910R93014A

United States
Environmental Protection
Agency

Region 10
1200 Sixth Avenue
Seattle WA 98101

Alaska
Idaho
Oregon
Washington

Water Division

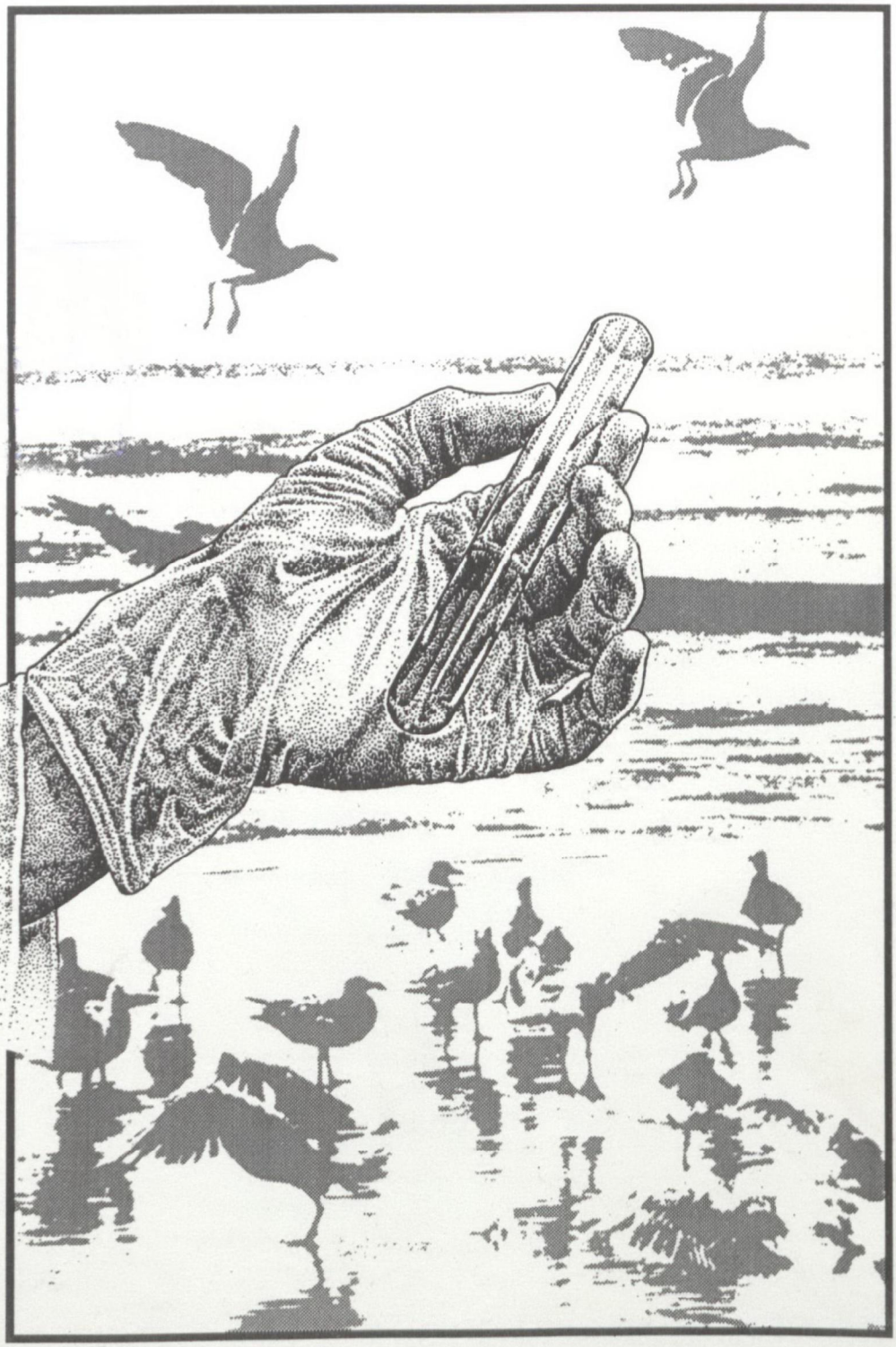
Surface Water Branch

August 1993

EPA

Refinements of Current PSDDA Bioassays

Final Report Summary



EPA DOCUMENT NUMBER: EPA 910/R-93-014 a

SUMMARY REPORT

REFINEMENTS OF CURRENT PSDDA BIOASSAYS

FINAL REPORT

EPA Contract No. 68-C8-0062
Work Assignment No. 3-57

March 19, 1993

Report No. 9210.003/B.016
SAIC Project No. 01-0098-03-1009

Submitted to:

U.S. Environmental Protection Agency
26 West Martin Luther King Drive
Cincinnati, Ohio 45268

Submitted by:

Science Applications International Corporation
Environmental Sciences Division
18706 North Creek Parkway, Suite 110
Bothell, WA 98011

An Employee-Owned Company

PROGRAM OVERVIEW

INTRODUCTION

Sediment larval bioassays are currently used under the Puget Sound Dredged Disposal Analysis (PSDDA) program to assist in the determination of proposed dredged material suitability for unconfined disposal at open water sites within Puget Sound. These bioassays are used as a screen for possible adverse biological effects that occur due to the presence of chemicals of concern within the test sediment. Included within the suite of tests employed within the PSDDA program are the larval sediment toxicity tests.

The Pacific oyster *Crassostrea gigas*, and the Northern Pacific sand dollar *Dendraster excentricus* account for most of the larval tests that have been conducted within the PSDDA program, as well as within other regulatory programs. Larval sediment toxicity tests are not only used in the PSDDA program, but also within Washington State's sediment quality standards (WAC 173-204) and as a part of dredged material testing programs throughout the U.S. Within the regulatory programs, both larval species are presumed to respond similarly to dredged material. There is no data concerning the relative response of these two organisms to varying conditions.

While larval test protocols have been well documented (ASTM, 1991, PSEP, 1992), these tests are thought to be sensitive (and may yield false positive effects) [1] to the entrainment of embryos by fine-grained test materials, and may be sensitive to the presence of ammonia that is frequently found in organic-rich Puget Sound sediments.

Suspended sediment effects include entrainment of test organisms by material settling in the chamber during the exposure, or any other potential suspended sediment-induced causes of larval mortality. The ideal sediment larval bioassay would measure the toxic response of a test organism to anthropogenic chemicals, and minimize or block effects from sediment conventional and physical influences. Within the PSDDA program, a reference sediment of similar grain size to the test material is included as a control for sediment grain size effects on the test organisms. However, these relatively "clean" reference sediments often have high mortalities exceeding recommended quality control standards for acceptable test results. This may in part, be due to physical effects such as interference in the test from larval entrainment by suspended solids, or to physiological stress due to small grain sizes. The use of varying grain sized sediments in exposures while maintaining the constancy of other variables could potentially contribute to the understanding of grain size effects.

Issues associated with false positive results from larval sediment bioassays were discussed at the PSDDA 1990 Annual Review Meeting (PSDDA Third ARM Minutes)[2]. Based on those discussions, a commitment was made by the PSDDA regulatory agencies to attempt to refine and resolve the issue of false positives in larval sediment toxicity tests.. The U.S. Environmental Protection Agency (EPA), Region 10, issued a Statement of Work to Science Applications International Corporation (SAIC), entitled *Refinements to Current PSDDA Bioassays*, dated July 31, 1991 that is intended to meet that commitment. The data presented in this report are the results of that SOW.

---

[1] A false positive condition occurs when the bioassay results indicate that a toxic response has occurred, but for reasons unassociated with sediment chemistry. Under these circumstances, the measured chemicals-of-concern in the sediment do not appear to be sufficiently high to explain the toxicological response, but the testing results indicate that significant mortality or abnormality have occurred within the test replicates.

[2] ARM Minutes, paragraph 9; Post-ARM Meeting Issue Resolution Summary, bullets on ML/SL adjustments and Effects of Grain Size, Ammonia, and Sulfides on AET Revisions.

SAIC was directed by EPA to focus its work on the two larval species that are frequently used in dredging programs; the Pacific oyster *Crassostrea gigas*, and the Northern Pacific sand dollar, *Dendraster excentricus*. Both of these organisms are found in Puget Sound, and occupy ecologically important niches. As such, the PSDDA program uses these two organisms as important indicators of possible deleterious effects due to dredged material disposal.

The objectives of the study were as follows:

1.) to determine the effect of ammonia on larval development. Determination of the LC50 and EC50 of ammonia to the two larval species will assist the PSDDA agencies in interpreting larval toxicity.

2.) to compare sensitivities of the sand dollar and oyster in both clean and contaminated sediments.

3.) to determine if a test protocol could be identified which minimizes the chance of false positive responses due to suspended sediment in the test chamber.

PROGRAM ORGANIZATION

The work plan was divided into a series of discrete phases that proceed in a linear fashion toward addressing EPA's objectives. These phases were as follows:

- **Phase I.** **Literature Search**
- **Phase II.** **Ammonia Effects on Bivalve and/or Echinoderm Species**
- **Phase IIIA.** **Species Sensitivity Comparison to Grain Size Effects**
- **Phase IIIB.** **Species Sensitivity Comparison to Contaminated Sediment Effects**

This report follows that format in presentation of test results. Each Phase is presented as a discrete document, with appropriate discussion of importance of the work, methods and materials, results, discussions, and recommendations based on the findings in the experiments. Each section builds on the data and information generated in the previous section.

Appendices for each phase are included with that section's report. Level II Quality Assurance Data for the analytical work conducted in Phase IIIB have been transmitted to EPA as a separate package.

The objectives of the study were as follows:

1.) to determine the effect of ammonia on larval development. Determination of the LC50 and EC50 of ammonia to the two larval species will assist the PSDDA agencies in interpreting larval toxicity.

2.) to compare sensitivities of the sand dollar and oyster in both clean and contaminated sediments.

3.) to determine if a test protocol could be identified which minimizes the chance of false positive responses due to suspended sediment in the test chamber.

PROGRAM ORGANIZATION

The work plan was divided into a series of discrete phases that proceed in a linear fashion toward addressing EPA's objectives. These phases were as follows:

- **Phase I.** **Literature Search**
- **Phase II.** **Ammonia Effects on Bivalve and/or Echinoderm Species**
- **Phase IIIA.** **Species Sensitivity Comparison to Grain Size Effects**
- **Phase IIIB.** **Species Sensitivity Comparison to Contaminated Sediment Effects**

This report follows that format in presentation of test results. Each Phase is presented as a discrete document, with appropriate discussion of importance of the work, methods and materials, results, discussions, and recommendations based on the findings in the experiments. Each section builds on the data and information generated in the previous section.

Appendices for each phase are included with that section's report. Level II Quality Assurance Data for the analytical work conducted in Phase IIIB have been transmitted to EPA as a separate package.

TABLE OF CONTENTS

PHASE I. LITERATURE SEARCH

PHASE II. AMMONIA EFFECTS ON BIVALVE AND/OR ECHINODERM SPECIES

List of Tables

List of Figures

PHASE IIIA. SPECIES SENSITIVITY COMPARISON TO GRAIN SIZE EFFECTS

List of Tables

List of Figures

PHASE IIIB. SPECIES SENSITIVITY COMPARISON TO CONTAMINATED SEDIMENT EFFECTS

List of Tables

List of Figures

REFINEMENTS TO CURRENT PSDDA BIOASSAYS

FINAL REPORT

PHASE I: LITERATURE REPORT

SAIC
An Employee-Owned Compa

PHASE I LITERATURE SEARCH

OVERVIEW

As part of its overall investigations concerning larval elutriate bioassays, the U.S. Environmental Protection Agency requested that SAIC conduct a review of the available literature prior to conducting the experiments described in Phases II and III. Specifically, the objective of this first phase was to gather information concerning recent findings on echinoderm and bivalve larvae comparability and sensitivity to ammonia, grain size or the existence of sediment in bioassay containers. Information gathered also included research relevant to sediment larval elutriate test concerns such as the possibility of false positive results due to the presence of suspended sediment in test chambers.

The literature review consisted of on-line literature searches, a survey of gray literature (unpublished data and reports), and a telephone survey in order to acquire information on recently published or unpublished reports and current research. This report documents the results of the surveys, and includes an annotated bibliography of relevant reports and a telephone inquiry list indicating the laboratories and individuals contacted and their relative responses. It is not intended to be an exhaustive search or review; but simply a presentation of information that is relevant to the subsequent experiments.

ON-LINE LITERATURE SEARCH

SAIC conducted an on-line literature search using the University of Washington Fisheries library system, the National Oceanic and Atmospheric Administration Library system, and the U.S. Environmental Protection Agency Library system in Seattle, Washington.

Key words used in the search are as follows:

ammonia, bioassay, bivalve, echinoderm, grain size, sediment elutriate test

Numerous references to ammonia toixicity to aquatic organisms were found in the literature. Selected references were downloaded from each library system and saved onto a personal computer disc. SAIC project toxicologists reviewed the literature search and obtained articles relevant to the Task Order. An annotated bibliography of references collected is presented below.

Bibliography

Ammonia-related Research

Ankley GT, Katko A, Arthur JW. 1990. **Identification of ammonia as an important sediment-associated toxicant in the lower Fox River and Green Bay, Wisconsin.** U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, Duluth, Minnesota 55804. Environmental Toxicology and Chemistry, Vol. 9. pp. 313-322.

Toxicity of sediment pore water from 13 sites in the lower Fox River/Green Bay watershed was assessed using a number of test species. Sediment pore water from the 10 lower Fox River sites exhibited acute toxicity to fathead minnows *(Pimephales promelas)* and *Ceriodaphnia dubia*, and pore water samples from all 13 sites were chronically toxic to *C. dubia*. Sediment pore water from seven of the sampling sites was toxic to *Selenastrum capricornutum*, but none of the samples were toxic to *Photobacterium phosphoreum*. Toxicity

characterization, identification and confirmation procedures indicated that a significant amount of the acute toxicity of the pore water to fathead minnows and *C. dubia* was due to ammonia. The identification of ammonia, a naturally occurring compound in sediments, as a potentially important sediment-associated toxicant has implications for sediment toxicity assessment and control, not only in the Fox River and Green Bay, but in other freshwater and marine systems as well.

Cardwell RD, Olsen S, Carr MI, Sanborn EW. 1979. **Causes of oyster larvae mortality in south Puget Sound.** Washington State Dept. of Fisheries, Olympia. NOAA Technical Memorandum ERL MESA-39, April 1979. 79 p.

Water samples were collected from the southern Puget Sound (SPS) basin in September 1977 and characterized for acute toxicity to Pacific oyster larvae (*Crassostrea gigas*), chemical composition, and biological composition. Certain receiving waters containing the dinoflagellates *Ceratium fusus* and *Gymnodinium splendens* were also tested specially to determine if they were toxic to oyster larvae, as was a laboratory culture of *C. fusus*. Toxicity tests of two sewage treatment plant effluents, ammonium chloride, and salinity were also conducted. The causes of oyster larvae mortality seemed clear from the laboratory and special receiving water bioassays of the dinoflagellates. Several multi-parameter statistical tests attempted to ferret and rank 16 biologic and chemical parameters in terms of their association with receiving water toxicity. Sewage plant effluents had such low toxicity that they could impart only localized toxicity in situ. The recurring receiving water toxicity problem in SPS is believed to affect, at a minimum, other species of bivalve molluscs. Evidence is presented suggesting the susceptibility of adult Pacific oyster, Olympia oyster (*Ostrea lurida*), and Manila littleneck clam (*Venerupis japonica*). (NOAA)

Fitt WK, Haymans DE, Coon SL. 1989. **Production and role of ammonia, an inducer of settlement of veliger larvae of oysters.** Dep. Zool., Univ. Georgia, Athens, GA. J. Shellfish Res.; vol. 8, no. 2, p. 456

Laboratory experiments with ammonium chloride have shown ammonia to be an inducer of settlement behavior of veligers of oysters in the genus *Crassostrea*. In spite of the fact that most animals and bacteria produce ammonia as a by-product of protein catabolism, natural levels of dissolved ammonia in seawater are typically low. This is confirmed in Georgia salt marshes, but increasing concentrations of ammonia/ammonium occur in proximity to the substrate. High concentrations have been documented from oyster beds in salt marshes. Eyed veligers exposed to oyster-conditioned seawater responded only to seawater containing >100 mu M ammonia/ammonium, suggesting that ammonia is a natural cue. As with other invertebrate larvae, veligers of oysters can be induced to settle by an adult-produced cue. A live and productive oyster bed, with its associated bacteria and assemblage of other invertebrates, has the potential of providing both settlement cues and appropriate substrate for veliger larvae.

Kingzett BC, Bourne N, Leask K. 1990. **Induction of metamorphosis of the Japanese scallop *Patinopecten yessoensis* Jay.** Dep. Fish. Oceans, Biol. Sci. Branch, Pacific Biol. Stn., Nanaimo, B.C. V9R 5K6, Canada. J. Shellfish Res.; vol. 9, no. 1, pp. 119-125.

Hatchery reared larvae of the Japanese scallop, *Patinopecten yessoensis*, were treated with different levels of neurotransmitters including, norepinephrine, epinephrine, L-DOPA, serotonin to test the ability of these compounds to increase percent metamorphosis in the absence of a suitable substrate. Thermal shock and the addition of ammonia were also tested for their effect on mature larvae. Norepinephrine, epinephrine and L-DOPA produced significant increases in percent metamorphosis. Results with ammonia were variable and significant increases in percent metamorphosis depended on concentration and exposure time. No consistent

significant increase in percent metamorphosis was observed when mature larvae were treated with serotonin or subjected to cold temperature shock.

Kobayashi N. 1980. **Comparative sensitivity of various developmental stages of sea urchins to some chemicals.** Biological Laboratory, Doshisha University; Kyoto, 602, Japan. Marine Biology 58, pp. 163-171.

The sensitivity to some chemical agents was examined comparatively at sperm, fertilization, cleavage, blastula, gastrula, pluteus and metamorphosis stages of a sand dollar from Japanese waters *(Peronella japonica)* and a sea urchin from the Pacific coast of Australia *(Heliocidaris erythrogramma).* These agents included Cu sulphate, ABS and NH_3 chloride. Responses observed included departures from control rates of fertilization and developmental reduction at the attainment of first cleavage, gastrula, pluteus or metamorphosis stages. Using minimum effective concentrations of the 3 chemicals at various developmental stages of *P. japonica,* it was found that sensitivity to chemicals varies from fertilization to metamorphosis. It seems that sperm activity is the most sensitive, and that fertilization and gastrulation are more sensitive than first cleavage, blastulation and pluteus formation. *H. erythrogramma* seems to show nearly the same responses to Cu, but is more sensitive at metamorphosis.

Pierson KB, Ross BD, Melby CL, Brewer SD, Nakatani RE. 1983. **Biological testing of solid phase and suspended phase dredged material from Commencement Bay, Tacoma, Washington.** Washington Univ., Seattle (USA). Fisheries Research Inst., 71 pp.

Sediments from nine sites in Blair and Sitcum Water-ways, Commencement Bay, were tested for potential acute chemical toxicity using chinook salmon *(Oncorhynchus tshawytscha)* smolts, Pacific oyster *(Crassostrea gigas)* larvae, and phoxocephalid amphipods. Survival of salmon smolts was not affected by 96 hr exposure to elutriates of up to one part per thousand by volume from 5 sites. Oyster larvae developed abnormal shells following 48 hr exposure using undiluted water drained from defrosted sediment from 4 sites, but were not affected by 1:5 dilutions; of artificially prepared elutriates. 240 hr exposure to sediments from each of the nine sites neither decreased survival of amphipods nor altered the time spent in the sediment or the amphipod's ability to rebury in sand. Ammonia-nitrogen concentrations in artificially prepared 1:5 elutriates at ambient pHs would be potentially toxic to salmonids and other fishes; therefore dredging methods that dilute the elutriate are recommended. An elutriate dilution of 1:1000 was shown to be safe; elutriate concentrations greater than 1:1000 could be toxic to salmonids and other fishes. Amphipod bioassays should not be used to assess potential chemical toxicity of dredged sediments until further research clarifies confounding factors such as anoxia and starvation.

PRC Environmental Management Inc., 1992. **Results of a spiked ammonia sediment porewater study.** Navy CLEAN Contract No. N62474-88-D-5086.

Ammonia spiked sediment bioassays were conducted with the polychaete *Nephtys caecoides;* and porewater elutriate bioassays with the mysid *Holmesimysis costata,* and the amphipod *Ampelisca abdita.* Results of the study indicate that sediment porewater ammonia levels have potential to cause toxicity to infaunal and epibenthic species. Porewater ammonia levels were highly toxic to mysids (less than 24-48 hours) and amphipods at 96 hours. The 96-hour LC_{50} as total ammonia was similar for both species, but the LC_{50} at 48 hours was significantly different between species. In spiked sediments under flow-through conditions, initial sediment ammonia levels were rapidly decreased and no significant toxicity was found in infaunal polychaete worms. In the future, it is suggested that polychaetes be tested under static renewal or static conditions to assess the toxic potential of sediment absorbed ammonia.

Sullivan BK, Ritacco PJ. 1985. **Ammonia toxicity to larval copepods in eutrophic marine ecosystems: A comparison of results from bioassays and enclosed experimental ecosystems.** Mar. Ecosystems Res. Lab., Grad. Sch. Oceanogr., Univ. Rhode Island, Narragansett, RI 02882. Aquat. toxicol.; vol. 7, no. 3, pp. 205-217.

In an experiment designed to simulate eutrophication of a shallow coastal ecosystem, nutrients were added to experimental ecosystems (MERL mesocosms) in six different treatment levels. The authors observed large reductions in the numbers of normally dominant copepods of the species *Acartia tonsa* and *A. hudsonica* associated with high concentrations of unionized ammonia (NH sub(3)) in the two most nutrient enriched treatments. Comparison of 48 h LC sub(50) values of 10-15 mu M multiplied by I super(-1) NH sub(3) obtained from laboratory bioassays with concentrations of NH sub(3) associated with increased mortality in the MERL tanks indicated that bioassay data correctly predicted trends of high and low mortality as well as fluctuations in the numbers of copepods in MERL tanks. Actual mortality rates of the mesocosm copepods was sometimes higher than predicted, however.

Sumathi VP, Chetty AN. 1990. **Ambient ammonia clearance by bivalve mollusc Lamellidens corrianus (Lea).** Dep. Zool., Sri Venkateswara Univ., Tirupati 517502, India. Environ. Ecol.; vol. 8, no. 4, pp. 1333-1334.

Freshwater mussels Lamellidens corrianus were used in ambient ammonia clearance for 5 days. The mussels cleared the ammonia efficiently during the 5 day period. The ammonia uptake may possibly be one of the indices of its biopurification potentials.

