830711, 3.39 +/- 0.47MM(N=25) SOLID PHASE REF
SEDIMENT BATCH 3, BOTTLES 3,4,12. MG/L DETERMINED BY DRY WEIGHT MEASUREMENTS.
(1) PERCENT FOR SOLID PHASE TESTS
(2) MILLIGRAMS/LITER FOR SUSPENDED PARTICULATE TESTS, DRY WEIGHTS OR COULTER COUNTS OR BOTH

TABLE B4
ACUTE TOXICITY DATA SHEET
COE/ERLN FVP

STUDY PLAN: 8 INVESTIGATOR: SCOTT/REDMOND

EXPERIMENT DESCRIPTION: SUSPENDED DATE OF TEST: 830711

TEST NUMBER: 2 CHRONIC CHAMBER SPECIES: AMPELISCA SP

** EXPERIMENTAL CONDITIONS **

TEMPERATURE: 20.00 DEGREES CENTIGRADE RANGE: 20.00 - 20.50
SALINITY: 32.00 PARTS PER THOUSAND RANGE: -
EXPOSURE DURATION: 4 DAYS
PHOTOPERIOD: 14 HOURS
FLOW RATE: 40 MLS/MIN VOLUME ADDITIONS/DAY 19
NUMBER OF ANIMALS/REPLICATE: 50
NUMBER OF REPLICATES/TREATMENT: 1
ANIMAL'S LIFE STAGE: JUVENILE AGE: DAYS SIZE: MILLIMETERS
CONTROLS: SEAWATER/SOUTH REFERENCE SOLID
FOOD USED NONE

EXPOSURE CONCENTRATIONS (1)		OXYGEN	ANIMALS USED PER TREATMENT		NUMBER DEAD AT DAY				
NOMINAL	MEASURED(2)	MG/L	4 DAY	10 DAY	1	2	3	4	10
100MG BRH/REF	92.00		50					25	
200MG BRH/REF	239.19		50					44	
25MG BRH/REF	33.79		50					10	
50MG BRH/REF	55.00		50					10	
SEAWATER/REF			50					2	

96 HOUR LC50 90.9(76.2-110.7)MG/L NO EFFECT CONCENTRATION LESS THAN 33.8 MG/L
ORGANISMS COLLECTED NARROW R. 830711, 3.39 +/- 0.47MM(N=25). SOLID PHASE REF
SEDIMENT [illegible]TCH 3,30TTLES 3,4,12. MG/L DETERMINED BY DRY WEIGHT MEASUREMENTS
(1) PERCENT FOR SOLID PHASE TESTS
(2) MILLIGRAMS/LITER FOR SUSPENDED PARTICULATE TESTS, DRY WEIGHTS OR COULTER COUNTS OR BOTH

APPENDIX C

Growth of *A. abdita* in Chronic Tests

Table C1

Mean Length (mm) and Standard Deviation of A. abdita
Exposed to BRH Suspended Particulates for 28 and 45 Days in Chronic Test 1*

Treatment	Replicate	FOV	FE	FDV	M	OTH
			28 Days			
REF	1	6.81 ± 0.58	7.32 ± 0.52	6.79 ± 0.49	6.54 ± 0.47	6.82 ± 0.61
	2	6.53 ± 0.58	7.03 ± 0.48	5.97 ± 0.69	6.56 ± 0.49	6.48 ± 0.45
		6.71 ± 0.58	7.15 ± 0.51	6.35 ± 0.72	6.55 ± 0.47	6.64 ± 0.55
5% BRH (1.9 mg/l)	1	7.30 ± 0.34	7.71 ± 0.67	6.35 ± 1.18	6.92 ± 0.43	7.06 ± 0.46
	2	6.26	5.81 ± 0.53	5.07 ± 0.59	6.15	5.20 ± 0.56
		7.09 ± 0.55	7.39 ± 0.96	5.18 ± 0.71	6.88 ± 0.45	5.95 ± 1.06
10% BRH (3.8 mg/l)	1	---	7.13 ± 0.52	6.10 ± 0.66	6.58 ± 0.08	6.45 ± 0.83
	2	---	6.18 ± 0.93	5.54 ± 0.56	---	5.76 ± 0.58
			6.94 ± 0.70	5.79 ± 0.67	6.58 ± 0.08	5.76 ± 0.79
			45 Days			
REF	3	7.55 ± 0.51	7.64 ± 0.44	7.32 ± 0.26	6.69 ± 0.50	7.74 ± 0.41
	4	7.34 ± 0.39	7.72 ± 0.76	5.53 ± 2.79	6.72 ± 0.49	6.66 ± 0.85
		7.46 ± 0.47	7.68 ± 0.63	6.43 ± 1.92	6.70 ± 0.49	7.38 ± 0.75
5% BRH (1.9 mg/l)	3	7.65 ± 0.44	8.14 ± 0.60	6.63 ± 0.89	6.92 ± 0.49	7.94 ± 0.57
	4	7.30 ± 0.40	7.66 ± 0.64	---	6.56 ± 0.45	---
		7.54 ± 0.46	7.97 ± 0.65	6.63 ± 0.89	6.81 ± 0.50	7.94 ± 0.57
10% BRH (3.9 mg/l)	3	6.92 ± 0.42	7.06 ± 0.59	6.16 ± 0.64	6.42 ± 0.37	6.36 ± 0.51
	4	7.07 ± 0.56	7.77 ± 0.67	6.08 ± 0.89	6.52 ± 0.41	6.98 ± 0.63
		7.01 ± 0.51	7.49 ± 0.72	6.15 ± 0.64	6.51 ± 0.40	6.55 ± 0.61