Viana ML de. 1987. Efecto de compuestos nitrogenados en el crecimiento de *Schizopera elatensis* (Copepoda: Harpaticoidea) **(Effect of nitrogenated compounds on the growth of *Schizopera elantensis* (Copepoda: Harpacticoidea)).** Univ. Nac. Salta, Buenos Aires 177, Salta, Argentina. An. Mus. Hist. Nat. Valparaiso.; vol. 18, pp. 21-27. Language - Spanish

The inhibitory effect of ammonia, nitrites and nitrates at different concentrations on the growth of *Schizopera elatensis* was analyzed. Compounds were added in two ways: at the beginning of the experiment and daily during the experiment. In the former, growth was inhibited; when the compounds were added daily, there was no growth inhibition. Ammonia inhibited growth at all concentrations. Nitrites affected mainly the nauplii stage. Ammonia and nitrates affected all development stages. The results are compared with other reports on crustaceans, fishes and larvae.

Walch M. Dagasan L, Coon SL, Weiner RM, Bonar DB, Colwell RR., 1988. **Mechanisms of microbial induction of oyster larval settlement behavior and metamorphosis.** Cent. Mar. Biotechnol., Univ. Maryland, Baltimore, MD. First International Symposium on Marine Molecular Biology, October 9-11, 1988, Baltimore Maryland.; vp

A close relationship exists between specific bacterial films on inert substrata and settlement of competent larvae of the oysters *Crassostrea gigas* and *C. virginica*. Products of one particular bacterium, *Alteromonas colwelliana*, are especially active in inducing spat set. Identification of the soluble metabolites of *A. colwelliana* that induce search behavior has revealed at least two different kinds of active compounds. One is ammonia, a product of amino acid degradation. The other is a group of closely related products of the enzyme tyrosinase, including L-dihydroxyphenylalanine (L-DOPA) and one or more trihydroxyphenylalanines.

Wang X-Y, Zhang D-H, Ji D-R, Zhang S-Q. 1985. **The toxic effect of ammonia on larvae and juveniles of oyster *(Crassostrea gigas)*.** Shandong Mar. Cultivation Inst., Qingdao, People's Rep. China. Trans. Oceanol. Limnol./Haiyang Huzhao Tongbao.; no. 4, pp. 66-71. Language - Chinese

Role of ammonia in toxicity tests used in evaluation of dredged material. 1992. U.S. Environmental Protection Agency, Task 3 of Work Assignment #13.

A 96 hour range find and two 96 hour definitive assays were completed as water-only assays to determine the level of toxicity of unionized ammonia to *Ampelisca abdita*. The geometric mean for the two 96 hour definiitive LC_{50} is 2.27 mg/l unionized ammonia. A 10 day solid phase assay with *Ampelisca abdita* shows a correlated dose response with toxicity of unionized ammonia. The LC_{50} of unionized ammonia for overlying water is 1.29 mg/l. Ammonia porewater values indicate that the amphipods were exposed to ammonia through both the porewater and overlying water. However, the lower pH of the sediment porewater (7.2-7.5) as compared to the overlying water (8.06-8.09) had a strong influence on the concentration of unionized ammonia. The LC_{50} calculated with the porewater unionized ammonia values was 0.21 mg/l.

Ambient aquatic life water quality criteria for ammonia. U.S. Environmental Protection Agency, Office of Research and Development, Environmental Research Laboratory, Duluth Minnesota.

This document presents a comprehensive report on the effects of ammonia on freshwater and marine organisms. Within this document, the following data pertaining to marine bivalves and echinoderms is presented: The LC_{50} for the Eastern oyster, *Crassostrea virginica* is 24-37 mg/L NH_3 at 46-62 mm in length, 8.3-13 mg/L at 13-17 mm. The mean acute value is reported at 18.3 mg/L NH_3. The LC_{50} for the Quahog clam, *Mercenaria mercenaria* is 3.2-5.0 mg/L NH_3 at 28-33 mm in length, 4.6-7.2 mg/L at 4.7-5.2 mm in length. The mean acute value is reported as 5.01 mg/L NH_3. For the mussel, *Mytilus edulis*, exposure of 0.097 mg/L NH_3 for ≤ 1 hour caused 50% reduction in ciliary beating rate. 0.11 mg/L exposure for ≤ 1 hour caused 90% reduction. 0.11-0.12 exposure caused complete inhibition of cilia.

Research Relevant to Sediment Grain Size and to Elutriate Tests

Daniels SA, Munawar M, Mayfield CI. 1989. **An improved elutriation technique for the bioassessment of sediment contaminants.** In Environment bioassay techniques and their application., pp. 619-631; Hydrobiologia., vol. 188-189. ed. by Munawar M. Dixon G., Mayfield CI, Reynoldson T, Sadar MH. Res. and Appl. Branch, Natl. Water Res. Inst., CCIW, P.O. Box 5050, 867 Lakeshore Rd., Burlington, Ont. L7R 4A6, Canada.

An improved method is proposed for the preparation of sediment elutriates which permits relatively realistic determination of bioavailable contaminants. It suggests the use of rotary tumbling in a cycle of 3-4 rpm to achieve sediment-water mixing. Experiments were undertaken to evaluate the mixing efficiency of the rotary tumbler as compared to that of the compressed air, wrist-action shaker, and reciprocal shaker methods. Sediment to water ratios of 0:1, 1:20, 1:10, and 1:4 were tested over 0.5, 1.0, 24, and 48-h elution periods. Elutriate evaluations were based on chemical, physico-chemical and gravimetric determinations; and also on super(14)C-phytoplankton bioassays using *Chlorella vulgaris* (Beyerinck). Results indicated that rotary tumbling produced the most consistent bioassay-supportable data. It was also the most efficient procedure when used for 1 h with 1:4 sediment-water mixtures.

Davis HC. 1960. **Effects of turbidity-producing substances in sea water on eggs and larvae of the clam *(Venus (Mercenaria) mercenaria)*.** U.S. Fish and Wildlife Service, Milford, Conn. Biological Bulletin; vol. 118, pp. 48-54.

The effects of several concentrations of silt, clay (kaolin), Fuller's earth, and chalk on development of the eggs of the hard clam *(Venus (Mercenaria) mercenaria)* and the effect on the survival and growth of their larvae are reported. Although some clam eggs developed normally in concentrations of 4.0 g/l of clay, chalk or finely ground Fuller's earth, the percentage decreased as the concentration of these suspended materials increased. In silt concentrations of 0.75 g/l or lower, the percentage of clam eggs developing normally was not significantly different from that in control cultures, but decreased progressively in successively higher concentrations. Results appear to indicate that larger particles (coarse silt 62-31 microns) have the greatest effect on clam egg development and larval growth.

Davis HC, Hidu H. 1969. **Effects of turbidity-producing substances in sea water on eggs and larvae of three genera of bivalve mollusks.** Bureau of Commercial Fisheries, Milford, Conn. Biological Lab. The Veliger; vol. 11, no. 4, pp. 316-323.

As little as 0.188 g/l of silt caused a significant decrease in the percentage of american oyster *(Crassostrea virginica)* eggs developing normally, as did 3 g/l of kaolin (silt, clay) or 4 g/l of Fuller's earth (dusting powder). The percentages of oyster eggs developing normally was not affected by concentrations of silicon dioxide of 4 g/l, regardless of particle size. Clam eggs were affected only at 4 g/l of the smallest particles (less than 5 microns). These smallest particles of silicon dioxide had, however, the greatest effect on survival and growth of clam and oyster larvae. Larger particles (5-25 microns and 25-50 microns) had little effect on survival of either species or on growth of clam larvae. Growth of oyster larvae decreased progressively as the size of silicon dioxide particles was decreased. Bivalve larvae grew faster in low concentrations of turbidity-producing substances than in clear seawater, possibly because the suspended particles chelate or adsorb toxins present in larval cultures. (Legore-Washington)

Kloechner K. Rosenthal H, Willfuehr J. 1985. **Invertebrate bioassays with North Sea water samples. 1. Structural effects on embryos and larvae of serpulids, oysters, and sea urchins.** Biol. Anst. Helgoland (Zent.), Notkest. 31, D-2000 Hamburg 52, FRG. Helgol. Meeresunters.; vol. 39, no. 1, pp. 1-19.

Structural effects of bottom and surface water samples from two dumping grounds in the inner German Bight on the development of three meroplanktonic organisms *(Pomatoceros triqueter:* Polychaeta, *Psammechinus miliaris:* Echinodermata, and *Crassostrea gigas:* Mollusca) were investigated. the titaniumdioxide dumping site was sampled immediately after dumping (within the visible waste trail 1 km behind the vessel), and 10 h after dumping. Samples were taken in the sewage sludge deposition area in the intervals between the usual dumping activities, regardless of the exact dumping schedule. The preserved bioassay test organisms were inspected microscopically to count percentages of "normal" larval hatch in test water samples, reference water samples, and laboratory aged control water samples (5 to 10 replicates). The relative water quality of various dumping sites was expressed in terms of "net risk" -values (Woelke, 1972) compared to hatching rates observed in the controls.

Meador JP, Ross BD, Dinnel PA, Picquelle SJ. 1990. **An analysis of the relationship between a sand-dollar embryo elutriate assay and sediment contaminants from stations in an urban embayment of Puget Sound, Washington.** NMFS, Environ. Conserv. Div., 2725 Montlake Blvd. E., Seattle, WA 98112. Mar. Environ. Res.; vol. 30, no. 4, pp. 251-272.

A sand-dollar embryo test was used to assess the toxicity of contaminants in sediment elutriate samples from Puget Sound, Washington. A synoptic chemical data set of priority pollutants was reduced and subjected to combinatorial clustering which grouped stations by the amount of chemicals present. Clustering was done for metals and organic compounds together and separately. Analysis of variance revealed that the embryo test was able to predict the group of stations considered least contaminated by organic chemicals but not for metals, although copper and lead could not be excluded due to confounding effects. The results generally support the additivity hypothesis of toxicity in that as total contamination increased toxicity increased. Due to a possible change in redox conditions or the release of bio-organically bound metals, it was concluded that the elutriate test may not be appropriate for assessment of metal contaminants associated with sediment.

Ramsdell KA, Strand JA, Cullinan VI. 1989. **Amphipod bioassay of selected sediments from Sequim Bay, Washington.** Northeastern Illinois Univ., Chicago, IL 60625. Marine Technology Soc., Washington, DC., Institute of Electrical and Electronics Engineers, New York, NY. Oceans '89: The Global Ocean. Volume 2: Ocean Pollution. pp. 443-448; Oceans '89.

Amphipod *(Rhepoxynius abronius)* bioassays performed in surveys of Sequim Bay suggested possible sediment toxicity at three sites. These findings were not supported by other biological analyses and tests (dominant infauna, oyster larvae test) nor by the finding of relatively low levels of priority pollutants. A re-examination of the sites demonstrated that the Sequim Bay sediments were clearly nontoxic. Mean survivorship ranged from 89 to 100%. It was hypothesized that earlier indications of toxicity may have been due to a relatively high percentage of fines (greater than or equal to 80%) and/or a relatively low interstitial salinity (24 ppt) encountered at one or more of the 1983-1984 sites. The continued use of Sequim Bay as both a reference bay and a source on control sediment in future marine research is recommended.

Dinnel, Paul A. 1990. **Annotated bibliography of bioassays related to sediment toxicity testing in Washington State.** Fisheries Research Institute, University of Washington, School of Fisheries. US Army Corps of Engineers, Seattle District, Seattle, WA: Contract No. E318900PD

Jones, R. Anne. 1978. **Evaluation of the elutriate test as a method of predicting contaminant release during open-water disposal of dredged sediments and environmental impact of open-water dredged material disposal.** Vol I: discussion: final report. US Army Corps of Engineers; US Army Engineer Waterways Experiment Station; University of Texas at Dallas. US Army Engineer Waterways Experiment Station.

Lee, G. Fred. 1978. **Evaluation of the elutriate test as a method of predicting contaminant release during open-water disposal of dredged sediments and environmental impact of open-water dredged material disposal.** Vol II: data report: final report. US Army Corps of Engineers; US Army Engineer Waterways Experiment Station; University of Texas at Dallas. US Army Engineers Waterways Experiment Station.

Factors influencing the development of Pacific oyster larvae in 48-hour bioassays of spent sulfite liquor. National Council for Stream Improvement, Inc.; EPA Dallas, TX: DOC NCASI 115

Development of a modified elutriate test for estimating the quality of effluent from confined dredged material disposal areas. US Army Engineer Waterways Experiment Station; EPA Boston, MA: DOC 01A0004899

Biological assessment of the soluble fraction of the standard elutriate test final report. 1977. US Army Corps of Engineers, Waterways Experiment Station, Environmental Effects Laboratory;

Other Relevant Studies

Okubo K, Okubo T. 1962. **Study on the bioassay method for the evaluation of water pollution-II. Use of the fertilized eggs of sea urchins and bivalves.** Bulletin of the Tokai Regional Fisheries Research Laboratory, No. 32, pp. 131-140. English Summary.

A method of bioassay has been developed by using artificially fertilized eggs of sea urchins and bivalves as test organisms. The procedures of bioassay with fertilized eggs of the test organisms are described and the results obtained for various pollutants are compared. The method proposed in this report is advantageous because the effects of pollutants on the development of eggs were easily recognizable in disturbed metamorphosis. Four species tested, two species each for sea urchins and bivalves, were similar in sensitivity to pollutants. The sensitivity of the present test organisms to pollutants was much higher than that shown by other test organisms. The morphologically ineffective concentration of some pollutants for the embryonic development of sea urchins and bivalves may be directly equal to the safe level of the pollutant concentration for littoral fishes. (Katz-Washington)

Phelps HL, Warner KA. 1990. **Estuarine sediment bioassay with oyster pediveliger larvae *(Crassostrea gigas)*.** Biol. Dep., Univ. District Columbia, Washington, DC 20008. Bull. Environ. Contam. toxicol.; , vol. 44, no. 2, pp. 197-204.

There are several standard bioassays for toxicants in water but few for sediment. the pediveliger larva of the Pacific oyster, *Crassostrea gigas,* was explored as a sediment bioassay organism because of nearly year round commercial availability, and common use in previous work with culture and toxicity testing. One comparison bioassay was also made with the native Chesapeake Bay species, *Crassostrea virginica,* which may become more readily available in the future.

Considerations in selecting bioassay organisms for determining the potential environmental impact of dredged material. 1981. US Army Engineers Waterways Experiment Station.

Development and validation of a field bioassay method with the Pacific oyster, *Crassostrea gigas,* embryo: a thesis submitted in partial fulfillment of the requirements for the degree Doctor of Philosophy. EPA HQ, Wash, DC: BKS QH91.57.B5W6

GRAY LITERATURE SEARCH

Gray literature is defined as federal, state, local or private-entity sponsored research that is not generally distributed to public library systems. Inquiries were made by phone and mail to the following institutions that are known to sponsor or be repositories of such work, using the key search words identified above: ammonia, bioassay, bivalve, echinoderm, grain size, sediment elutriate test

- Woods Hole Marine Biological Library

  Susan Bertraux
  Woods Hole Oceanographic Institute
  Woods Hole, MA 02543
  508-543-1400 ext.2269

  Informed that use of library requires fee and search must be done in person. Was suggested that local SAIC office near Woods Hole be contacted to do search.

- Environment Canada

  Telephone inquiries have not been acknowledged.

- Environmental Protection Agency

  Literature search performed at the Seattle EPA Library using the EPA's Online Library System (OLS), National Catalog

No information was found in this effort.

TELEPHONE INQUIRIES

Telephone inquiries were made to both public and private laboratories in order to gather information concerning recent findings on echinoderm or bivalve larval comparability or sensitivity to ammonia, grain size, or the existence of sediment in bioassay containers. Questions asked of each individual contacted are as follows:

1. Has your organization been involved in any research associated with the following topics?

   a. The effects of ammonia on either bivalve or echinoderm larval species (especially development).

   b. The effects of grain size distributions or the amount of suspended sediment in sediment elutriate tests using either bivalve or echinoderm larval species.

   c. Research comparing the relative sensitivities of bivalve vs. echinoderm larval species.

   d. Any other research relevant of the issue of sediment larval elutriate tests (i.e., the possibility of false positive results due to the presence of suspended sediment in the test chamber).

2. If yes are copies of those reports available?

3. If those reports represent private clients, to whom would EPA need to address a request for information release?

4. Are you aware of any other person or institute conducting relevant or related research to whom we could address an inquiry?

For each inquiry, a telephone log containing the above questions was completed. A list of the organizations and individuals contacted is provided below. A brief synopsis of conversations with each individual is also included.

Batelle Pacific Northwest Laboratories, Sequim, WA.

Dr. Jack Word - 206-683-4151

prefers the Green Book methods - found that sediment in the chambers hinders an accurate count of the organisms for survival and abnormality assessments - performed a "quick and dirty" evaluation using oyster larvae - found low survival when using the PSEP method and a much higher survival using the Green Book method for the same sediment tested - with respect to sensitivity, they found that there was a greater sensitivity (higher mortality and abnormality) in the controls, during certain periods of the year, if water was collected near shore - this occurred even if water was filtered - suspected toxins from dinoflagellates - if water was collected off shore in the straights (where diatoms were prevalent instead of the dinoflagellates) during these periods, there was no toxic response in the controls

California Marine Pollution Lab, Granite Canyon, Monterey, CA.

Mr. Brian Anderson - 408-624-0947

generally have used sediment toxicity tests to indicate contamination - have made crude ammonia measurements using HACH kit - found that in some of the controls which exhibited a toxic response, there were high levels of ammonia - this data was submitted to the San Francisco Regional Water Resources Control Board (project manager Karen Tabersky)have done sediment elutriate tests using oyster larvae and a small number of pore water extraction tests using echinoderms

EBASCO Environment, Bellevue, WA.

Frank S. Dillon - 206-451-4500

has studied freshwater molluscs conducting filtering assays using environmental samples - have found a correlation between filtering rates and presence of ammonia - found detrimental effects from low levels of ammonia over long periods - have used pH levels as an indicator of the amount of the non-ionized ammonia fraction - has not done work on sediments (suspended particles or grain size) - generally uses centrifuge extraction to obtain the interstitial water. Mr. Dillon provided copies of the following articles:

Zischke JA, Arthur JW. 1987. Effects of elevated ammonia levels on the fingernail clam, *Musculium transversum*, in outdoor experimental streams. Arch. of Environ. Contam.

Toxicol., 16:225-231.

Arthur JW, West CW, Allen KN, Hedtke SF. 1987. Seasonal toxicity of ammonia to five fish and nine invertebrate species. Bull. Environ. Contam. toxicol., 38:324-331.

Environmental Protection Agency (EPA)

Athens, Georgia, EPA Region 4; Bill Peltier - 404-546-2296, main office number - 404-546-2294
mainly deals with bioassays strictly as a regulatory measure - does not really work with research aspects

Environmental Research Lab, Duluth, MN: Gerald T. Ankley - 218-720-5603
did not have information regarding these issues

Gulf Breeze Laboratory, FL: Barbara Albrecht - 904-934-9351
currently not doing any work with echinoderm or bivalve larvae; has been aware of effects on urchin larvae in the past, although is not aware of specific reports from their laboratory documenting this concern

Manchester laboratory, WA: Joe Cummins - 206-895-4347 and Margaret Stinson - 206-871-8821
did not have information regarding these issues

Newport, OR: Gary A. Chapman - 503-867-4041 and Rick Swartz - 503-867-4031
did not have information regarding these issues - not involved at this time with any work pertaining to echinoderm and bivalve larvae, or elutriate tests

EVS Consultants, Ltd., North Vancouver, B.C.

Dr. Peter M. Chapman - 604-986-4331
involved in research concerning some of the topics although the information was confidential - expects it to be released in June 1993

Fisheries Research Institute, University of Washington, Seattle, WA.

Dr. Paul Dinnel - 206-543-7345
currently involved in revising/writing ASTM bioassay protocols - has been more involved in effluent studies and water column assays at this time - is not aware of current research being performed regarding the issues of concern

Gulf Coast Research Lab, Ocean Springs, MS.

Dr. Tom Lytle - 601-875-2244
is expecting to begin studies in association with the Gulf Breeze Labs including field validation and sediment tests to improve bioassay techniques, and revising and developing laboratory and field sediment bioassays - some of the work will focus on bio-availability of particular compounds and sediment structure as it affects bioavailability - will also be examining sediment in association with the organisms's life stages

Dr. William Walker - 601-872-4261

working with water column chemicals and effects on early life stages of fish, as well as shell deposition and its effects on oysters - is not aware of anyone currently conducting research on the topics of concern

Jo Ellen Hose, Shell Beach, CA - 805-773-6715

has researched the development of echinoderm and bivalve larvae, but has not examined larval sensitivity to ammonia or grain size

MEC Analytical Systems, Inc., San Rafael, CA.

Mark J. Burke - 415-435-1847

currently involved in TIE form using echinoderms - sanitation effluent studies - is predicting that ammonia is a problem

National Council for Air and Stream Improvement (NCASI), Shannon Point Marine Center, Anacortes, WA.

Mr. Tim Hall - 206-293-4748

generally have been working with effluent studies and not sediments - has done some work with methodology concerning sperm and egg ratios, fertilization, and stocking density - also found that if they used on-line filter systems in which there is dead water for a period of time, their echinoderms did not do well - suspected toxicity was due to hydrogen sulfide - was not a problem when used flow through systems

National Oceanographic and Atmospheric Administration (NOAA), Seattle, WA.

Dr. Ed Casillas - 206-553-7740

currently not doing research with larvae - have done some studies with the reproductive stages - juvenile to adult - have monitored ammonia and sulfides

Mr. Ed Long - 206-526-6338

involved in studies using the fertilization success of sea urchins as a toxicity test - extremely sensitive - studies using undiluted pore water - results of analysis of ammonia in the pore water indicated that there was a poor correlation (R^2 = .100) with toxicity (using undiluted pore water) and sea urchin fertilization - unpublished information at this time - will be released in spring of 1993 - this work is with Dr. R. Scott Carr, U.S. Fish and Wildlife, Corpus Christi, Texas

does not feel ammonia is the problem - feels it is more of a regional or site specific issue other work has/will included studies in San Francisco Bay using the Green Book methods (reference provided below), and other urchin fertilization tests in Tampa Bay and Galveston (EPA, Region 6) - mentioned work by Jo Ellen Hose with echinoderm and bivalve larvae looking at subcellular abnormality endpoints for bioassays

Long ER, Markel R. 1992. An evaluation of the extent and magnitude of biological effects associated with chemical contaminants in San Francisco Bay, California. NOAA Technical Memorandum NOS ORCA 64. Seattle, WA.

South California Coastal Water Research Project, Long Beach, CA.

Steven M. Bay - 310-435-7071

has done some work with echinoderm larvae and sperm in interstitial water has a report in press which includes ammonia studies with echinoderm larvae - is sending this information as well as a report completed for NOAA comparing 5 bioassays in San Francisco:

Long ER, Buchman MF, Bay SM, Breteler RJ, Carr RS, Chapman PM, Hose JE, Lissner AL, Scott J, Wolfe A. 1990. Comparative evaluation of five toxicity tests with sediments from San Francisco Bay and Tomales Bay, California. Environmental Toxicology and Chemistry, 9:1193-1214

Bay S, Burgess R, Nacci D. in press. Environmental toxicology and risk assessment (1st symposium). Status and applications of echinoid (phylum Echinodermata) toxicity test methods.

Mr. Bruce Thompson - 310-435-7071

is involved in some work with echinoids and ophiuroids and their sensitivity to contaminants in sediments; current research includes examining sensitivity of urchins to interstitial sulfides - doing ammonia work with urchin sperm in straight water tests - is not aware of research occurring with bivalves or sand dollars in southern California related to sediment bioassays

Toxscan, Inc., Watsonville, CA.

Dr. Phil Carpenter - 408-724-4522

have performed some studies concerning grain size effects, in particular with *Ampelisca*

U.S. Fish and Wildlife, Corpus Christi, TX.

Dr. Scott Carr - 512-888-3366

have performed work with echinoderms and their sensitivities to ammonia and grain-size mentioned sperm cell test - prefer use of pore-water rather than elutriate

WES, Vicksburg, MS.

Mr. Tom Dillon - 601-636-3111

not currently involved with any work concerning echinoderm and bivalve larvae - have done work in fresh water with elutriate testing - trying to determine physical and chemical factors in mortality

Telephone Inquiry List

Batelle Pacific Northwest Laboratories, Sequim, Wa.
Dr. Jack Word - 206-683-4151

California Marine Pollution Lab, Granite Canyon, Monterey, CA.
Mr. Brian Anderson - 408-624-0947

EBASCO Environmental, Bellevue, WA.
Frank S. Dillon - 206-451-4500

Environmental Protection Agency (EPA)

Athens, Georgia, EPA Region 4:	Bill Peltier - 404-546-2296, main office number - 404-546-2294
Environmental Research Lab, Duluth, MN:	Gerald T. Ankley - 218-720-5603
Gulf Breeze Laboratory, Florida:	Barbara Albrecht - 904-934-9351
Manchester Laboratory, WA:	Joe Cummins - 206-895-4347 Margaret Stinson - 206-871-8821
Newport, OR:	Gary A. Chapman - 503-867-4041 Rick Swartz - 503-867-4031

EVS Consultants, Ltd., North Vancouver, B.C.
Dr. Peter M. Chapman - 604-986-4331

Fisheries Research Institute, University of Washington, Seattle, WA.
Dr. Paul Dinnel - 206-543-7345

Gulf Coast Research Lab, Ocean Springs, MS.
Dr. Tom Lytle - 601-875-2244
Dr. William Walker - 601-872-4261

Jo Ellen Hose, Shell Beach, CA - 805-773-6715

MEC Analytical Systems, Inc., San Rafael, CA.
Mark J. Burke - 415-435-1847

National Council for Air and Stream Improvement (NCASI), Shannon Point Marine Center, Anacortes, WA.
Mr. Tim Hall - 206-293-4748

National Oceanographic and Atmospheric Administration (NOAA), Seattle, WA.
Dr. Ed Casillas - 206-553-7740
Mr. Ed Long - 206-526-6338

South California Coastal Water Research Project, Long Beach, CA.
Steven M. Bay - 310-435-7071
Mr. Bruce Thompson - 310-435-7071

Toxscan, Inc., Watsonville, CA.
Dr. Phil Carpenter -408-724-4522

U.S. Fish and Wildlife, Corpus Christi, TX
Dr. Scott Carr - 512-888-3366

WES, Vicksburg, MS.
Mr. Tom Dillon - 601-636-3111

REFINEMENTS TO CURRENT PSDDA BIOASSAYS

FINAL REPORT

PHASE II: AMMONIA EFFECTS ON BIVALVE AND/OR ECHINODERM SPECIES

SAIC
An Employee-Owned Company

List of Tables

List of Figures

PHASE II AMMONIA TOXICITY TO LARVAL SPECIES

INTRODUCTION

Ammonia has long been recognized as a toxicant to aquatic organisms. Numerous experimental studies and reviews concerning ammonia toxicity exist in the literature (see EPA, 1985). The Environmental Protection Agency utilized many of these published results to establish a water quality criterion for ammonia (EPA, 1985). The criterion document, and published values for ammonia toxicity to a wide range of organisms, are useful for interpreting water-column toxicity effects in regulatory effluent bioassays.

By contrast, the role of ammonia in sediment toxicity bioassays has only recently begun to be investigated. In a literature survey, only the paper by Ankley et al. (1990) discussed ammonia as an important sediment-associated toxicant in freshwater sediments.

Within the context of regulatory dredged sediment testing, understanding the conditions under which sediment-associated ammonia can cause significant bioassay responses, is of particular importance. Biological testing of dredged sediments for disposal suitability decisions is based on the assumption that positive (mortality) responses are correlated with the presence of contaminants of concern in the test sediments. Positive responses in the bioassays trigger a regulatory decision that the material is unsuitable for either open ocean or deep water disposal. Guidelines for making a suitability decision for open water disposal are defined under the Puget Sound Dredged Disposal Analysis program (PSDDA) based on statistically significant percentage differences as compared to reference sediment responses. Guidelines defined in EPA's manual for *Evaluation of Dredged Material Proposed for Ocean Disposal* (1991) utilize statistical difference, relative to the control or reference sediment.

Ammonia is not considered to be a chemical of concern. Failure of a bioassay due to the presence of ammonia, is not viewed by the resource agencies as sufficient reason for rejecting dredged sediment for open water disposal. However, in the absence of information on the conditions under which ammonia can be identified as causal agent, the regulatory agencies will reject for open-water disposal tested material that has exceeded the bioassay interpretive guidelines for suitability.

The U.S. Army Corps of Engineers Dredged Material Management Office, Seattle District conducted statistical analyses on echinoderm larval data submitted by bioassay laboratories under the PSDDA program. In that study, the test sediments were found not to have elevated levels of the 58 PSDDA chemicals-of-concern, but yet demonstrated significant biological responses, exceeding the PSDDA guidelines. Their study found statistical correlations (α = 0.05, r^2 = 0.91) between levels of echinoderm mortality, and reported levels of aqueous total ammonia (USACE, 1991). Furthermore, those analyses indicated that when test vessels were aerated, the apparent toxic effects of ammonia were ameliorated. Those relationships have subsequently been used in the PSDDA program in interpreting anomalous false positive bioassay data. In addition, all larval bioassays are currently conducted using aeration in test vessels.

The resource agencies implementing the PSDDA[1] program identified the need to have corroborative, experimentally-derived, ammonia toxicity data to support their decision making process. Furthermore, data are required for the two larval tests organisms principally used in dredged sediment characterization programs, the Pacific oyster *Crassostrea gigas*, and the sand dollar *Dendraster excentricus*. While other molluscs and echinoderms are used (eg., mussels or sea urchins), oysters and sand dollars to date have made up the bulk of sediment testing programs.

---

[1]Those agencies include the Environmental Protection Agency, the Corps, and the Washington Departments of Ecology, and Natural Resources.

The objectives of this study were as follows:

- To establish the No Observed Effect Concentration (NOEC) and both the Lethal Concentration (LC) and Effective Concentration (EC) to 20%, 30%, and 50% of oyster and sand dollar larvae.

- To determine if simple aeration of the experiment vessel during the test could affect the test results.

METHODS AND MATERIALS

TEST OVERVIEW

Unless otherwise specified, ammonia will be taken to mean the total measured complex of NH_3 and NH_4. Maintaining that convention simplifies comparisons to the selected range of nominal spiked concentrations. However, it must be emphasized that the unionized form (NH_3) is comprises most of the toxicity, and that the amount of unionized ammonia present in the system is dependent upon the temperature, pH, and salinity of the test system. Unionized values will be given for the NOEC, LC, and EC discussions.

While it was the original intent of this study to conduct the oyster and sand dollar exposures concomitantly, gravid broodstock oysters were unavailable, or larval survival was poor during the late winter/early spring, when the program was initiated. The initial experiment that provided the sand dollar data was set up with both species, but oyster larval survival did not meet the PSEP-prescribed survival guideline of 50%.

The sand dollar larval exposures were conducted at the laboratory of Parametrix, Inc., at its facilities in Bellevue, WA in January, 1992. The oyster exposures were performed at SAIC's Environmental Testing Center in Narragansett, Rhode Island in June, 1992. The basic protocols followed at both facilities were those described in the *Puget Sound Estuary Protocols (PSEP, 1991)* for oyster and sand dollar larvae. Procedures followed during the testing at each facility, are described below.

Dendraster excentricus

Sample Preparation

The test dilution series was set at a range of nominal 0.0, 0.28, 0.63, 1.25, 2.5, 5.0, and 10 mg/L as NH_3-N. This dilution series was based on previous in-house work that had found an EC50 of between nominal 0.275 and 0.625 mg/L NH_3-N. Ammonium chloride was obtained from the Sigma Chemical Corporation (ACS Reagent Grade). A 10,000 ppm NH_3-N stock solution was prepared by dissolving the ammonium chloride in distilled water, using a magnetic stir bar to ensure complete dissolution. The stock solution was prepared fresh the day prior to the exposures.

Seawater was collected from deep, upwelled water approximately 800 meters offshore of Duwamish Head in Seattle, Washington. Water was collected via a submersible pump from a depth of 15 meters, and transported back to the laboratory in well-seasoned polyethylene containers. At the lab the seawater was filtered to 0.22 μ, passed through an ultra-violet sterilizer, and the salinity adjusted to 28 ppt using deionized water. Water for testing was used within 8 hours of collection. Quarterly priority pollutant scans conducted at the lab have shown no chemicals of concern detected in the seawater.

Borosilicate glass jars were used for the exposure containers, with 900 mL of the filtered-adjusted seawater. In these exposures, the ammonium dilutions were prepared by dispensing a pre-determined

volume of the stock solution into each replicate container. To approximate conditions that would occur under PSDDA testing, the test solutions were prepared four hours prior to inoculation of the embryos into the test vessels. For each test concentration, six replicates were prepared: five of those were for organism exposures, and the sixth was used to withdraw aliquots for ammonia measurements at time points 0 (test solution preparation), 4 hours (point of organism inoculation), 24 hours, and 48 hours (test termination).

Source of Broodstock and Spawning Conditions

Adult sand dollars were collected by divers off of West Beach on Whidbey Island. Approximately 200 adults were collected, and transported in water to the laboratory. At the lab, the organisms were placed into a temperature controlled growth chamber held in a 15° C growth chamber in fresh seawater with vigorous aeration. The organisms were collected 48 hours prior to testing.

Spawning of the sand dollars was conducted by injection of between 0.5 and 1.0 milliliter (mL) of a 0.5 molar solution of potassium chloride prepared in de-ionized water. Spawning occurred in the growth chamber at 15° C. Adults were inverted over cups with fresh seawater to collect the gametes. Eggs were selected by examination of the each individual females discharge under the microscope. Only those eggs that had dense, non-vacuolate cytoplasm, and were fully-rounded, were selected for use in the experiments. Sperm from five males were selected by examination under the microscope for vigorously swimming cells. Counts were made of both the egg and sperm prior to fertilization.

Fertilization was controlled by dispensing a volume of sperm solution to deliver approximately 100 sperm cells per egg. Post-fertilization, the developing embryos were washed twice by allowing the eggs to settle to the bottom, decanting the overlying water, and replacing it with fresh seawater. Thereafter, until inoculation, the embryos were kept in solution by frequent agitation with a perforated plunger.

Counts of the embryos were made, and a determination made as to the volume of embryo stock solution to be dispensed to deliver a final density of between 20 and 30 organisms per mL. Volumetric pipettes were gravimetrically calibrated to deliver the determined volume of embryos. Spawning occurred three hours prior to, and fertilization two hours prior to test inoculation. Embryos had developed to approximately the 16 cell stage at inoculation.

Experimental Procedure

For each test concentration, five test replicates were run without aeration, and five replicates with gentle aeration. Aeration was accomplished by dispensing the air through a glass pipette set at a rate of less than 100 bubbles per minute. As noted above, samples for ammonia measurement were taken for each concentration from a separate sixth replicate, in aerated and unaerated treatments.

Post inoculation, physical monitoring measurements (pH, salinity, dissolved oxygen, and temperature) were taken for each test series. Those measurements were taken again at 24 and 48 hours.

Inoculation of embryos was performed using a volumetric pipette, calibrated to deliver between 20 - 30 embryos/mL. To ensure a homogeneous distribution of embryos in the stock solution, a perforated plunger was use to mix the solution. Post-inoculation, two 10 mL aliquots were withdrawn from each of five seawater controls, and counted to determine the actual number of larvae dispensed into replicates. All subsequent mortality determinations were made by comparison to the initial seawater control counts.

To ensure consistency with previous echinoderm tests, a reference toxicant was run in a gradient series using cadmium chloride. The series included 0, 1.5, 3, 6, and 12 mg/L as cadmium.

The end of the test is taken as the point at which greater than 90% of the organisms in the seawater control reach the pluteus larval stage, as defined by the PSDDA program. For each test replicate, two 10 mL aliquots were withdrawn, fixed with 5% buffered formalin, and scored microscopically as normal (pluteus larvae) or abnormal. It should be noted here that abnormality can include larval forms that may be embryologically correct (eg., prism stage larvae). Failure to achieve the same developmental state as the controls is scored as abnormal - the test is interpreted as having a deleterious effect on larval development.

Ammonia Measurements

The method for ammonia analysis are those found in the *Standard Methods for the Examination of Water and Wastewater*, (1989). Ammonia, as NH_3-N, was measured using an Orion Model 95-12 ion specific electrode coupled with an Orion model 720A meter. Ammonium chloride standards "certified traceable to National Bureau of Standards material" were purchased from the manufacturer. All dilutions of standards were performed with volumetric glassware, using deionized water. A calibration slope of the probe response is made using dilutions of the calibration standard, and compared to established Control Chart values. The 720A meter, once calibrated, provides a direct read-out of NH_3-N values.

Crassostrea gigas

Sample Preparation

The test dilution series was set at a range of nominal 0.0, 1, 5, 10, 20, and 40 mg/L as NH_3-N. This dilution series was based upon previous published data by Cardwell, et al (1979) who reported an EC50 of 15.0, and an LC50 of 21.7 m/L as NH_4Cl. Ammonium chloride for these experiments was obtained from Aldrich Chemical Company (Reagent Grade). In contrast to the echinoderm experiments, the concentration exposures were made in batch (6 L), as opposed to each individual replicate. A stock solution of NH_4Cl was made up in seawater immediately prior to making the appropriate dilutions. This method proved to be more accurate in approximating the nominal values, than did the previous method.

Seawater was obtained from the University of Rhode Island's Graduate School of Oceanography seawater intake from Narragansett Bay. This intake is proximal to the EPA's Environmental Research Lab at Narragansett seawater intake (within approximately 300 ft.), and has been used successfully as control water for numerous saltwater criterion work for bivalve larvae. Seawater was filtered to 0.45 μ , and transported to the laboratory in polyethylene carboys. Water for the experiments was used within eight hours of collection.

Glass Mason jars were used for the exposure containers, with 800 mL of the filtered-adjusted seawater. To standardize the initial ammonia for both aerated and unaerated treatments, the test concentrations were first made up in batch, and then dispensed into the replicate vessels. To approximate conditions that would occur under PSDDA testing, the test solutions were prepared four hours prior to inoculation of the embryos into the test vessels. For each test concentration, six replicates were prepared: five of those were for organism exposures, and the sixth was used to withdraw aliquots for ammonia measurements at time points 0 (test solution preparation), 4 hours (point of organism inoculation), 24 hours, and 48 hours (test termination).

Source of Broodstock and Spawning Conditions

Gravid shellfish were obtained from two sources: Coast Oyster Company of Quilcene, WA, and from Hog Island Oyster Company in Bodega Bay, California. This was done to ensure maximum opportunity for acquiring gravid animals. Animals were shipped dry on overnight freight in blue-ice cooled containers. Upon arrival to the lab, the blue ice was removed, and the shellfish allowed to come to room temperature prior to being placed back in the water. During that approximately one-hour period,

shells were cleaned of fouling organisms (barnacles, mussels) and detritus to the best extent possible. After coming to room temperature, the animals were placed in a 20° C re-circulating saltwater bath, and allowed to siphon for one hour before thermal stimulation. While both sets of shellfish spawned, only the Coast Oyster parental stock was used for the ammonia exposures.

Thermal stimulation to induce spawning was accomplished by quickly raising the temperature of the water bath to approximately 30°. The oysters were closely observed, and when an individual commenced spawning, it was transferred to a shallow tray to complete discharge of gametes.

Eggs were collected by first pouring the egg suspension gently through a 64 μ Nitex screen to remove excess gonadal or fecal material. Prior to fertilization, the eggs were examined microscopically to ensure that they had the normal "tear-drop" shape, and that there were no other unusual features which would preclude the use of the eggs (e.g., large vacuoles within the eggs). Sperm was also examined microscopically to ensure that the cells were actively swimming. Sperm density was determined and a volume of solution that yielded approximately 100 sperm cells/egg was used for fertilization.

As was done for the echinoderms, spawning took place 3 hours before inoculation, with fertilization occurring just two hours prior to test exposures.

Experimental Procedure

The experimental procedure was identical to that described for the echinoderms. For each test concentration, five test replicates were run without aeration, and five replicates with gentle aeration accomplished by dispensing the air through a pipette set at a rate of less than 100 bubbles per minute. Samples for ammonia measurement were taken for each concentration from a separate sixth replicate, in aerated and unaerated treatments.

Post inoculation, physical monitoring measurements (pH, salinity, dissolved oxygen, and temperature) were taken for each test series. Those measurements were taken again at 24 and 48 hours. The pH of each nominal concentration was measured at the time of each ammonia sampling effort (0, 4, 24, and 48 hours).

A reference toxicant of cadmium chloride was run using a concentration series of 0, 7.5, 1.5, 3, and 6 mg/L as cadmium.

The end of the test is taken as the point at which > 90% of the organisms in the seawater control reach the prodissoconch I, or "D-shaped", larval stage, as defined by the PSDDA program. For each test replicate, two 10 mL aliquots were withdrawn, fixed with 5% buffered formalin, and scored microscopically as normal (prodissoconch) or abnormal. As noted previously, abnormal larvae could include developmentally correct stages; failure to achieve prodissoconch within the same time frame as the controls was interpreted as abnormal.

Ammonia Measurements

Ammonia measurements were measured using an Orion Model 95-12 probes, coupled to an Orion model 250A meter. Ammonium chloride standards "certified traceable to National Bureau of Standards material" were purchased from the manufacturer. All dilutions of standards were performed with volumetric glassware, using diluent adjusted to the salinity of test samples with reagent sodium chloride. Ammonia was measured using the standard addition method; the probe response to each unknown sample is measured before and after addition of a known volume of standard. Because the slope of the probe response is an important term in the calculation of results for the standard addition method, it was checked with duplicate observations of standard before, and duplicate observations after each set of unknown samples was read, or every two hours, whichever came first.

Data Analyses

Calculations for determining the unionized ammonia component in the samples were based on the methods of Whitfield (1974). A simple spreadsheet program for determination of unionized ammonia in seawater was written by Dr. Glen Thursby of SAIC, and utilized in this report. By Dr. Thursby's permission, the spreadsheet is provided to EPA with this report, and may be used by EPA at its discretion.

Larval response data were first tabulated in a spreadsheet, percentage mortality and abnormality determined by replicate, and the mean of all replicates for an exposure treatment was reported in the data summary. Calculations are those recommended by ASTM (1991) for larval bioassays:

% Mortality = [1-(number of normal plus abnormal larvae/mean initial seawater control counts)]*100

% Abnormality = [number of abnormal larvae/number of normal plus abnormal larvae]*100

This method of data expression was selected over the PSDDA combined mortality (mortality plus abnormality) endpoint. This was specifically done to distinguish be able to distinguish the lethal concentration (LC) from the effective concentration (EC). Data were then normalized to the seawater control mortality and abnormality responses by simple subtraction of the respective seawater values from the associated exposure concentration.

There were insufficient intermediate data points to calculate the regression line for determination of oyster LC and EC responses As such, a geometric mean for the median response was calculated (Zar, 1989). To calculate the equation of the line for the echinoderm EC responses, least squares regression was used (Zar 1984). Calculation of the regression equation was done using Microsoft's Excel 4.0, with confirmation using equations defined in Zar (1984). All other statistics were calculated by formulae entered into the spreadsheet.

Determination of the probability of encountering a Type II (beta) error in the regression was performed using the Fisher *z* transformation of the critical value of *r*, following the methods of Zar (1984).

To determine if significant differences occurred between the aerated and unaerated treatment, the method described by Zar for the testing of two sample regression coefficients was followed. Determination of the NOEC was by visual examination of the data - statistical analyses were not necessary.

For calculation of LC, EC, and NOEC concentrations, the total ammonia, and un-ionized ammonia, values recorded at the initiation of the experiments (T_0) were used. EC and LC 20 and 30 values were determined as they correspond to the PSDDA regulatory guidelines for suitability determinations in dredged sediment testing.

RESULTS

Results of the ammonia effects experiment appear in Table II-1. Larval data tables, the physical monitoring data, and ammonia measurements may be found in the Phase II appendix tables (Appendix A).

Data overall exceeded PSEP quality assurance requirements for larval bioassays. For the oysters, control mortality was less than 20% for the unaerated treatment, and 24% for the aerated treatments. Abnormality was 1.9% and 0.6%, respectively. Mortality for the unaerated echinoderm control was

5.6%, and 2.4% for the aerated control. Abnormality in the unaerated control was 11.3%, which exceeded the PSEP-specified limit by 1.3%. The effect on overall data quality was interpreted to be negligible. Abnormality for the aerated control was 2.1%. The physical monitoring data also show that for both species tests, all parameters were well within established guidelines.

Table II-1 presents the results of Phase II, with data normalized to final seawater mortality and abnormality values. It should be re-emphasized that % mortality is the percent larvae **not** recovered, relative to the number of larvae in the seawater control. Abnormality is the number of abnormal larvae, divided by the total number of larvae recovered in that replicate.

For the oyster ammonia solutions, the actual measured values were close to the nominal values. Table II-1, and Figure II-1 and II-2 show that the levels of ammonia remained constant throughout the course of the experiment, in both un-aerated and aerated treatments. In contrast with the oyster results, the ammonia levels in both the echinoderm aerated and unaerated treatments dropped over time (Figures II-3, II-4); by as much as 1.6 mg/L in the nominal 10 mg/L vessel.

Larval oysters were not affected by ammonia concentrations up to 4.68 mg/L (0.13 mg/L un-ionized NH_3), but showed high mortality and abnormality responses to concentrations at 9.79 mg/L, and above. It is interesting to note that above 9.79, the response was asymptotic Figure II-5; there was no further increase in mortality or abnormality at higher concentrations. All of the surviving larvae in concentrations $\geq$ 9.79 mg/L were truly abnormal; i.e., were non-identifiable cellular masses that did not resemble any developmental stage. No differences were observed in the unaerated and aerated treatments.

In contrast to the oysters, echinoderm larval mortality was negligible at all concentrations tested (Figure II-6). However, larval development was impacted in concentrations as low as 1.82 mg/L (0.01 mg/L unionized NH_3). As with the oyster larvae, the response appears to become asymptotic at 4.26 mg/L. During larval scoring, most of the abnormal larvae scored appeared to be embryologically distinct forms. The preponderance of abnormal larvae scored at 1.82 mg/L were still at the pre-pluteus prism form (see Dinnel and Stober, 1985). Larvae exposed to 4.26 mg/L were still at the gastrulation phase.