*N for each measurement is shown in Table 12.
Initial size of 100 A. abdita is 3.30 mm ± 0.51.

Table C2

Mean Length (mm) and Standard Deviation of A. abdita
Exposed to BRH Suspended Particulates for 32 and 58 days in Chronic Test 2*

Treatment	Replicate	FOV	FE	FDV	M	OTH
			32 Days			
REF	1	6.09 ± 0.24	6.39 ± 0.70	5.33 ± 0.27	5.51 ± 0.41	5.80 ± 0.52**
	2	6.53 ± 0.38	6.33 ± 0.77	5.28 ± 0.38	5.33 ± 0.31	5.67 ± 0.54**
		6.14 ± 0.29	6.37 ± 0.73	5.29 ± 0.36	5.50 ± 0.41	5.74 ± 0.53
5% BRH (2.0 mg/1)	1	6.67 ± 0.64	6.40 ± 0.65	5.21 ± 0.50	5.60 ± 0.43	5.49 ± 0.58**
	2	6.15 ± 0.78	6.35 ± 0.85	5.24 ± 0.41**	5.57 ± 0.31	5.46 ± 0.53**
		6.34 ± 0.76	6.39 ± 0.72	5.22 ± 0.46	5.59 ± 0.36	5.47 ± 0.55
10% BRH (4.5 mg/1)	1	6.50 ± 0.63	5.96 ± 0.50	4.60 ± 0.53	5.25 ---	4.67 ± 0.63**
	2	7.24 ---	6.49 ± 0.85	4.76 ± 0.51	---	4.27 ± 0.74**
		6.75 ± 0.62	6.04 ± 0.58	4.67 ± 0.53	5.25 ---	4.42 ± 0.73
			58 Days			
REF	3	7.16 ± 0.62	7.44 ± 0.63	7.02 ± 0.83	6.31 ± 0.60	7.37 ± 0.45
	4	7.26 ± 0.65	7.66 ± 0.51	7.32 ± 0.48	6.58 ± 0.63	7.26 ± 0.51
		7.21 ± 0.63	7.58 ± 0.56	7.18 ± 0.67	6.40 ± 0.62	7.33 ± 0.47
5% BRH (2.3 mg/1)	3	6.65 ± 0.79	6.96 ± 0.63	6.82 ± 0.89	6.54 ± 0.10	7.38 ± 0.99
	4	6.89 ± 1.05	7.01 ± 0.41	6.96 ± 0.64	6.13 ± 0.58	7.15 ± 0.48
		6.73 ± 0.87	6.99 ± 0.48	6.88 ± 0.77	6.24 ± 0.53	7.23 ± 0.69
10% BRH (5.0 mg/1)	3	6.65 ± 0.38	6.85 ± 0.58	6.36 ± 0.54	5.84 ± 0.45	6.56 ± 0.56
	4	6.05 ---	5.90 ± 0.69	5.45 ± 0.53**	5.43 ---	5.46 ± 0.56**
		6.57 ± 0.42	6.69 ± 0.69	5.80 ± 0.69	5.83 ± 0.45	6.15 ± 0.77

* N for each test is shown in Table 13.
**Indicates those samples which were subsampled with the Folsom plankton splitter.

END

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