In these exposures, the NOEC for oyster larvae can be set at 4.68 mg/L total ammonia, corresponding to an unionized value of 0.08 mg/L. Calculation of lethal or effective concentration values is dependent upon observing intermediate values on a dose/response continuum. Unfortunately, in the case of oysters, the response was "all or nothing" for both mortality and abnormality. As such, the best estimate for both LC50 and EC50 is the geometric mean of the two ammonia values associated with the low and high responses, which was calculated as 6.83 mg/L, which would correspond to an unionized value of 0.13 mg/L.

For echinoderm larvae, there was no test concentration that produced lethality during the 48 hour exposure. For the abnormality response, the NOEC was established as 1.24 mg/L (0.014 mg/L unionized ammonia). Calculation of the regression equation for the two treatments yielded different equations of the line, but virtual similarity in expression of EC50 values (Tables II-2,3). When tested, the two regression equations were found to be statistically different at an alpha level of 0.05 (Table II-4).

Testing for a Type II (beta) error indicated that the probability of a beta error is less than 0.01% for both aerated and unaerated treatments (see Tables II-2 and II-3).

Table II - 1. Results of Ammonia Effects Experiment

Crassostrea gigas

	Un-Aerated		Measured Total NH3 (mg/L)				Aerated		Measured Total NH3 (mg/L)			
Nominal NH3			Time						Time			
Concentration (mg/L)	% Mortality	% Abnormality	To	T4	T24	T48	% Mortality	% Abnormality	To	T4	T24	T48
0	0.00	0.00	0.00	N.D.	N.D.	N.D.	0.00	0.00	0.00	N.D.	N.D.	N.D.
1	1.70	0.20	0.89	1.06	1.11	1.02	0.00	0.30	0.89	1.04	0.99	1.07
5	0.00	1.00	4.68	5.02	4.92	4.88	8.60	1.70	4.68	5.08	4.88	4.58
10	69.30	94.50	9.79	10.31	10.02	9.94	74.30	96.90	9.79	10.20	9.77	10.61
20	77.60	98.10	19.06	19.52	18.26	19.20	75.10	99.40	19.06	18.94	17.26	18.90
40	79.70	98.10	37.81	40.65	39.21	41.00	75.30	99.40	37.81	41.83	37.35	41.40

Dendraster excentricus

	Un-Aerated		Measured Total NH3 (mg/L)				Aerated		Measured Total NH3 (mg/L)			
Nominal NH3			Time						Time			
Concentration (mg/L)	% Mortality	% Abnormality	To	T4	T24	T48	% Mortality	% Abnormality	To	T4	T24	T48
0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.00	0.00	0.00	----	0.02	0.03
0.28	0.00	3.50	0.21	0.21	0.24	0.20	5.10	0.50	0.27	0.22	0.20	0.20
0.63	2.00	1.00	0.63	0.45	0.51	0.48	1.50	1.60	0.52	0.48	0.43	0.34
1.25	6.30	1.60	1.14	1.01	1.01	0.94	0.00	1.00	1.24	0.92	0.84	0.76
2.50	6.40	48.30	1.82	1.92	1.92	1.82	3.90	31.80	2.32	1.93	1.66	1.52
5.00	4.80	88.50	4.26	4.07	3.29	3.15	2.30	97.60	3.86	3.79	3.66	3.07
10.00	2.60	88.40	7.64	7.84	8.00	6.05	3.20	97.70	7.32	8.12	7.11	6.08

FIGURE II-1 OYSTER AMMONIA & TIME
AERATED TREATMENTS

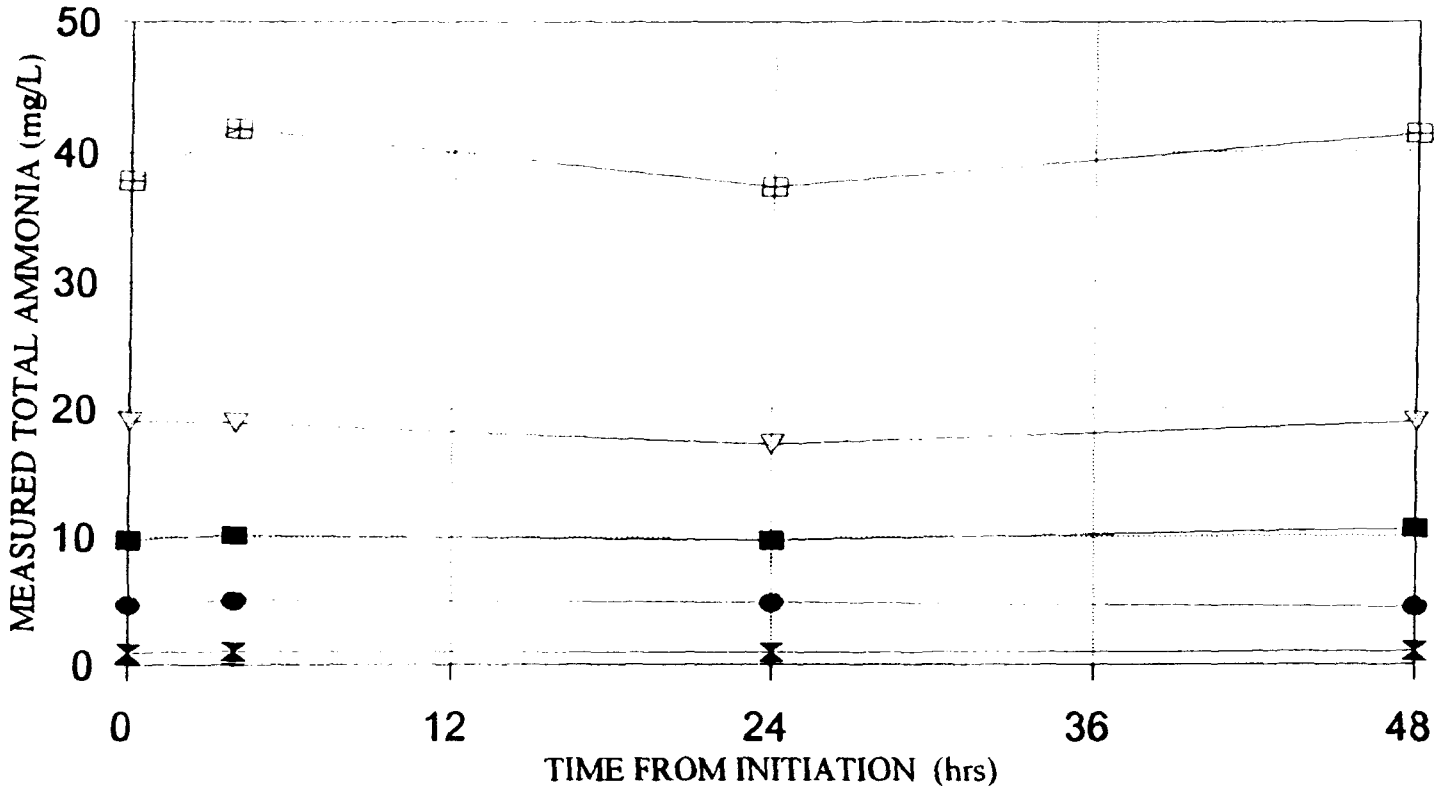


FIGURE II - 2 OYSTER AMMONIA VS. TIME
UNAERATED TREATMENTS

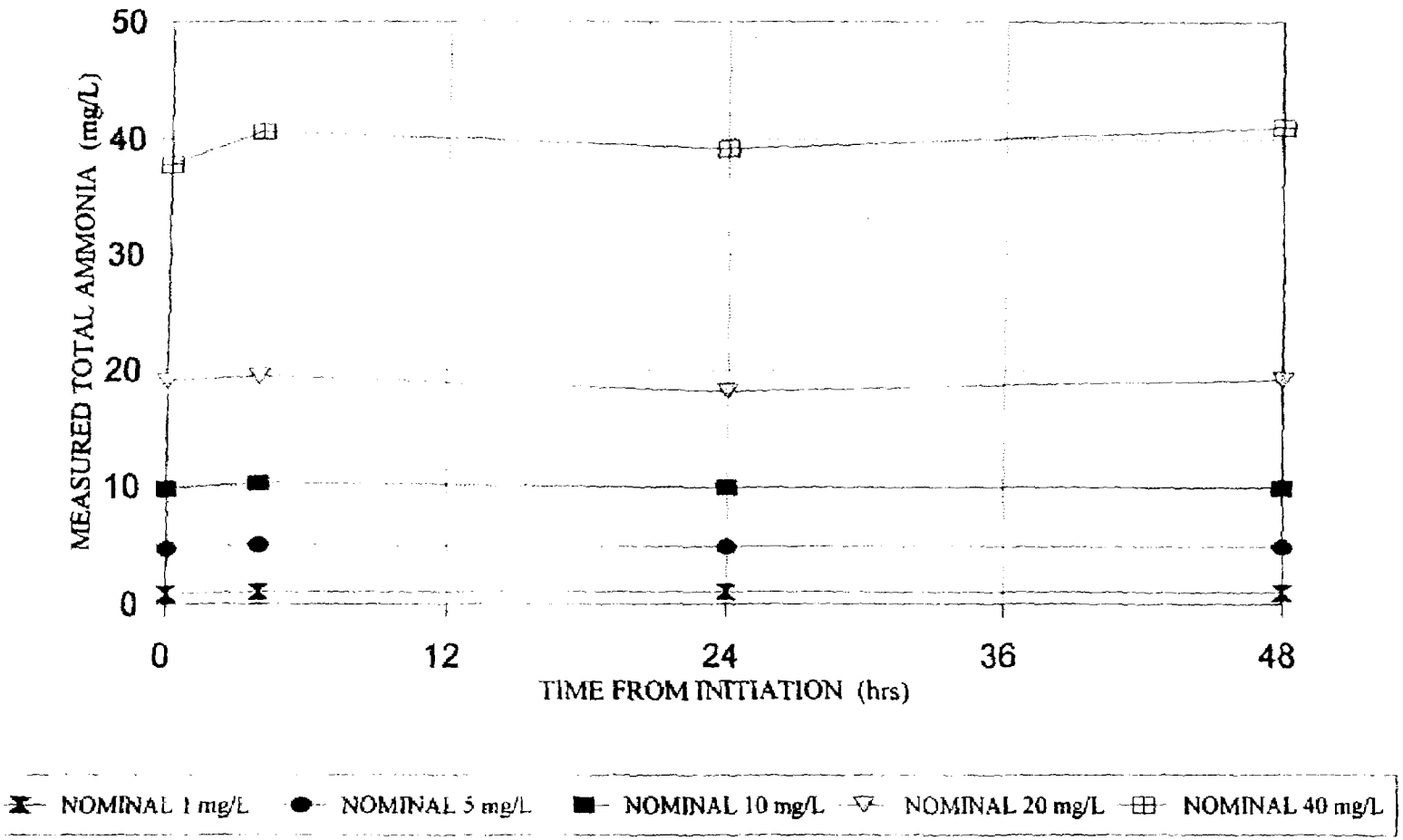


FIGURE II-3 ECHINODERM AMMONIA &. TIME
UNAERATED TREATMENTS

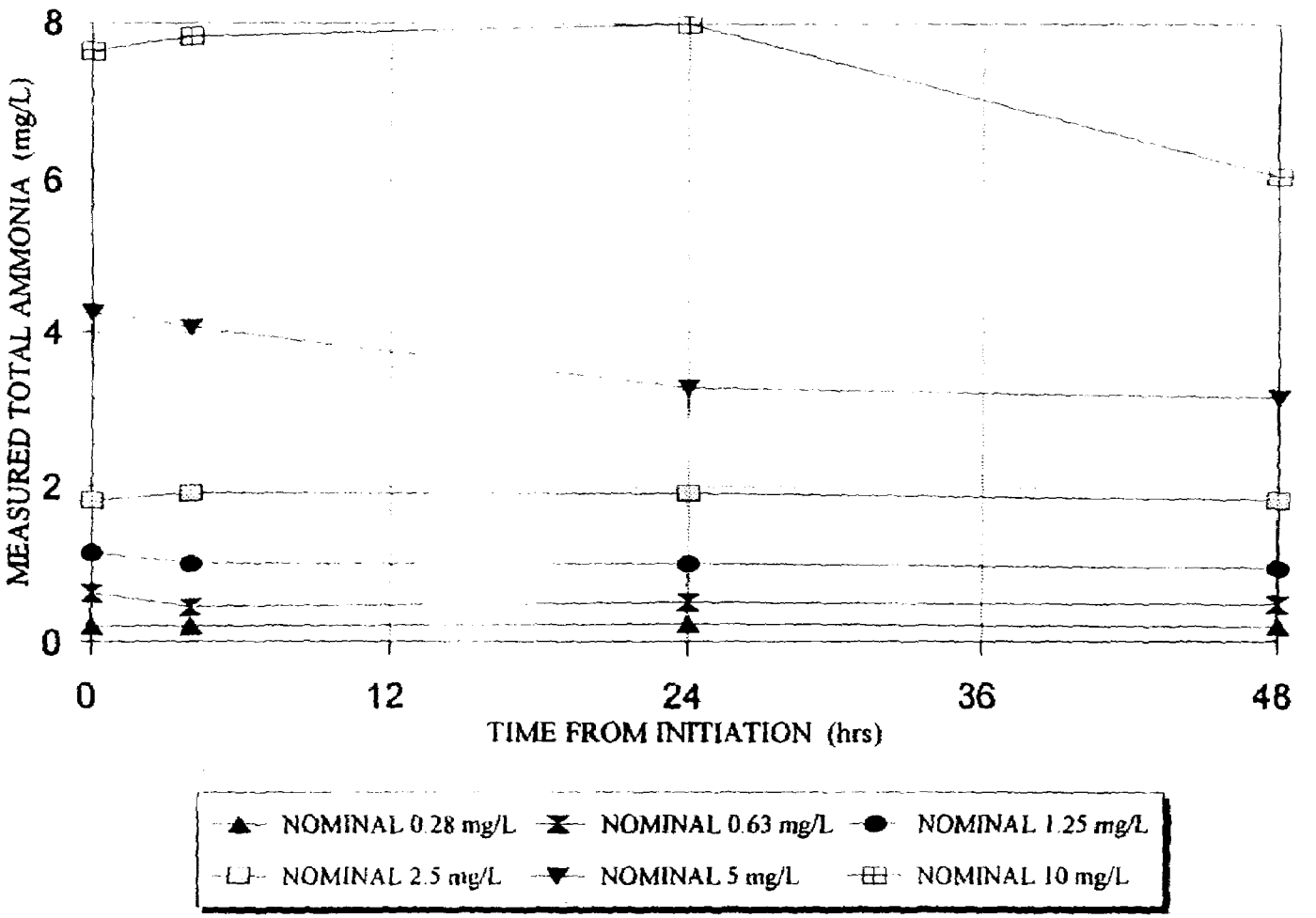


FIGURE II - 4. ECHINODERM AMMONIA VS.
UNAERATED TREATMENTS

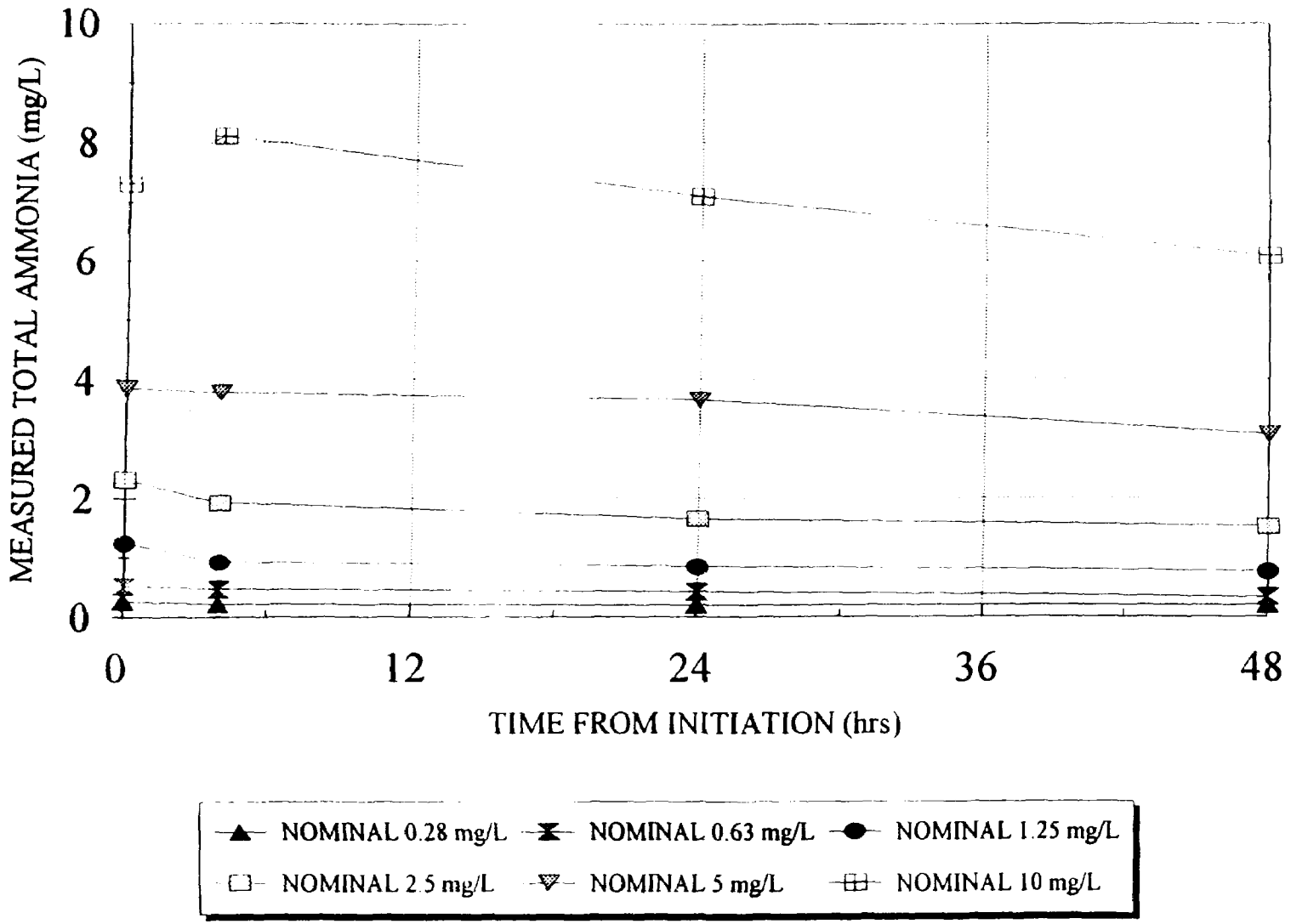


FIGURE II-5. OYSTER AMMONIA EFFECTS
AERATED VS. UNAERATED TREATMENTS

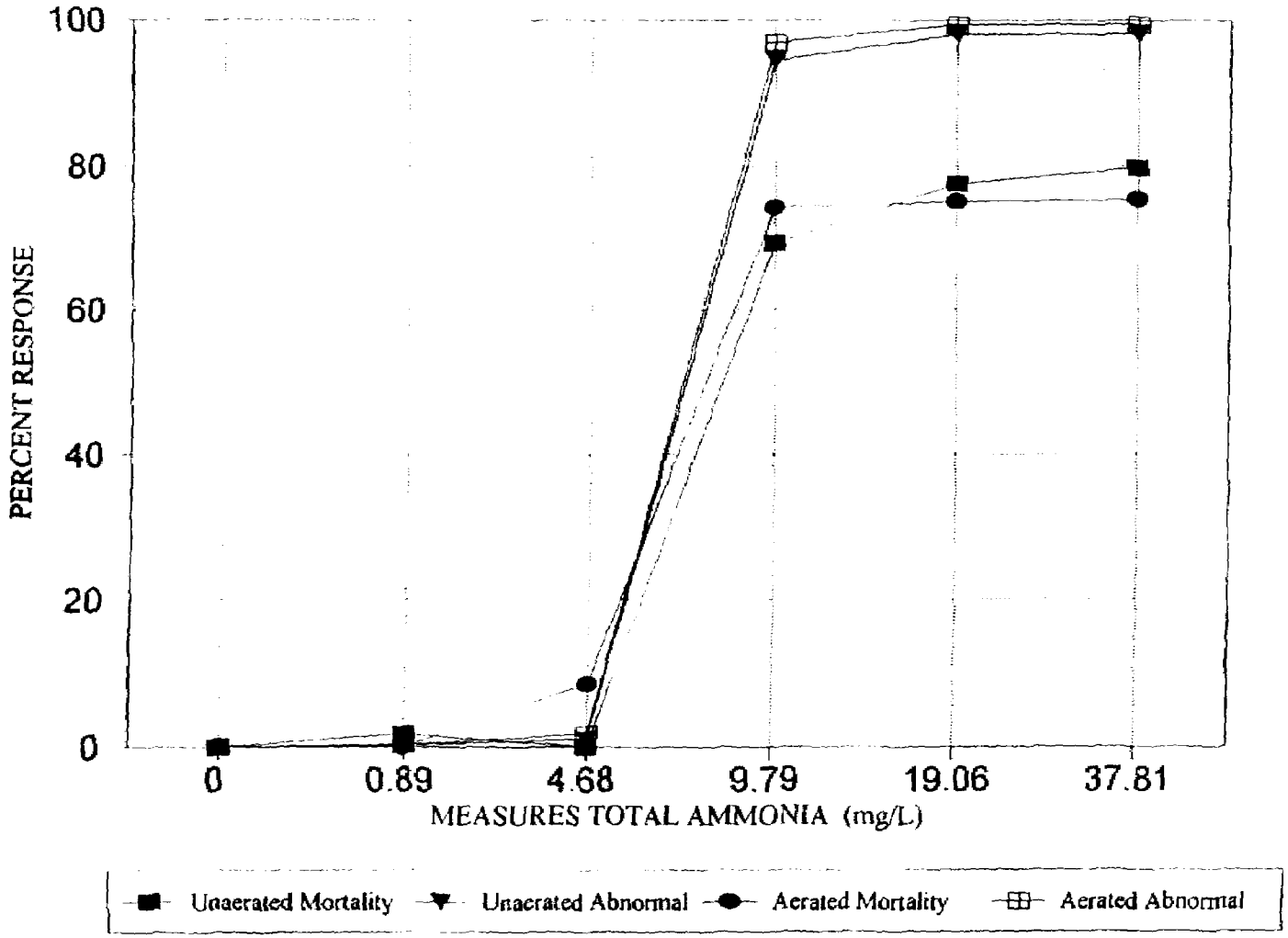
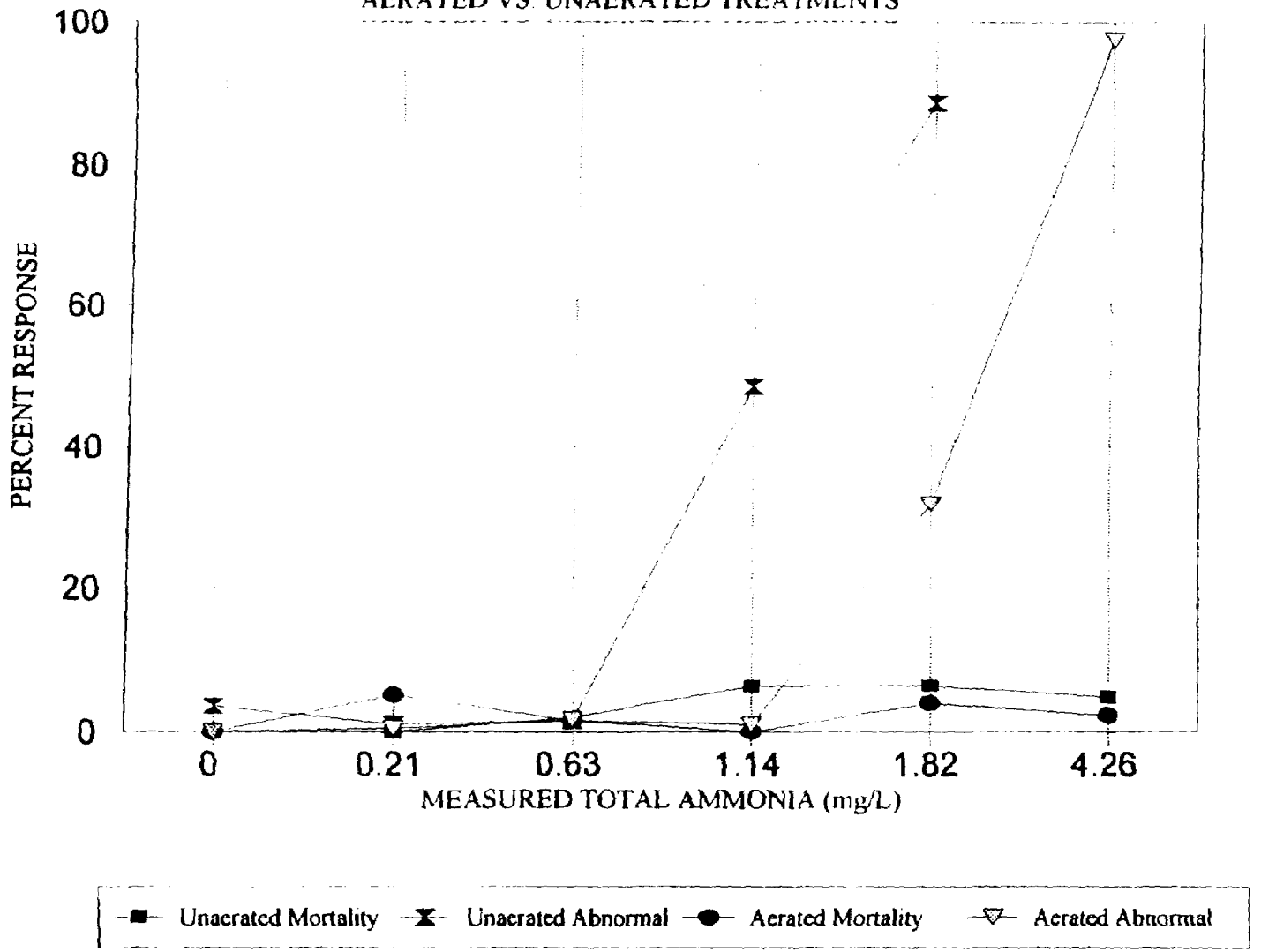


FIGURE II-6 ECHINODERM AMMONIA EFFECTS
AERATED VS. UNAERATED TREATMENTS



DISCUSSION

The results of the ammonia stability over time between the two species appear on the surface to be dichotomous. Ammonia levels in the oyster exposures remained stable, while the higher nominal concentrations in the echinoderm dropped over time. Re-examination of the laboratory analysis records for the echinoderm exposures revealed that the standards measured were off by 10 - 20% of the expected value (eg., 10 mg/L standard measured as 9 mg/L), and the T_{48} matrix spike run with the batch recovered only 80% of the spike. The measurements made for the oyster tests did not show a similar variance. While this is a modest degree of imprecision that is within laboratory QA requirements, a 20% difference could account for the apparent drop. Therefore, it is concluded that ammonia concentrations remained stable over the course of the 48 hour exposure in both unaerated and aerated treatments.

Based on the limited data, it appears that for oysters, the mortality response is not different from the abnormality response. The appearance of misshapen larval forms indicates that the oyster development is severely impacted by the levels of aqueous ammonia. By contrast, the sand dollar larvae were not acutely affected by ammonia, but the chronic effect was readily apparent. The main effect observed during these experiments was an inhibition of normal larval development. It should also be pointed out that the sand dollar larvae were more sensitive to the effects of ammonia, than were oyster larvae.

The effects of ammonia toxicity on sand dollars are in general agreement with those found by Kobayashi (1977) for the Japanese sand dollar, *Peronella japonica*, and the sea urchin *Stronglyocentrotus droebachiensis* (Kobayashi, 1981). For both of those echinoderm species, ammonia as low as 2 mg/L caused a retardation of the developmental processes, and at 5 mg/L, the larvae developed to the blastula stage where development was arrested.

That there were statistical differences noted between the echinoderm aerated and unaerated treatments is consistent with the Corps' findings (1991). However, the findings of this study are not in complete agreement with the Corps'. In addition to different regression equations ($y = 15.09 + 35.46x$ vs. this study), the Corps' data suggest 40% mortality at 0.6 mg/L total-ammonia. Under salinity, temperature, and pH conditions prescribed by PSEP, the level of unionized ammonia would be 0.01, a value that this study suggests should have no effect. However, it must be emphasized that the data used by the Corps was taken in whole sediment exposures, as opposed to the spiked seawater exposures in these experiments.

These findings are only useful if a threshold level for evaluating potential ammonia false positive responses can be set. In order to strengthen the data set for setting a critical ammonia value, we elected to combine the aerated and unaerated data sets. This decision was based on evaluating the expected EC values, and determining that for values of EC50 and greater, the level of unionized ammonia under standard conditions was virtually the same. The summation of both species NOEC and EC values is given in Table II-5, the combined regression is given in Table II-6.

There was sufficient statistical power within the experimental design to set with confidence the levels of unionized ammonia associated with 20 and 30% effects. What limits the application of these conclusions in the regulatory environment would be the lack of power associated with only one ammonia reading associated with five replicate larval readings per test sediment. With these few data, the difference in effects would need to be approximately 50% in order to detect a significant difference at an $\alpha = 0.05$. As such, the EC50 values for echinoderm, set at 0.03 mg/L unionized ammonia, would be the proposed ammonia criterion value.

However, the percent variance allowed for in the specific ion probe must be accounted for in setting the criterion. In this study, the testing laboratories allow for a variance of $\pm$ 15 in the calibration slope. Thus, two values may be off by as much as 30%, and still reported as an allowable detect by the analytical operator.

Based on the above argument, it is proposed that an echinoderm effect threshold be set at 0.04 mg/L as unionized ammonia. This value is derived by taking the minimum detectable difference of 50% (0.03 mg/L unionized), and allowing for a 30% variation on the analytical measurements. It is of interest to note that EPA's Ammonia Criterion Document sets as its 4-day criterion concentration for pH = 8.0, 15°C, in freshwater at 0.036 mg/L.

It is further proposed that the NOEC unionized ammonia levels be employed as a warning level. Unionized ammonia measurements of 0.014 mg/L (NOEC) would be used as to indicate that additional ammonia monitoring during the test would be required.

Insufficient dose/response data was generated for the oyster larvae to develop an oyster ammonia threshold value. However, a value for echinoderms was proposed that corresponded to the EC50. As such, an interim value equal to the oyster larval LC/EC50 of 0.13 mg/L unionized ammonia is proposed, until such time as additional work is conducted to more rigorously define the number.

Table II-7 presents a compilation of theoretical values for unionized ammonia that were determined over the range of parameters allowed for a PSDDA echinoderm bioassay. Total ammonia values are presented in the left-hand column, while unionized ammonia values for the allowable temperature (15°C ± 1°) and pH (7.5 - 8.5) at a constant salinity of 28 ppt. Values shaded represent those concentrations of unionized ammonia exceeding the proposed criterion. Mortality results falling within those shaded areas, could be considered for false positive effects due to the levels of unionized ammonia.

RECOMMENDATIONS

- An ammonia testing criterion of 0.04 mg/L unionized ammonia is proposed for the echinoderm test. Data may be qualified as a possible false positive response if un-ionized ammonia values in echinoderm tests are greater than or equal to 0.04 mg/L.

- The above criterion value relates specifically to echinoderm abnormality, not mortality. For an acute criterion to be set for echinoderm larval mortality, additional work is necessary.

- A warning level of 0.014 mg/L unionized ammonia is recommended. The warning level would be used as to indicate that additional ammonia monitoring during the test would be required.

- An interim oyster-specific criterion is proposed as 0.13 mg/L unionized ammonia. Some caution is recommended in using this number for interpretation, as it is an estimate. Additional work is recommended to better define that number.

- Aeration appears to have an effect on ammonia toxicity on echinoderm. PSDDA should continue to use aeration in the larval bioassays.

- All laboratories performing PSDDA bioassays should be required to express all ammonia values as the un-ionized form. The SAIC spreadsheet format could be made standard for data submittal.

Table II - 2. Calculation of Regression and Power for the Determination of the Unaerated Ammonia EC Values for Echinoderms

DATA

Measured Total Ammonia	Proportional Abnormality	Adjusted Abnormality
1.14	0.13	0.00
1.14	0.09	-0.04
1.14	0.10	-0.02
1.14	0.13	0.00
1.14	*0.10*	*-0.03*
1.14	0.10	-0.02
1.14	0.17	0.04
1.14	0.16	0.03
1.14	0.15	0.02
1.14	0.15	0.02
1.82	0.71	0.58
1.82	0.65	0.53
1.82	0.57	0.44
1.82	0.65	0.53
1.82	0.66	0.53
1.82	0.67	0.54
1.82	0.76	0.63
1.82	0.72	0.59
1.82	0.28	0.15
1.82	0.29	0.16
4.26	1.00	0.87
4.26	1.00	0.87
4.26	0.99	0.86
4.26	1.00	0.87
4.26	1.00	0.87
4.26	1.00	0.87
4.26	1.00	0.87
4.26	1.00	0.87
4.26	0.99	0.86
4.26	1.00	0.87

Regression Statistics

Multiple R	0.90
R Square	0.82
Adjusted R Square	0.81
Standard Error	0.16
Observations	30.00

Analysis of Variance

	df	*Sum of Squares*	*Mean Square*	*F*	*Significance F*
Regression	1.00	3.32	3.32	125.32	0.00
Residual	28.00	0.74	0.03		
Total	29.00	4.06			

	Coeffici	*Standard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.15	0.06	-2.49	0.02	-0.28	-0.03
x1	0.25	0.02	11.19	0.00	0.20	0.29

Equation of the line = ***y = mx + b =*** **0.25x - 0.15**

EC20 =	(0.2+0.15)/0.25 =	1.40
EC30 =	(0.3+0.15)/0.25 =	1.80
EC50 =	(0.5+0.15)/0.25 =	2.60

Calculation of β Error using Fisher's z Transformation (Zar, 1984)

Zβ(2) = (z-z,alpha)*sqrt(n-3)

n = 30.00 degrees of freedom = 29

r = 0.9 $\alpha = 0.05$

z = 0.5*(LN((1+r)/(1-r))

= 1.49

r 0.05(2),39 = 0.308

z 0.05 = 0.3183

Zβ(2) = (1.49-0.3183)*(sqrt(30-3)) = 6.088331793685

β = P(Z>6.088) < 0.0001

Therefore, the power of the test = 1 - β = 0.9999

Table II - 3. Calculation of Regression and Power for the Determination of the Aerated Ammonia EC Values for Echinoderms

DATA

Measured Total Ammonia	Proportional Abnormality	Adjusted Abnormality
1.24	0.04	0.01
1.24	0.04	0.01
1.24	0.03	-0.00
1.24	0.02	-0.02
1.24	0.02	-0.02
1.24	0.03	-0.00
1.24	0.03	-0.01
1.24	0.03	-0.00
1.24	0.04	0.01
1.24	0.04	0.01
2.32	0.42	0.39
2.32	0.29	0.26
2.32	0.31	0.28
2.32	0.41	0.38
2.32	0.29	0.26
2.32	0.29	0.26
2.32	0.35	0.32
2.32	0.34	0.31
2.32	0.30	0.27
2.32	0.39	0.36
3.86	1.00	0.97
3.86	1.00	0.96
3.86	1.00	0.96
3.86	1.00	0.97
3.86	1.00	0.96
3.86	1.00	0.97
3.86	1.00	0.97
3.86	0.99	0.96
3.86	1.00	0.97
3.86	1.00	0.96

Regression Statistics

Multiple R	0.99
R Square	0.98
Adjusted R Square	0.98
Standard Error	0.05
Observations	30

Analysis of Variance

	df	*Sum of Squares*	*Mean Square*	*F*	*Significance F*
Regression	1.00	4.82	4.82	1726.78	0.00
Residual	28	0.08	0.00		
Total	29	4.90			

	Coeffici	*Standard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.50	0.02	-20.56	0.00	-0.55	-0.45
x1	0.37	0.01	41.55	0.00	0.35	0.39

Equation of the line = ***y = mx + b =*** **0.37x - 0.5**

EC20 = (0.2+0.5)/0.37 = 1.89

EC30 = (0.3+0.5)/0.37 = 2.16

EC50 = (0.5+0.5)/0.37 = 2.70

Calculation of **β** **Error using Fisher's *z* Transformation (Zar, 1984)**

Zβ(2) = (z-z,alpha)*sqrt(n-3)

n = 30 degrees of freedom = 29

r = 0.99 α = 0.05

z = 0.5*(LN((1+r)/(1-r))

= 2.76

r 0.05(2),39 = 0.31

z 0.05 = 0.32

Zβ(2) = (2.76-0.3183)*(sqrt(30-3))

12.69

β = P(Z>12.69) < 0.0001

Therefore, the power of the test = 1 − β = 0.9999

Table II - 4. Testing for the Difference between the Aerated and Unaerated Regression Coefficients

Null Hypothesis: Unaerated Regression Coefficient = Aerated Regression Coefficient

Unaerated		Aerated	
n=	30	n=	30
Mean Sum X =	2.41	Mean Sum X =	2.47
Mean Sum Y =	0.45	Mean Sum Y =	0.43
Sum Squares X =	227.596	Sum Squares X =	218.196
Sum Square Y =	10.0056	Sum Square Y =	10.30806
Sum X*Y =	85.6848	Sum X*Y =	86.9808
b =	-0.15	b =	-0.5
m =	0.25	m =	0.37
residual SS =	0.74	residual SS =	0.08
residual df =	28	residual df =	28

1. Pooled Residual Mean Square

= (residual SS Unaerated + residual SS Aerated)/(residual df of Unaerated + residual df Aerated)

(0.74 + 0.08)/(28 + 28) = 0.014643

2. Standard error of the difference between regression coefficients

= square root [(pooled residual mean square/sum x-squared unaerated) + (pooled residual mean square/sum x-squared aerated)]

sqrt((0.014643/227.6)+(0.014643/218.2)) = 0.011465

3. Calculation of t statistic

= (slope of unaerated - slope of aerated)/combined standard error

-0.15--0.5/0.014643 = 33.99601

4. Critical t for two -tailed test at alpha 0.05 2.005

Calcualted t > Critical t

Therefor, reject the null hypothesis

Table II-5. Summary of No Observed Effect Concentration, and Effective Concentration values.

SPECIES	MORTALITY		ABNORMALITY							
	NOEC		NOEC		EC20		EC30		EC50	
	Total	Union.	Total	Union.	Total	Union	Total	Union	Total	Union
OYSTER	4.68	0.08	4.68	0.08	N.D.	N.D.	N.D.	N.D.	6.83	0.13
SAND DOLLAR	N.D.	N.D.	1.24	0.014	1.63	.019	1.97	.022	2.63	.03

Table II - 6. Calculation of Regression Equation Using Combined Echinoderm Aerated/Unaerated Data Sets.

Measured Total Ammonia	Adjusted Abnormality
1.14	0.00
1.14	-0.04
1.14	-0.02
1.14	0.00
1.14	-0.03
1.14	-0.02
1.14	0.04
1.14	0.03
1.14	0.02
1.14	0.02
1.24	0.01
1.24	0.01
1.24	-0.00
1.24	-0.02
1.24	-0.02
1.24	-0.00
1.24	-0.01
1.24	-0.00
1.24	0.01
1.24	0.01
1.82	0.58
1.82	0.53
1.82	0.44
1.82	0.53
1.82	0.53
1.82	0.54
1.82	0.63
1.82	0.59
1.82	0.15
1.82	0.16
2.32	0.39
2.32	0.26
2.32	0.28
2.32	0.38
2.32	0.26
2.32	0.26
2.32	0.32
2.32	0.31

Regression Statistics

Multiple R	0.93
R Square	0.87
Adjusted R Square	0.87
Standard Error	0.14
Observations	60

Analysis of Variance

	df	*Sum of Squares*	*Mean Square*	*F*	*Significance F*
Regression	1.00	7.79	7.79	385.82	0.00
Residual	58	1.17	0.02		
Total	59	8.96			

	Coeffici	*Standard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-0.29	0.04	-7.01	0.00	-0.37	-0.21
x1	0.30	0.02	20	0.00	0.27	0.33

Equation of the line = ***y = mx + b =*** **0.30x - 0.29**

EC20 =	(0.2+0.29)/0.3 =	1.63
EC30 =	(0.3+0.29)/0.3 =	1.97
EC50 =	(0.5+0.29)/0.3 =	2.63

Measured Total Ammonia	Adjusted Abnormality
3.86	0.97
3.86	0.96
3.86	0.96
3.86	0.97
3.86	0.96
3.86	0.97
3.86	0.97
3.86	0.96
3.86	0.97
3.86	0.97

Measured Total Ammonia	Adjusted Abnormality
4.26	0.87
4.26	0.87
4.26	0.86
4.26	0.87
4.26	0.87
4.26	0.87
4.26	0.87
4.26	0.87
4.26	0.86
4.26	0.87

Table II-7. Theoretical values for unionized ammonia determined for PSDDA echinoderm bioassays.

Measured Total Ammonia ppm	Unionized Ammonia at 14 degrees C			Unionized Ammonia at 15 degrees C			Unionized Ammonia 16 degrees C		
	pH = 7.5	pH = 8.0	pH = 8.5	pH = 7.5	pH = 8.0	pH = 8.5	pH = 7.5	pH = 8.0	pH = 8.5
1	0.01	0.02	0.06	0.01	0.02	0.07	0.01	0.02	0.07
2	0.01	0.04	0.13	0.01	0.04	0.14	0.02	0.05	0.15
3	0.02	0.06	0.19	0.02	0.07	0.20	0.02	0.07	0.22
4	0.03	0.08	0.25	0.03	0.09	0.27	0.03	0.10	0.29
5	0.03	0.10	0.32	0.04	0.11	0.34	0.04	0.12	0.36
6	0.04	0.13	0.38	0.04	0.13	0.41	0.05	0.14	0.44
7	0.05	0.15	0.44	0.05	0.16	0.47	0.05	0.17	0.51
8	0.05	0.17	0.51	0.06	0.18	0.54	0.06	0.19	0.58
9	0.06	0.19	0.57	0.06	0.20	0.61	0.07	0.22	0.65
10	0.07	0.21	0.63	0.07	0.22	0.68	0.08	0.24	0.73
11	0.07	0.23	0.69	0.08	0.25	0.74	0.09	0.27	0.80
12	0.08	0.25	0.76	0.09	0.27	0.81	0.09	0.29	0.87
13	0.09	0.27	0.82	0.09	0.29	0.88	0.10	0.31	0.94
14	0.09	0.29	0.88	0.10	0.31	0.95	0.11	0.34	1.02
15	0.10	0.31	0.95	0.11	0.34	1.02	0.12	0.36	1.09
16	0.11	0.33	1.01	0.12	0.36	1.08	0.12	0.39	1.16
17	0.11	0.35	1.07	0.12	0.38	1.15	0.13	0.41	1.23
18	0.12	0.38	1.14	0.13	0.40	1.22	0.14	0.43	1.31
19	0.13	0.40	1.20	0.14	0.43	1.29	0.15	0.46	1.38
20	0.13	0.42	1.26	0.14	0.45	1.35	0.16	0.48	1.45

Shaded values are those for which an echinoderm abnormality response could be predicted.

REFERENCES

APHA 1989. *Standard Methods for the Examination of Water and Wastewater.* American Public Health Association. 1015 Fifteenth Street NW. Washington, D.C. 20005

Ankley, G.T., A. Katko, and J.W. Arthur. 1990. Identification of Ammonia as an Important Sediment-Associated Toxicant in the Lower Fox River and Green Bay Wisconsin. Environmental Toxicology and Chemistry 9:313 - 322.

American Society For Testing Materials. 1991. Standard Guide for Conducting Static Acute Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Molluscs. Method E 724-89. *1991 Annual Book of ASTM Standards* Volume 11.04. ASTM 1916 Race Street, Philadelphia, PA 19103-1187.

Cardwell,R.D., C.E. Woelke, M.I. Carr, and E. W. Sanborn. 1979. Toxic Substance and Water Quality Effects on Larval Marine Organisms. State of Washington Department of Fisheries, Technical Report No. 45.

Dinnel, P.A., and Q.J. Stober, 1985. Methodology and Analysis of Sea Urchin Embryo Bioassays. Circular No.85-3. Fisheries Research Institute, University of Washington. Seattle, WA.

Kobayashi, N. 1977. Preliminary experiments with sea urchin pluteus and metamorphosis in marine pollution bioassays. Publ. Set Marine Biol. Lab 24:9-21

Kobayashi, N. 1981. Comparative toxicity of various chemicals, oil extracts, and oil dispersant extracts to Canadian and Japanese sea urchin eggs. Publ. Seto Marine Biol. Lab 26: 123 - 133.

PSEP, 1991. *Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments.* U.S. EPA, Region 10, Office of Puget Sound. Seattle, WA.

USACE, Seattle District. 1991. Memorandum for Record. Decision on the Suitability of Dredged Material Tested under PSDDA Guidelines for Bellingham Maintenance Dredging in Whatcom Creek Waterway, Squalicum Creek Waterway, and I&J Street Waterway to be Disposed of at the Bellingham Bay Nondispersive Open Water Disposal Site and Rosario Straits Dispersive Site. CENPS-OP-DMMO. 3 June 1991.

U.S. Environmental Protection Agency, 1991. *Evaluation of Dredged Material Proposed for Ocean Disposal, Testing Manual.* United States Environmental Protection Agency and the U.S. Army Corps of Engineers. EPA - 503 /8-91 / 001.

U.S. Environmental Protection Agency, 1985. Ambient Aquatic Life Water Quality Criteria for Ammonia. NTIS PB85-227114.

Whitfield, M. 1974. The hydrolysis of ammonium ions in sea water -- a theoretical study. J. Mar. Biol. Assoc. U.K. 54:565 - 580.

Zar, J.H. 1984. *Biostatistical Analysis*, Second Edition. Prentice-Hall, Inc., Englewood Cliffs, N.J. 07632. *xv* + 718 pp.

REFINEMENTS TO CURRENT PSDDA BIOASSAYS

FINAL REPORT

PHASE IIIA: SPECIES SENSITIVITY COMPARISON TO GRAIN SIZE EFFECTS

SAIC®
An Employee-Owned Company

TABLES

FIGURES

PHASE IIIA: SPECIES SENSITIVITY COMPARISON TO CLEAN REFERENCE SEDIMENTS

INTRODUCTION

Sediment larval bioassays are currently used under regulatory dredged sediment testing programs to help determine the suitability of proposed dredged material for unconfined open water disposal. These elutriate bioassays are used as a screen for possible adverse biological effects that occur due to the presence of chemicals of concern in the dredged test sediment.

One problem with the larval sediment bioassays is that the physical presence of fine-grained material in the test vessels may entrain embryos, which could result in a false positive response.[1] Within the context of the current PSDDA regulatory environment, exceedences of the regulatory guidelines in bioassays are interpreted as being a toxic response to the presence of detected, or non-detected, chemicals of concern. Disposal at the open-water sites is not allowed when such exceedences occur.

Within the PSDDA program, a reference sediment of similar grain size to the test material is included to account for potential sediment grain size effects on the test organisms. However, these relatively "clean" reference sediments often have high mortalities exceeding recommended quality standards for acceptable test results. This may, in part, be due to physical effects such as interference in the test from larval entrainment by suspended solids, or to physiological stress due to small grain sizes.

The PSDDA agencies identified the need for sponsoring research in defining the conditions under which grain-size effects could cause larval mortality or abnormality using regulatory dredged sediment testing protocols. The study was constructed so as to determine if criteria can be identified that indicate when the chance of false positive responses due to suspended sediment in the test chamber could occur. This would necessarily include the two classes of organisms most frequently used in dredged sediment elutriate testing; bivalves and echinoderms.

One further variable that the agencies identified was to compare the effects of the different exposure protocols that have been used for dredged sediment testing. There are two principal means of elutriate preparation: 1) the Puget Sound Estuary Program (PSEP, 1991) procedure used under the PSDDA program, and 2) the "Green Book" method (EPA, 1991) for evaluating dredged material for open ocean disposal. Within the PSDDA program, three variations have been employed on the PSEP protocol. These are:

- Allow suspended materials 4 hours to settle prior to testing, with no aeration during testing (standard PSEP)
- Allow suspended materials 4 hours to settle, using aeration during testing
- Allow suspended materials 24 hours to settle, with no aeration during testing.

---

[1] A false positive condition occurs when the bioassay results indicate that a toxic response has occurred, but for reasons un-associated with sediment chemistry. Under those circumstances, the measured chemicals-of-concern do not appear to be sufficiently high to explain the toxicological response, but the testing results indicate that significant mortality or abnormality has occurred within the test replicates.

The intent was to compare the methods to determine if there were any differences in organism responses, and if not, to select a method for regulatory testing that would retain environmental sensitivity, but potentially would minimize the possibility of false positives (eg., aeration to maintain high levels of dissolved oxygen, longer settling times - 24 hours - to minimize fine sediment entrainment of larvae).
The objectives of this study were as follows:

- Compare the sensitivity of oyster and sand dollar embryo exposures to clean reference sediment of varying grain sizes, and test procedures.
- Within a single species exposure, compare the responses to varying grain sizes and test procedures.
- Determine the conditions under which either the bivalve or echinoderm larval bioassay methods would be susceptible to false positive results due to the presence of suspended sediment in the test chamber.

METHODS AND MATERIALS

TEST OVERVIEW

The Pacific oyster, *Crassostrea gigas*, and the eastern Pacific sand dollar, *Dendraster excentricus*, were selected for study. While sand dollars are the most frequently used species for testing under this program, bivalve larvae have been used in other Puget Sound programs, as well as in testing conducted under the EPA/USACE Ocean Disposal program.

A target range of grain-sizes was pre-selected in consultation with the PSDDA agencies. The four grain-size distributions were chosen to reflect the range of materials most frequently seen within Puget Sound dredged sediments. The targeted test range was less than 30% fines, 45 - 60% fines, 65 - 75% fines, and greater than 85% fines.

Larvae were exposed for 48 hours to the test sediments/test conditions, and the responses compared to that observed in a seawater control.

All testing was conducted at SAIC's Environmental Testing Center, in Narragansett, Rhode Island.

REFERENCE SEDIMENT COLLECTION AND ANALYSES

Reference sediment samples were collected in Carr Inlet using 3 reference stations that have been previously used under the PSDDA testing program.[2] Reference sediments within Carr Inlet have been documented to be relatively free of the PSDDA chemicals of concern and represent a wide range of grain sizes (PTI, 1991). The three reference stations, identified as CRR 2, CRR 4, and CRR 6, have

[2]These site designations are independent from those identified in PTI (1991), and bear no relationship to stations identified by PTI as CARR 2, 4, or 6.

been shown to possess 45, 65, and 85% fines, respectively. During the collection trip, an additional station possessing less than 30% fines was located, and assigned the station name CRR A.

Samples were collected using the Washington State Department of Natural Resources' 0.1 m^2 stainless steel van Veen grab, aboard the research vessel Brendan D II. Station locations were determined using both the Global Positioning System (GPS) and Loran-C. When a sample was collected, the station's position and water depth along with the time of sample collection were recorded in a field log.

All sample collection, handling, processing, and chain of custody procedures were conducted in accordance with PSEP (1986, 1989). Prior to deployment, the van Veen was thoroughly washed with Alconox soap, followed by a sequence of nitric acid (10%), deionized water, and methanol rinses. Between stations, the sampler was washed with Alconox and rinsed with seawater.

Once a grab sample was taken, the overlying water was siphoned off from one side of the sampler. Surface sediments were inspected for disturbance, and depending on their acceptability, sampled or rejected. As 8 liters of material were required for analytical and biological testing, multiple grabs were necessary at each station to achieve the required volume. To avoid possible adverse effects associated with high sediment sulfate levels, the sampling was restricted to the oxic layers (i.e., less than or equal to 2 cm). This was especially important at CRR 2, where the anoxic layers smelled strongly of hydrogen sulfide. Samples for total sulfide were collected from one, randomly selected grab and preserved in zinc acetate to minimize oxidation. Samples were homogenized on-board with stainless-steel, decontaminated utensils. After compositing, sediments were transferred to appropriate glass containers, labelled, and taped shut. Proper chain-of-custody procedures were followed in all transfers.

To ensure that the grain-size distribution required from each station was met, care was taken in the field to verify the percent fines using a wet sieving technique. This involved measuring 100 mL of the sediment into a graduated cylinder. The 100 mL of sediment was then carefully transferred into a 62 μ screen, and thoroughly washed to push the fine materials through the screen. After washing, the retained material was transferred back into the graduated cylinder, and the volume recorded. The percent fines was inferred by subtracting the retained volume of sand from the original 100 mL.

For this study, only PSDDA sediment conventional analyses were required. Conventional analyses (i.e., total solids, total organic carbon, total volatile solids, ammonia, and total sulfide) were conducted by Analytical Resources Incorporated. Grain size analyses were performed by Soil Technology. Analyses for PSDDA conventional parameters were performed in accordance with those methods employed during Phase IIIB.

Sample Preparation

Each of the four test sediments were prepared according to the following procedures:

- PSDDA 4-Hour Settlement. Twenty grams of test sediment per liter were measured into pre-labelled glass Mason jars. Filtered seawater was added, and the contents vigorously shaken for approximately 10 seconds. The test vessels were then placed in the temperature-controlled water bath and allowed to settle for 4-hours prior to inoculation of test embryos. One set of five replicates prepared this way was aerated; a second set of five replicates was run without aeration.

- PSDDA 24-Hour Settlement. These treatments were prepared exactly as above, with the exception of an allowance for 24 hours of settling time. These replicates were prepared a day ahead of time, so that the end of the settling time would coincide with the inoculation. All replicates prepared this way were not aerated during the test.

- Green Book. The method for preparing an elutriate as defined by the EPA involved making a 1 part sediment to four parts seawater mixture, that is vigorously stirred for 30 minutes. While the Green Book recommends use of a magnetic stirrer, the volume of material prepared in batch (8 L) prevented effective use of a stir bar. In these experiments, vigorous aeration was used. Test treatments were thoroughly mixed manually at the initiation of the mixing, and at 10 minutes intervals thereafter. The resultant slurry was allowed to settle for 30 minutes, and the liquid portion was carefully poured off so as to not disturb the settled material. The retained liquid was then dispensed into the test vessels.

  For each sediment, a total of 10 replicate samples were prepared. All 10 replicates were inoculated and held under the same conditions during testing. As defined by the Green Book protocol, aeration was not used unless dissolved oxygen levels fell to less than 40% saturation. At the end of the exposure, aliquots for larval counts were taken from one set of five replicates by carefully decanting off the overlying water into a second container, taking care not to include any of the bottom material (in accordance with the "PSDDA counts" method). In the second set of five replicates, the entire vessel contents were first mixed, and then aliquots were withdrawn for larval counts (in accordance with the Green Book method). This latter procedure was an effort to determine if larvae could be identified that had been entrained in the sediment during exposure.

Source of Broodstock and Spawning Conditions

Gravid sand dollar and Pacific oyster broodstock were obtained from the same sources as described for Phase II. All organisms were transported to the SAIC Testing Center in Narragansett on overnight freight, and were used on the day of arrival. Prior to spawning, the organisms were acclimated to test temperature.

Spawning procedures were identical to those described in Phase II.

Experimental Procedure

For each organism, and test procedure, an individual set of five seawater control replicates was set up to duplicate the sediment test procedure. For example, for the four-hour settling-aerated treatments, there were five aerated seawater controls that were placed under the same conditions as the test replicates prior to inoculation. The controls for the 24-hour settling test were set up at the same time as the test treatments, and allowed to "settle" for 24 hours. Green Book controls were mixed and handled identical to the test treatments.

For each organism and test procedure, six replicates were prepared. Where required, aeration was accomplished by dispensing the air through a glass pipette set at a rate of less than 100 bubbles per minute. The sixth replicate was used for taking physical monitoring and ammonia measurements. Post inoculation, physical monitoring measurements (pH, salinity, dissolved oxygen, and temperature) were

taken for each test series. Those measurements were taken again at 24 and 48 hours. Ammonia was taken at the time of inoculation, and at test termination.

Inoculation of embryos occurred using a volumetric pipette, calibrated to deliver between 20 and 30 embryos per mL. To ensure homogenous distribution of embryos in the stock solution, a perforated plunger was use to mix the solution. Post-inoculation, two 10 mL aliquot were withdrawn from each of five seawater controls, and counted to determine the actual number of larvae dispensed into replicates. All subsequent mortality determinations were made by comparison to the initial seawater control counts. Separate control counts were made for the "Green Book" procedures, due to the difference in test volume, as described above.

For both organisms, a single set of reference toxicant was run in a gradient series using cadmium chloride. The series was identical to that identified in Phase II. To confirm cadmium levels, a sample of the highest concentration was analyzed.

The end of the test is taken as the point at which > 90% of the organisms in the seawater control reach the prodissoconch (oyster), or pluteus larval (sand dollars) stage, as defined by the PSDDA program. Beginning at T = 45 hours, laboratory staff withdrew 10 mL aliquots from each treatment controls, and counted the number of normal and abnormal larvae. The procedure was repeated hourly until the 90% criterion was achieved, at which time the test was terminated.

For each test replicate, two 10 mL aliquot were withdrawn, fixed with 5% buffered formalin, and scored microscopically as normal (pluteus larvae) or abnormal. As a quality assurance procedure, 20% of all larval counts were re-scored by a second counter. In the event of a discrepancy between the counters, a third count was made, and procedures reviewed prior to proceeding further with counts.

Data Analysis

Larval response data were first tabulated in a spreadsheet, and then percentage mortality and abnormality determined by replicate, and then the mean of all replicates for an exposure treatment reported in the data summary. All data in the spreadsheets were checked and confirmed against the original data sheets, prior to proceeding with analyses.

For each treatment, the seawater control final counts were taken, and then compared to the initial inoculum counts for determination of percent control survival. Mortality in these exposures is expressed as the PSDDA combined mortality/abnormality endpoint. All sediment treatment comparisons are made against the number of surviving, normal larvae in the controls.

RESULTS

Reference sampling locations, depth of sampling, and conventional analyses results for the four Carr Inlet stations are found in Table IIIA-1. The analytical laboratory report may be found in Appendix B. The field efforts were successful in collecting material within the desired grain size ranges. In this effort, the wet-sieving method was an effective predictor of the laboratory determined percent fines. In these four cases, the field-predictive method was generally within 10% of the actual value. While bulk ammonia in CRR2 was relatively high, the aqueous unionized ammonia level measured in the experimental beakers didn't exceed 0.01 mg/L.

Table IIIA-1. Sampling location, conventional and grain size data for reference sediment samples.

Station	Location		Depth (m)	% Field Retained Sand*	% Lab Fines	Total Solids %	Tvs (mg/kg)	TOC (%)	Ammonia (mg/kg)	Sulfide (mg/kg)
	GPS	Loran TD								
CRR 2	47° 19.94'N 122° 40.74'W	27951.1 42214.0	23	60	28	64.74	12,700	0.4	74.85	0.13
CRR 4	47° 19.99'N 122° 40.48'W	27953.4 42214.9	14	40	51	62.59	13,700	0.4	3.72	0.11
CRR 6	47° 21.98'N 122° 38.82'W	27960.6 42223.1	18	10	87	32.49	21,800	1.2	9.87	0.15
CRR A	47° 20.18'N 122° 40.88'W	27956.4 42214.0	13	80	6	78.32	10,100	0.6	2.59	0.1

* = Percent material retained on the 62μ screen in field wet-sieving of test material.

Data, overall, were judged to be within acceptable PSDDA quality assurance parameters. In the oyster exposures, control mortality was less than 30%, with less than 10% abnormality. One exception was the control for the four-hour, aerated group, which had a mortality of 34.3%; this value was still within PSEP guidelines. Salinity was between 28 - 30 ppt, pH and temperature at acceptable levels. The levels of unionized ammonia were less than 0.04 mg/L, accept for in the Green Book preparations, where significantly higher levels were encountered. Additional problems occurred in the dissolved oxygen (DO) levels in the Green Book elutriate preparations. On Day 2 (24 hours) after inoculation, it was found that DO levels had fallen below 40% saturation in all of the Green Book elutriate vessels, and in some cases to less than 1 mg/L.

Echinoderm mortality for the seawater controls ranged between 8 - 13.5%, with abnormality exceeding the PSEP 10% maximum in one set (13.5%). Salinity was between 28 - 30 ppt, with pH range of 7.8 - 8.3. Temperature mean for all replicates was 16° C, which is higher than the PSEP-required limit of 15° C. However, the overall effect on data quality is thought to be negligible. Unionized ammonia values were less than 0.04 mg/L, except for the Green Book elutriates. DO levels were within acceptable limits, except for the Green Book treatments, where the DO again fell to less than 40% saturation.

Intra-sample variability for some treatments within these experiments is somewhat problematic. Within both oyster and sand dollar treatments, standard deviations in some replicates exceeded 33%, with corresponding Coefficients of Variance greater than 20%. There are no specific PSDDA, PSEP, or ASTM guidance on maximum allowable intra-replicate variability. Exercising best professional judgement, these data cannot provide definitive guidance on the relationship between grain size and mortality. However, they are judged to be of sufficient quality to indicate overall trends in that relationship.

Table IIIA-2 presents a summation of the experimental treatments. Larval data tables, physical monitoring data, and ammonia measurements may be found in Appendix B.

Table IIIA-2. Results of Phase IIIA oyster and echinoderm larval tests with varying grain-size reference sediment.

Phase IIIA Oyster Mortality and Abnormality

	PSDDA 4 Hour Settling (Aerated)		PSDDA 4 Hour Settling (Unaerated)		PSDDA 24 Hour Settling (Unaerated)		Elutriate Green Book protocol[1]		Elutriate Green Book w/PSDDA Count[2]	
	Mortality %	Abnormality %	Mortality %	Abnormality %	Mortality %	Abnormality %	Mortality %	Abnormality %	Mortality %	Abnormality %
Carr 6 (87% fines)	87.5	70.2	47.0	8.7	39.5	16.0	99.9	99.8	98.6	20.2
Carr 4 (51% fines)	17.1	1.4	20.5	0.7	-1.9	0.3	83.0	80.1	92.4	5.5
Carr 2 (28% fines)	11.0	2.1	9.8	1.0	-10.1	0.5	87.7	59.8	94.5	11.1
Carr A (6% fines)	14.1	2.7	14.9	1.6	-2.8	0.4	99.9	99.4	93.6	10.5

Phase IIIA Sand Dollar Mortality and Abnormality

	PSDDA 4 Hour Settling (Aerated)		PSDDA 4 Hour Settling (Unaerated)		PSDDA 24 Hour Settling (Unaerated)		Elutriate Green Book protocol[1]		Elutriate Green Book w/PSDDA Count[2]	
	Mortality %	Abnormality %	Mortality %	Abnormality %	Mortality %	Abnormality %	Mortality %	Abnormality %	Mortality %	Abnormality %
Carr 6 (87% fines)	16.8	8.7	45.5	23.6	-23.8	8.0	100.0	100.0	100.0	100.0
Carr 4 (51% fines)	-2.3	8.3	47.2	25.2	-2.1	7.8	100.0	100.0	94.6	8.6
Carr 2 (28% fines)	13.3	17.8	31.6	13.7	-10.4	7.1	100.0	100.0	92.6	9.5
Carr A (6% fines)	9.8	9.6	57.9	12.8	-21.0	7.6	100.0	100.0	96.9	61.2

1 This treatment followed Green Book elutriate protocol, including counting technique, abnormality, mortality.

2 This treatment followed Green Book elutriate protocol, however, water was poured off similar to PSDDA solid phase bioassay, so that larvae in the bottom of the jar are not counted, including abnormality, mortality.

Figure IIIA-1. Oyster Mortality
Grain Size and Aeration Effects

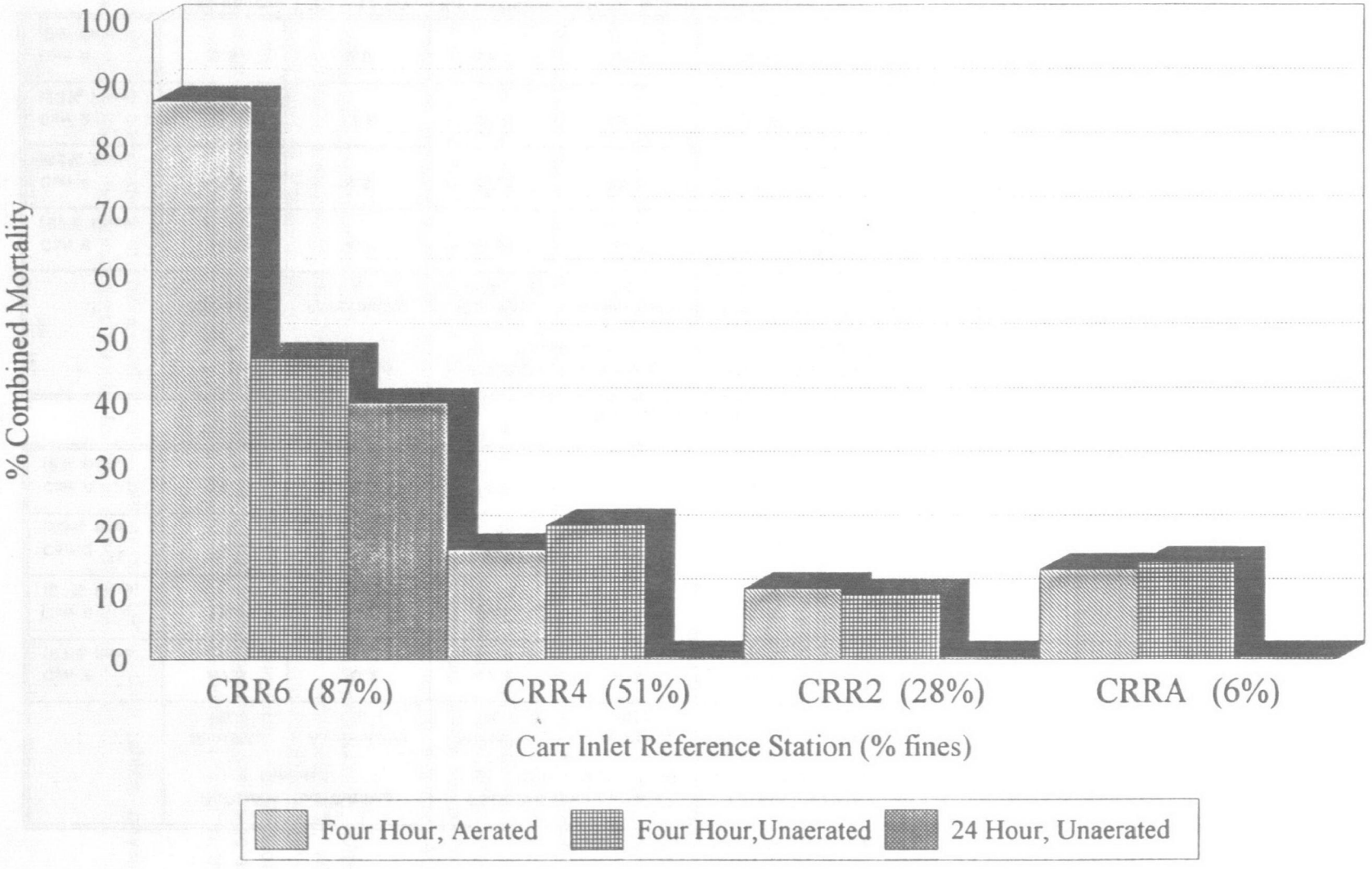


Figure IIIA-2. Oyster Abnormality

Grain Size and Aeration Effects

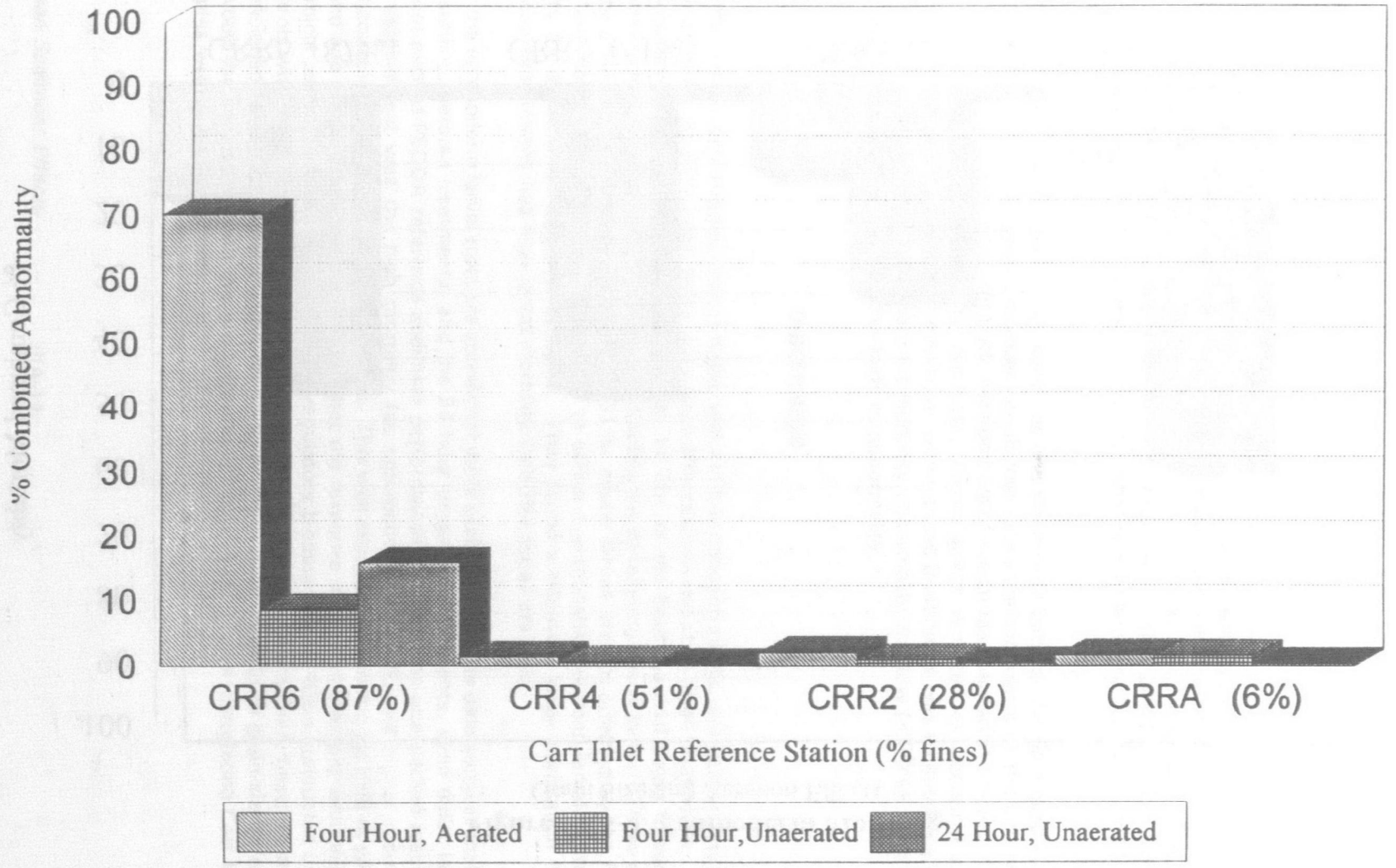
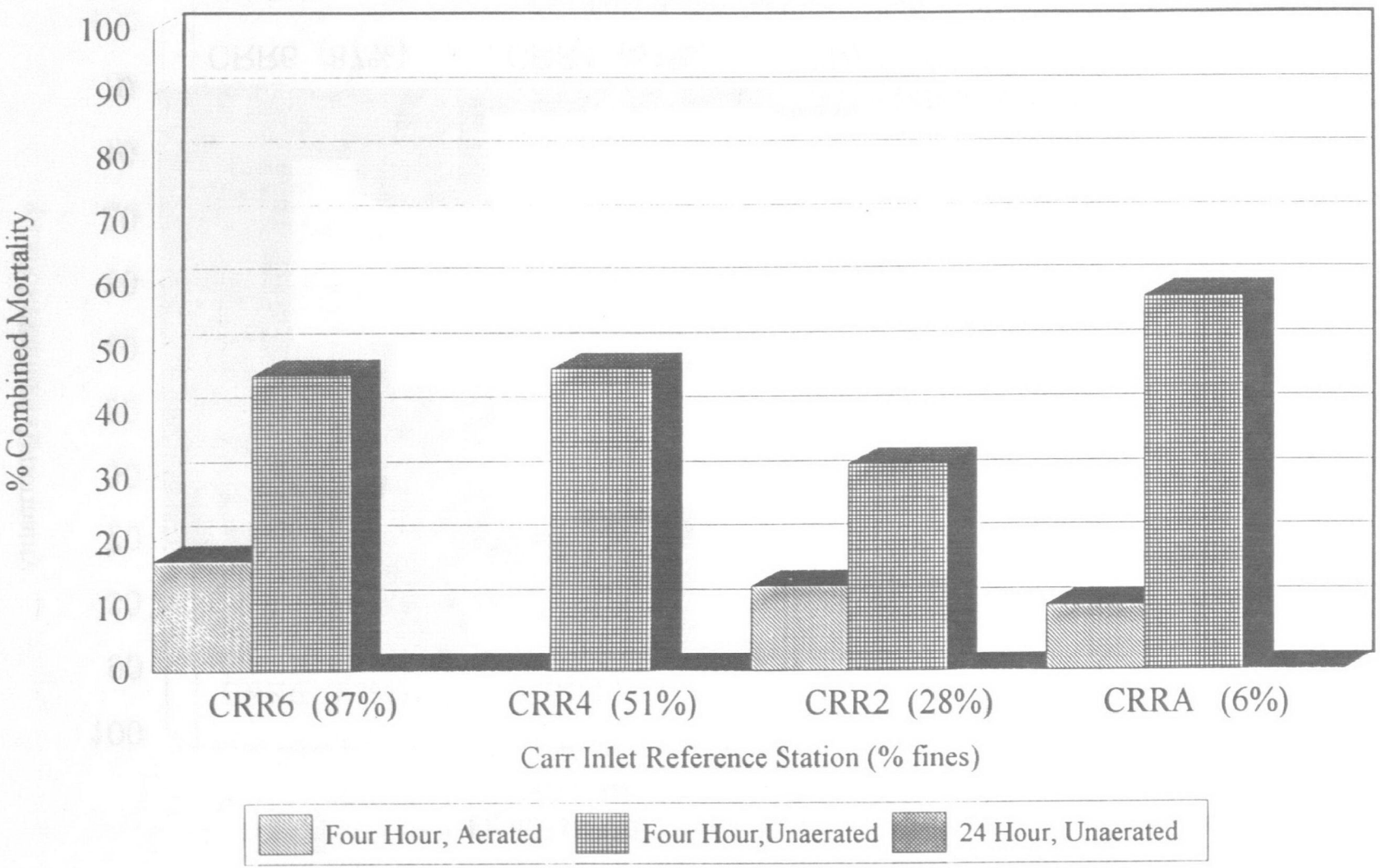


Figure IIIA-3. Echinoderm Mortality

Grain Size and Aeration Effects



Mortality in all Green Book elutriate treatments for both species was virtually 100%. The principal reason for the high mortality appears to be low levels of dissolved oxygen in all the Green Book elutriate procedures. There is likely a compounding factor due to grain size and entrainment, but low DO is believed to be the principal cause.

As a result of the loss of all the Green Book larval data, the remaining comparisons and discussions will focus on the three PSDDA-treatments only.

Figure IIIA-1 presents a bar chart summation of the oyster larval mortality data by stations. In all treatments, the CRR 6 reference sediment showed significantly greater responses, relative to the other three Carr stations. CCR4, CRR2, and CRRA were not significantly different from each other, within the respective treatments. Figure IIIA-2 shows the effect of grain size and treatment on oyster abnormality. As with mortality, the greatest effect was observed in the CRR6, 4-hour aerated treatment. Percent abnormality for CRR6 was low for the other two treatments, but higher than that observed for the other three stations.

The response of the sand dollar larvae to the exposures and treatments can be seen in Figure IIIA-3. Of immediate note is the higher apparent mortality in all four reference sediments of the unaerated treatments. These high responses represent both low numbers of live larvae counted at the end of the exposure, and a relatively high abnormality response. Those larvae reported as abnormal were developmentally retarded, suggesting that some factor within those treatments was inhibiting cellular development. For the aerated 4-hour settlement the mortality/abnormality was low ($<20\%$). In all four 24-hour treatments, there was virtually no mortality or abnormality.

DISCUSSION

The results for the oyster tests suggest that at higher percent fine concentrations, oyster mortality and abnormality are effected. At 87% fines, there were large effects observed at all three treatments. The high response observed in the aerated treatment may be due to the finer material being forced to remain in suspension longer, entraining or otherwise affecting the developing oyster larvae. These data suggest that for grain-sizes of fines percentages $\leq 51\%$, oyster larvae are not affected by the finer material under any treatment. PTI (1991) attempted to define a relationship between larval mortality and abnormality for oyster with Carr Inlet samples. They found a relatively weak correlation ($r = 0.557$), but those experiments had high larval mortality ($>50\%$) for all stations.

The results for the echinoderm larval tests are somewhat more problematic. The data suggest that for the four-hour settling, aerated treatments, and the 24 hour unaerated treatments, sand dollar larval responses do not exceed PSDDA reference sediment performance criteria. The unexpected result in the four-hour settling test was the high mortality (as non-recovered larvae), and higher retarded develoment, encountered for all the reference stations. This was especially surprising for CRR A, which was largely sand and shells. There is no indication that the response was related to any ammonia or other test chemical parameters. Numerous PSDDA regulatory bioassays have been conducted using unaerated controls with West Beach sand, and had virtual 100% survival. At the very best, the most that can be concluded from those data is that the mortality effect is not related to grain-size effects. That finding would be consistent with the work conducted by PTI (1991) for correlating Carr Inlet reference samples with grain size effects.

To date, there has been little work addressing the effects of fine grained material on larval survival in sediment elutriate bioassays. The effects of particulate matter on bivalve larval development were addressed by Davis (1960) and by Davis and Hindu (1969). In those papers, the authors described inhibition of larval development to the prodissoconch stage (hinged or "D"-shaped) by silt/clay concentrations in as low as 3-4 gms/L.

While direct extrapolation of those data to elutriate testing is not possible (owing to major differences in exposure procedure) the ability to express inhibitory effects in gms/L of silts and clays has some utility. In order to compare these results to the findings of Davis, the grams of silt and clay present in the test vessels for each test sediment was calculated based on the percent solids, and percent fractional components, according to the following formula:

Test Sediment (gms) x Percent Solids x Percent Silt and/or Clay

These calculations are presented in Table IIIA-3.

Table IIIA-3. Estimates of Silt and Clay Fractions Present in Bioassay Vessels Based on Grain Size Results.

Reference Station	Test Sediment (gms)	Total Silt (gms)	Total Clay (gms)	Clay Fractions by Particle Size		
				<3.9	<1.9	<0.9
CRR 6	20	4.16	1.56	0.39	0.31	0.84
CRR 4	20	5.63	0.88	0.10	0.10	0.68
CRR 2	20	2.83	1.16	0.34	0.00	0.82
CRR A	20	0.47	0.94	0.16	0.00	0.78

Carr 4 has a total fines concentration of 6.51 grams/L, while Carr 6 has a fines concentration of 5.7 g/L.. Based on the hypothesis that total higher fines loads correspond to higher mortality, Carr 4 should have had a higher percent mortality than Carr 6. When compared to the data, Carr 6 had mortality in the oysters, but was not significantly different from Carr 4 in the echinoderm exposures. When compared to the values published by Davis, both Carr 4 and Carr 6 should have had no surviving larvae to the prodissoconch stage.

As an example of how this procedure could be applied for data interpretation, the grain size data from the *Reference Area Performance Standards* (PTI, 1991) were re-expressed as grams of material per liter in Table IIIA-4. In reporting the percent fines for stations SM30 (96%) and HM05 (58%), there is a mathematical difference of 38%. However, when the percent solids are factored into the calculation, the total amount of silt and clay in an elutriate test vessel is only 0.26 gms different. When HM05 is compared to SM34 (74% fines), the calculated grams of material is actually higher for HM05 (6.5 vs. 5.55 gms) for HM05.

Table IIIA-4. Comparison of reported grain size distributions vs. mass of material in bioassay test vessel. Data taken from PTI (1991).

Station	Test Sediment	Solids	Reported Grain Size Distribution			Actual Material In Test Replicate		
			Fines	Silt	Clay	Silt	Clay	Total
	(gms)	(%)	(%)	(%)	(%)	(gms)	(gms)	(Total)
SM30	20	35	96	63	34	4.41	2.38	6.79
SM34	20	37	74	48	27	3.55	2	5.55
HM05	20	56	58	40	18	4.48	2.02	6.5
CR23	20	58	49	43	6	4.99	0.7	5.68
SM33	20	60	29	19	10	2.28	1.2	3.48
CR21	20	71	14	9	5	1.28	0.71	1.99
SM31	20	89	13	8	5	1.42	0.89	2.31

It is suggested that the more important component in this evaluation is the clay content. Table IIIA-4 presents the predicted settling rates of silt and clay particles in the bioassay chambers, based on the physical principal of settling, known as Stoke's Law. The assumptions made to generate these settling rates (eg., spherical particles, seawater having a viscosity of 1) are under ideal circumstances, and are probably not equivalent to those observed in bioassay chambers. However, the table is useful in demonstrating what materials should theoretically remain in suspension after 4, 24, and 48 hours of settling. Actual rates are probably slower.

Table IIIA-5 indicates that the only particles that are of concern after four hours of settling would be the clay fraction of particle size $\leq 1.9\ \mu$, and after 24 hours the $\leq 0.9\ \mu$ fraction. Based on the data in Table IIIA-3, Carr 6 would have the greatest amount of material in suspension at 4 hours, but that all stations would have an equivalent amount of material in suspension after 24 hours settling. It should be noted that changes to the viscosity of the seawater with the addition of the sediment, coupled with non-ideal behavior of sediment particles in test suspension, would likely result in the settling rates being much slower than shown here. Aeration of the test vessels would likely create small currents in the test chambers that also would effect the rate of settling.

Table IIIA-5. Predicted Settling Rates of Silt and Clay Particles Sizes in Bioassay Chambers, based on Stoke's Law.

Particle Size	Settling Rate	Height of Vessel	Settled Distance of Particles		
			4 hours	24 hours	48 hours
(μ)	(cm/s)	(cm)	(cm)	(cm)	(cm)
62	0.1400	15	2016.00	12096.00	24192.00
31	0.0500	15	720.00	4320.00	8640.00
3.90	0.0023	15	32.61	195.69	391.37
1.90	0.0005	15	7.36	44.15	88.30
0.90	0.0001	15	1.65	9.91	19.81

We caution that at this level, the relationship between clay particles and oyster mortality is still not clearly defined. More data is necessary to evaluate the strength of the relationship, and to define the level at which relative amounts of silts or clays in bioassay vessels becomes a problem. We do suggest, however, that the traditional means of examining the data in terms of "percent fines" is probably invalid, and that to do comparative analyses between stations, the data must be expressed as grams of fines/Liter.

RECOMMENDATIONS

- Caution should be exercised in utilizing *Crassostrea gigas* larvae in sediments known to have a high proportion of clays and silts.

- Larvae of *Dendraster excentricus*, when tested under current PSDDA protocols, do not show an adverse response to increasing silt and clay fractions. Under conditions of expected high silts/clay, the sand dollar test should be utilized.

- For the purposes of selecting suitable reference material for larval bioassay comparisons, the percent grain-size data should be converted to grams/Liter of silts and clays.

- Aeration is recommended for use in the Green Book larval bioassay.

REFERENCES

Davis, H., 1960. Effects of Turbidity-Producing Materials in Sea Water on Eggs and Larvae of the Clam *Venus (Mercenaria) mercenaria*. Biol. Bull. 118: 48 - 54.

Davis, H., and H. Hindu. 1969. Effects of Turbidity-Producing Substances in Sea Water on Eggs and Larvae of Three Genera of Bivalve Mollusks. The Veliger 11 (4): 316 - 323.

PSEP. 1986. Puget Sound Estuary Program. *Recommended Protocols for Measuring Selected Environmental Variables in Puget Sound.* Final Report. Prepared for the U.S. Environmental Protection Agency Region X, Office of Puget Sound, and the U.S. Army Corps of Engineers. Tetra Tech Inc., Bellevue, Washington.

PSEP. 1989. Puget Sound Estuary Program. *Recommended Guidelines for Measuring Metals/Organic Compounds in Puget Sound Sediment and Tissue Samples.* Prepared for the U.S. Environmental Protection Agency Region X, Office of Puget Sound, and the U.S. Army Corps of Engineers. PTI Environmental Services, Inc., Bellevue, Washington.

PSEP. 1991. Puget Sound Estuary Program. *Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments.* U.S. EPA, Region 10, Office of Puget Sound, Seattle, WA.

PTI Environmental Services (1991). *Reference Area Performance Standards for Puget Sound.* U.S. EPA, Region 10, Office of Coastal Waters, Seattle, WA

U.S. EPA 1991. *Evaluation of Dredged Material Proposed for Ocean Disposal, Testing Manual.* United States Environmental Protection Agency and the U.S. Army Corps of Engineers. EPA - 503 /8-91 / 001.

REFINEMENTS TO CURRENT PSDDA BIOASSAYS

FINAL REPORT

PHASE IIIB: SPECIES SENSITIVITY COMPARISON TO CONTAMINATED SEDIMENT EFFECTS

An Employee-Owned Company

List of Tables

List of Figures

PHASE IIIB: SPECIES SENSITIVITY COMPARISON TO CONTAMINATED SEDIMENTS

INTRODUCTION

Sediment larval bioassays are currently used under regulatory dredged sediment testing programs to help determine the suitability of proposed dredged material for unconfined open water disposal. These elutriate bioassays are used as a screen for possible adverse biological effects that occur due to the presence of chemicals of concern in the dredged test sediment.

Both the Puget Sound Dredged Disposal Analysis (PSDDA) program, and the Ocean Dumping Testing Manual (EPA/USACE,1991) allow a project proponent to select the test organism for the larval test. Most frequently, the Pacific oyster *Crassostrea gigas*, and the North American sand dollar *Dendraster excentricus*, are used in the programs. The regulatory agencies with jurisdiction in dredged testing programs currently regard all larval species as being equivalent in their response to test materials. However, to date, there have been no definitive data comparing the responses of sand dollars and oysters to the same sediments.

The Environmental Protection Agency identified the need to characterize and compare the responses of these two species to both clean and contaminated sediments. Phase IIIA of this document compares the response of the two species to clean reference sediments, the experiments of Phase IIIB compares the responses to sediments of known contamination.

The PSDDA agencies also wanted to compare the same test protocols described for Phase IIIA, with the contaminated sediments in these exposures. The intent was to compare the methods to determine if there were any differences in organism responses, and if not, to select a method for regulatory testing that would retain environmental sensitivity, but potentially would minimize the possibility of false positives (eg., aeration to maintain high levels of dissolved oxygen, longer settling times - 24 hours - to minimize fine sediment entrainment of larvae). Finally, the issue of whether the protocols developed for elutriate testing under the PSEP program were as effective as the procedures for elutriate testing in the Green Book in indicating sediment toxicity.

The objectives of this study are as follows:

- To determine if oysters and sand dollars have equivalent responses to the same level of contaminants in a sediment.
- To determine if oysters and sand dollars responses to test sediments are equivalent in predicting sediment toxicity under the PSDDA program.
- To determine how the various test protocols compare in terms of predicting sediment toxicity to both organisms.

METHODS AND MATERIALS

TEST OVERVIEW

The preliminary experimental design was to evaluate six different contaminated test sediments over the five test procedures. In order to try and minimize confounding influences of different grain sizes, sediment total organic carbon, sulfide, or other sediment conventional parameters, it was decided to collect just 2 contaminated sediments with known chemical distributions and positive bioassay responses. Those sediments would then be "diluted" by 50% and 75% with the clean Carr Inlet reference sediments identified in Phase IIIA. If similar conventional parameters could be matched, the only effect that would be measured would be varying contaminant load. This approach is similar to that employed by Pastorok and Becker (1989) who had attempted to examine both larval species responses in the same study. In that study, however, oyster larval survival was poor, preventing an effective comparison of the two species.

The Pacific oyster, *Crassostrea gigas*, and the eastern Pacific sand dollar, *Dendraster excentricus*, were selected for study. These two species are the most frequently tested under the elutriate sediment testing programs. Larvae were exposed for 48 hours to the test sediments/test conditions, and the responses compared to that in a seawater control, and a reference sediment.

All testing was conducted at SAIC's Environmental Testing Center, in Narragansett, Rhode Island.

SEDIMENT COLLECTION AND ANALYSES

Contaminated Sediment Site Selection

Prior to selecting the appropriate stations for field collections, a review was conducted of sediment data sets from both EPA's and the Corps' data bases. The objective was to locate stations with sufficiently high levels of contaminants, and a past history of having a positive, partial larval bioassay response [1]. The review included stations in Commencement Bay, Elliott Bay, Eagle Harbor, and Bellingham Bay. Upon evaluation of the data, two stations were identified from a data set associated with a former shipyards on the West Waterway and Elliott Bay. Location and justification for utilization of these two sites is as follows:

Site D1: This site is located at the western edge of the shipyard property line, in proximity to a pole-creosote facility, approximately 450 ft. offshore. Previous data indicated that the site had high levels of low and high molecular weight polyaromatic hydrocarbons (LPAH, HPAH), but no exceedances of the PSDDA SL for metals. The grain size was reported as 68% fines, and previous bioassays on sediment collected from D1 indicated an echinoderm response of 27.7% combined effects mortality, and *Rhepoxynius abronius* mortality of 42.5%.

---

[1] The stations used by Pastorok and Becker were not used in this study due to the high level of mortality observed in those sediments. In the present study, stations were sought for which partial larval mortality responses were observed.

Site M1: Site M1 is located on the West Waterway, approximately 525 ft. south of the north end of Pier 21, and 75 ft. eastward of the Pier. Data for M1 showed a mixture of contaminants, with high concentrations of copper, lead, and zinc. For copper and lead, the MLs were exceeded by approximately 2 and 3 times, respectively. Total LPAH, but not HPAH, exceeded the ML. Previous echinoderm larval bioassay data showed a response of 25% combined effects.

Contaminated Site Sample Collection

Samples were collected using a stainless steel 0.1 m^2 Young van Veen grab, aboard the research vessel KITTIWAKE. Station location was determined using both a Global Positioning System, and a Loran C system. Location, depth, and time of sample were recorded for each grab. PSEP protocols were followed for sampling procedures and sample acceptability. The van Veen sampler was thoroughly washed with Alconox detergent and seawater before the start of sampling, and between each sampling station. To obtain 20 L of sediment required to formulate the dilution series, it was necessary to take multiple grabs. All of the sediment from each acceptable grab was retained, except the sediment touching the sides of the sampler. Samples were collected with decontaminated stainless steel spoons and 19 L stainless pans.

For chemical analysis of the whole parent sediment, volatile organic and total sulfides samples were taken from a randomly selected grab from each station. Samples were placed into appropriate glass containers according to PSEP procedures. For total sulfide analyses, the samples were fixed in the field with zinc acetate. Samples were stored in coolers on ice at 4° C, and taken back to the SAIC laboratory to composite and prepare the dilution series.

Reference Sediment Collection

Reference sediments necessary to complete the sediment dilution series were collected in Carr Inlet. Carr Inlet has been recognized as a reference sediment site (PTI, 1991), and has been previously used under the PSDDA testing program. Two stations, CRR2 and CRR4 were identified to represent 45% and 65% fines for sediment grain size, respectively. As with Phase IIIA, CRR2 and CRR4 are site designations independent from those identified in PTI 1991. Samples were collected using a stainless steel 0.1 m^2 Young van Veen grab, aboard the University of Washington's 17 foot Boston whaler. Two field events were necessary to collect sufficient sediment to make the composites. Of note is that on the first collection trip the Loran C receiver, a Micrologic ML-3000, was faulty making positioning of the vessel at the proper Loran coordinates questionable. For the second field event, a Northstar 800 Loran C navigation system was acquired for positioning, which proved much more reliable. At each station, the Boston whaler was positioned according to the proper Loran C time delays (TDs), and for each replicate grab, the TDs never exceeded 0.1 from the original TDs.

To ensure that the grain size distribution required for each station was met, the wet sieving technique used and described in Phase IIIA was also used. All sampling procedures and judgement of sample acceptability followed the PSEP protocols, unless stated otherwise. To avoid possible adverse effects associated with high sediment sulfide levels, only the top 2 cm were collected from each sediment grab. Volatile organics and total sulfides samples were taken from a randomly selected grab from each station. A total of 20 L of sediment was collected at each station in the two trips, stored in clearly labeled polyethylene bags, and brought back to the SAIC laboratory to be homogenized. Samples were transported in coolers with ice to keep sediments at 4 degrees C.

Construction and Analyses of Contaminated/Reference Site Composites

The dilution series was prepared as 50%, and 25% dilutions of the parent contaminated sediment. Dilutions were constructed on a volume to volume basis, with a final volume of 10 L per test sediment. An industrial mixer was used to thoroughly mix each sample, before creating the dilutions. The stainless steel bowl and mixer were decontaminated between each sample. For each 100% contaminated sediment, the sample was thoroughly homogenized, and then sub-sampled for PSDDA sediment conventional chemistry and chemicals of concern analyses. The remaining sediment was then put into 2 L glass jars, purged with nitrogen gas, and refrigerated at 4° C until bioassay testing. To create the dilutions, samples were measured volumetrically with 4 L decontaminated glass beakers and thoroughly mixed with the industrial mixer. After thorough mixing of the dilution sample, samples for PSDDA sediment conventionals and chemicals of concern were collected. The remaining sediment was then put into 2 L glass jars, purged with nitrogen gas, then refrigerated until bioassay testing.

Analytical Methods

All of the chemical analytical procedures used in this program were performed in accordance with the most current PSEP (1986, 1989) and PSDDA (1989) documentation with modifications specified in the annual reviews (PSDDA, 1990 and 1991). Sediments were analyzed for the PSDDA chemicals of concern and conventional parameters specified in the PSDDA *Management Plan Report (MPR) Unconfined, Open-water Disposal of Dredged Material, Phase II (North and South Puget Sound)*, September 1989. Appropriate PSDDA/PSEP QA/QC analyses (i.e., matrix spike/matrix spike duplicate) were performed for both Batch B442 and B656 samples. A QA2 data package was also prepared by the laboratory and was submitted with the final data reports under separate cover.

Sediment samples for contaminant chemistry were submitted to Analytical Resources, Inc., in two batches. The first batch, B442, consisted of Samples M1, D1, M1C2-50/50, and D1C4-50/50 for contaminant chemistry analysis. Two Carr Inlet samples (Carr2 and Carr4) were also submitted in this batch for conventional chemistry testing. The second batch, B656, was composed of Samples D1C4-25/75 and M1C2-25/75, and samples were analyzed for the PSDDA chemicals of concern. Raw analytical results are presented by batch as are the QA1 review forms (see appendices).

Batch B656 sediments were submitted to the analytical laboratory beyond holding times for some parameters (see QA1 review) following an unplanned change in the work plan. For this reason, samples were not analyzed for volatile organic compounds.

Specific analytical methodologies and problems encountered during analyses are described in the following paragraphs.

Organics

Batch B442 samples were analyzed for volatile organics (VOAs) using Method 8240 (purge and trap). VOAs were analyzed using approximately 5.0 grams of sample (wet weight); no problems were noted during analysis.

Semivolatile organic compounds were prepared using extraction Method 3550 and analyzed by Method 8270 (GC/MS). Both PSEP-recommended modifications to the extraction method and the use of calibration standards of lower dilution concentrations were implemented to lower detection limits. In Batch B442, some surrogate recoveries were reported out of the recommended control limits. The laboratory postulated that twice the amount of surrogate/spike compound mixture was added at the time of extraction, however, the analytical results as reported were not affected. In addition, all samples required analysis at diluted concentrations because analytes were detected above the linear

calibration range. Sample D1C4-25/75, in batch B656, required re-analysis at a 1 to 10 dilution due to several analyte levels above the linear calibration range.

Pesticides and PCBs were extracted using Method 3550 and analyzed by Method 8080 (GC/ECD). The presence of pesticides and PCBs were verified using dual column confirmation. For both batches, matrix interferences caused the laboratory to raise detection limits for pesticides and PCBs.

Metals

Sediment samples were analyzed for the PSDDA metals of concern (except mercury) using the total acid digestion (TAD) method described in the PSEP (1989) documentation. Sediments were digested in a Teflon bomb using nitric, hydrochloric, and hydrofluoric acids. ARI utilized a pressure-controlled microwave heating technique to complete the digestion process. The digestion products were analyzed for arsenic, antimony, cadmium and silver using graphite furnace atomic absorption (GFAA). Copper, lead, nickel, and zinc were analyzed by inductively coupled plasma emission spectroscopy (ICP). Mercury was digested using nitric and sulfuric acids and oxidized using potassium permanganate and potassium persulfate. The digestate for mercury was analyzed using Cold Vapor Atomic Absorption (CVAA).

No problems during the metals analyses were reported by the laboratory.

Conventionals

The PSDDA conventional parameters include grain size distribution, total solids, total organic carbon, total sulfides, ammonia, and total volatile solids. Methods for conventional parameters followed those provided in PSEP (1986) and PSDDA (1989) with modifications specified in the annual reviews (PSDDA, 1990 and 1991). Ammonia measurements were determined using Plumb (1981). Particle grain size distribution for each sample was determined in accordance with ASTM D 422 (modified). Wet sieve analysis was used for the sieve sizes U.S. No. 4, 10, 18, 35, 60, 120, and 230. (Hydrogen peroxide was not used in preparations for grain size analysis which may break down organic aggregates causing an overestimation of the percent fines found in undisturbed sediment.) Hydrometer analysis was used for particle sizes finer than the 230 mesh, and water content was determined using ASTM D 2216. Sediment classification designation was made in accordance with U.S. Soil Classification System, ASTM D 2487.

No analytical problems were noted by the laboratory for all conventionals analyses.

BIOASSAY PROCEDURES

Test Sample Preparation

Each of the six test sediments were prepared according to the following procedures:

- PSDDA 4-hour Settlement, Aerated. Twenty grams per liter of test sediment were measured into pre-labeled glass Mason jars. Filtered seawater was added, and the contents vigorously shaken for approximately 10 seconds. The test vessels were then placed in the temperature-controlled water bath, and allowed to settle for 4 hours prior to inoculation of test embryos. This treatment was aerated throughout the entire 48 hour exposure.

- PSDDA 4-hour Settlement, Unaerated. These treatments were prepared identical to that described above, but were not aerated during exposures.

- PSDDA 24-Hour Settlement. These treatments were prepared exactly as above, with the exception of an allowance of 24 hours of settling time. These replicates were prepared a day ahead of time, so that the end of the settling time would coincide with the inoculation. All replicates prepared this way were not aerated during the test.

- Green Book. The method for preparing an elutriate involves making a 1 part sediment to four parts seawater mixture, that is vigorously stirred for 30 minutes. While the Green Book recommends use of a magnetic stirrer, the volume of material prepared in batch (8 L) prevented effective use of a stir bar. In these experiments, vigorous aeration was used, with a complete manual mixing at the start, and every 10 minutes. The resultant slurry was allowed to settle for 30 minutes, and the liquid portion was carefully poured off so as to not disturb the settled material. The retained liquid was then dispensed into the test vessels.

  For each sediment, a total of 10 replicate samples were prepared. All 10 replicates were inoculated and held under the same conditions during testing. Based on the experiences of Phase IIIA, where the dissolved oxygen levels fell below acceptable levels, a decision was made to begin aerating these sediments from the point of inoculation. At the end of the exposure, aliquots for larval counts were taken from one set of five replicates by carefully decanting off the overlying water into a second container, taking care not to include any of the bottom material ("PSDDA counts"). In the second set of five replicates, the entire vessel contents were first mixed, and then aliquots were withdrawn for larval counts.

Source of Broodstock and Spawning Conditions

Gravid sand dollar and Pacific oyster broodstock were obtained from the same sources as described for Phase II. All organisms were transported to the SAIC Testing Center in Narragansett by overnight freight, and were used on the day of arrival. Prior to spawning, the organisms were acclimated to test temperature.

Spawning procedures were identical to those described in Phase II.

Experimental Procedure

For each organism, and test procedure, an individual set of five seawater control replicates were set up to duplicate the conditions of the procedure. For example, for the 4-hour settling/aerated treatments, there were five aerated seawater controls that had sat under the same conditions as the test replicates prior to inoculation. The controls for the 24-hour settling were set up at the same time as those treatments, and allowed to "settle" for 24 hours. Green Book controls were mixed and handled identically to the test treatments.

For each organism and test procedure, six replicates were prepared. Where required, aeration was accomplished by dispensing the air through a glass pipette set at a rate of less than 100 bubbles per minute. The sixth replicate was used for taking physical monitoring and ammonia measurements. Post inoculation, physical monitoring measurements (pH, salinity, dissolved oxygen, and temperature) were

taken for each test series. Those measurements were taken again at 24 and 48 hours. Ammonia was taken at the time of inoculation, and at test termination.

Inoculation of embryos occurred using a volumetric pipette, calibrated to deliver between 20 - 30 embryos/mL. To ensure homogenous distribution of embryos in the stock solution, a perforated plunger was used to mix the solution. Post-inoculation, two 10 mL aliquots were withdrawn from one set of five seawater controls, and counted to determine the actual number of larvae dispensed into replicates. All subsequent mortality determinations were made by comparison to the initial seawater control counts. To ensure that all the various treatment controls were identical, an additional two 10 mL aliquots were taken from two replicates of each seawater control. These counts were made and compared to the other 10 initial counts.

To compare responses of the two organisms to a metal and organic reference toxicant, two sets of reference toxicants were run in a gradient series using cadmium chloride and phenol. The cadmium series was identical to that identified in Phase II. For the oysters, a phenol series of 0, 15.6, 31.25, 62.5, and 125 mg/L was run, and for the echinoderms 31.25, 62.5, 125, and 250 mg/L.

The end of the test is taken as the point at which > 90% of the organisms in the seawater control reach the prodissoconch (oyster), or pluteus larval (sand dollars) stage, as defined by the PSDDA program. For each test replicate, three 10 mL aliquots were withdrawn, fixed with 5% buffered formalin, and two were scored microscopically as normal (pluteus larvae) or abnormal. As a quality assurance procedure, 20% of all larval counts were re-scored by a second counter. In the event of a discrepancy between the counters, a third count was made, and procedures reviewed prior to proceeding further with counts. If further resolution was needed, the third aliquot was scored for that replicate.

Data Analysis

Larval response data were first tabulated in a spreadsheet, percent mortality and abnormality determined by replicate, and then the mean of all replicates for an exposure treatment reported in the data summary. All data in the spreadsheets were checked and confirmed against the original data sheets, prior to proceeding with analyses.

For each treatment, the seawater control final counts were taken, and then compared to the initial inoculum counts for determination of percent control survival. Mortality in these exposures is expressed as the PSDDA combined mortality/abnormality endpoint. All sediment treatment comparisons are made against the number of surviving, normal larvae in the controls.

Each of the test sediments and treatments were analyzed to determine how the results would compare to a PSDDA open-water disposal determination. The mortality data were compared to the control, and the appropriate reference sediment data from IIIA, using the arcsin/square root transformation of proportional data, and conducting one-way *t*-test analyses. The 20% and 30% guideline values were then applied, to determine "suitability" under PSDDA.

To evaluate differences between the two species by station and treatment, multiple two-way *t*-tests were run on the transformed mortality data using Excel 5.0. An alpha level of 0.05 was used for all comparisons.

To compare differences between test treatments, an Analysis of Variance (ANOVA) was conducted, followed by using Tukey's Wholly Significant Differences test on the transformed mortality data. The Statgraphics statistical package was used to run the ANOVA and derive the pooled standard error, and

the differences determined by following the procedures of Zar (1984) to determine the q statistic and critical q values.

Determination of reference toxicant LC50 and EC50 values was done using EPA's probit program on proportional mortality and abnormality data.

RESULTS

SEDIMENT COLLECTION AND ANALYSES

Sediment Conventionals

Station coordinates, conventional, and grain size analyses are presented in Table IIIB-1. The blended sediments for the D1 series achieved a fair agreement on grain size distribution (58 - 68% fines), and percent total solids (approximately 52 to 65%). However, the analysis of CRR2 showed that there was a much lower level of percent fines than was anticipated (14% vs. the 31% measured during Phase IIIA). As a result, the M1 dilution series ranged between 14 and 43% fines, and total solids ranging from 43 to 76%.

Table IIIB-1. Sampling location, conventional and grain size data for IIIB sediment composites.

Station	Location		Water Depth (m)	% Fines	Total Solids	Tvs (mg/kg)	TOC (%)	Ammonia (mg/kg)	Sulfide (mg/kg)
	GPS (North by West)	Loran TD							
M1	47°35.11N 122°21.67 W	27989.3 42296.1	16	43	42.94	6.06	2.90	17.60	238.00
M1C 25/50	---	---	--	25	64.77	1.36	1.00	9.72	275.00
M1C2 25/75	---	---	--	13	73.48	2.50	0.80	18.60	73.00
CRR2	NONE	27954.1 42214.4	21-23	14	74.55	6.74	0.40	5.41	<1.66
D1	47°35.15N 122°22.02 W	27991.1 42295.3	14	58	51.85	2.54	1.80	11.00	297.00
D1C4 50/50	---	---	--	63	55.63	3.25	1.30	13.10	99.60
D1C4 25/75	---	---	--	66	62.12	11.50	0.80	33.12	175.00
CRR4	NONE	27953.1 42215.4	13	68	64.65	12.70	0.50	11.9	<1.81

Sediment Analyses

The results of the screening for PSDDA chemicals of concern are presented in Table IIIB-2. PSDDA screening levels, maximum levels, and the 1988 oyster Apparent Effect Threshold (AET) values (PTI, 1988) are also shown on Table IIIB-2. The original laboratory data sheets, chain-of-custody records, and QA1 memoranda are included in the appendix of this section. QA2 data have been provided to EPA under separate cover.

In general, the mixing of contaminated sediments with the clean reference sediment achieved the desired effect of diluting the chemicals of concern. Table IIIB-2 shows that for metals, and most of the LPAH and HPAH, the chemicals of concern were decreased proportionally. Some exceptions were noted in the D1 series for napthelene and acenapthene at the 50% dilution. For napthelene, the measured value doubled, while the acenapthene level remained diluted. Review of the laboratory procedures indicated that the measured values were valid data points. One possible explanation is that while every effort was made to achieve thorough mixing of the test sediment, "pockets" of undiluted sediment remained, which may have had higher levels of these compounds.

All test sediments exceeded at least the PSDDA SL for several analytes, and Station M1 and all of the D1 series dilutions exceeded the ML and AET for some of analytes. As expected, M1 had a high level of copper,lead, mercury, and zinc, and exceeded the SL for most of the low polyaromatic hydrocarbons (LPAH) and high PAH. The 50% and 25% dilution samples for M1 had no ML or AET exceedences, but several SL exceedences for metals, and the LPAH and HPAH fractions. Station D1 had lower levels of metal contaminants that were reduced to at or below the SL in the 50 and 25% dilution series. By contrast, the D1 series had extremely high levels of LPAH and HPAH at all dilutions. The total LPAH level for D1 exceeded the AET value by 3 times. Oyster AET exceedences were measured at all D1 series dilutions.

Both M1 and D1 showed SL exceedences for phenols, miscellaneous extractables, and Total PCB. D1 and D1C4 50/50 had dibenzofuran levels exceeding the ML and AET. Neither series showed any detected chlorinated hydrocarbons, phthalates, volatile organics, or pesticides.

Based on the chemical data alone, all of the D1 dilution series, and Station M1, would not be suitable for open water disposal under the PSDDA program. M1C2 50/50 and M1C2 25/75 had numerous SL, but no ML exceedances, and would be eligible for biological testing under the PSDDA program. All test stations exceeded the Washington State Sediment Quality Standards and the Impact Zones Maximum Chemical Criteria for total LPAH and HPAH (WAC 173-204-320 and 420).

BIOASSAY RESULTS

Data Acceptability

Oyster larval survival to the prodissoconch stage was >95% for all seawater controls, with abnormality less than 4%. Unionized ammonia levels were less than 0.04 mg/L for all controls and most treatments at inoculation and conclusion. Noted exceptions were many of the Green Book preparations which had exceeded the guideline value at the end of the test.

Echinoderm larval survival to the pluteus larval stage in the seawater controls was >70%, with less than 10% abnormality, with the exception of the Green Book controls, where the abnormality was recorded as 12.1%. The effect on data quality by a 2% exceedance is thought to be negligible. Unionized ammonia levels were all less than 0.04 mg/L at the test initiation, but values for two of the Green Book M1 treatments exceeded that value at test termination.

Table IIIB 2. Concentrations of PSDDA Chemicals of Concern Found in Test Sediments.

PSDDA PARAMETER	PSDDA SL	PSDDA ML	1988 OYSTER AET	STATION M1	M1C2 50/50	M1C2 25/75	D1	D1C4 50/50	D1C4 25/75
CONVENTIONALS									
Total Solids (%)				42.94	64.77	73.48	51.85	55.63	62.12
Total Volatile Solids (%)				6.06	1.36	2.50	2.54	3.25	11.50
Total Organic Carbon (%)				2.90	1.00	0.80	1.80	1.30	0.80
Total Sulfides (mg/kg)				238.00	275.00	73.00	297.00	99.60	175.00
Ammonia (mg/kg)				17.60	9.72	18.60	11.00	13.10	33.12
Grain Size (Percent Fines)				43.00	25.00	13.00	58.00	63.00	66.00
METALS (ppm, dry weight)									
Antimony	20	200		149.00	60.10	19.90	7.30	3.40	1.15
Arsenic	57	700	700	200.00	77.40	28.80	20.70	9.78	7.36
Cadmium	0.96	9.6	9.6	0.95	0.40	0.21	1.43	0.72	0.50
Copper	81	810	390	881.00	355.00	171.00	174.00	80.50	50.30
Lead	66	660	660	324.00	141.00	69.30	139.00	66.00	42.10
Mercury	0.21	2.1	0.59	0.99	0.18	0.09	0.43	0.21	0.08
Nickel	140			39.00	29.00	22.80	36.00	35.00	39.40
Silver	1.2	6.1	>0.56	0.81	0.42	0.25	0.72	0.34	0.23
Zinc	160	1600	1600	909.00	366.00	178.00	290.00	126.00	86.00
ORGANICS (ppb, dry weight)									
LPAH									
Napthelene	210	2100	2100	140.00	76.00	23.00	1700.00	3400.00	840.00
Acenapthylene	64	640	>560	46J	21.00	15.00	260.00	120.00	88.00
Acenaphthene	63	630	500	360.00	170.00	59.00	1900.00	1900.00	830.00
Fluorene	64	640	540	380.00	170.00	79.00	1600.00	1400.00	710.00
Phenanthrene	320	3200	1500	3400.00	1300.00	500.00	6500.00	5000.00	1500.00
Anthracene	130	1300	960	800.00	350.00	110.00	3300.00	1300.00	1000.00
2-Methylnaphthalene	67	670	670	100.00	46.00	24.00	530.00	490.00	290.00
Total LPAH	610	6100	5200	5180.00	2133.00	810.00	15790.00	13610.00	5258.00

Boxes around the values indicates SL exeedences.
***Shading** of the values indicates ML exceedences*

Table IIIB 2. Concentrations of PSDDA Chemicals of Concern Found in Test Sediments.

		PSDDA		STATION					
PSDDA PARAMETER	SL	ML	1988 OYSTER AET	M1	M1C2 50/50	M1C2 25/75	D1	D1C4 50/50	D1C4 25/75
HPAH									
Fluoranthene	630	6300	2500	5900.00	2500.00	1100.00	15000.00	9300.00	6300.00
Pyrene	430	7300	3300	6200.00	1800.00	1200.00	24000.00	9900.00	11000.00
Benzo(a)anthracene	450	4500	1600	2600.00	1200.00	470.00	6100.00	2700.00	2100.00
Chrysene	670	6700	2800	3800.00	1500.00	570.00	11000.00	4800.00	3300.00
Benzofluoranthenes	800	8000	3600	4800.00	2200.00	1000.00	20000.00	7700.00	4100.00
Benzo(a)pyrene	680	6800	1600	2500.00	1100.00	470.00	8400.00	3300.00	1900.00
Indeno(1,2,3-c,d)pyrene	69	5200	690	1900.00	1000.00	280.00	3700.00	1500.00	880.00
Dibenzo(a,h)anthracene	120	1200	230	450.00	320.00	75.00	2000.00	820.00	280.00
Benzo(g,h,i)perylene	540	5400	720	1200.00	620.00	150.00	2500.00	1300.00	490.00
Total HPAH	1800	51000	17000	29350.00	12240.00	5315.00	92700.00	41320.00	30350.00
CHLORINATED HYDROCARBONS									
1,2-Dichlorobenzene	19	350	50	1.7U	1.3U	NO DATA	2.4U	1.4U	NO DATA
1,3-Dichlorobenzene	170		>170	1.7U	1.3U	NO DATA	2.4U	1.4U	NO DATA
1,4-Dichlorobenzene	26	260	120	1.7U	1.3U	NO DATA	2.4U	1.4U	NO DATA
1,2,4-Trichlorobenzene	13	64	64	1.7U	1.3U	NO DATA	2.4U	1.4U	NO DATA
Hexachlorobenzene (HCB)	23	230	230	72U	14U	13U	23U	17U	15U
PHTHALATES									
Dimethlyl phthalate	160		160	72U	14U	13U	23U	17U	15U
Diethyl phthalate	97		>73	72U	14U	13U	23U	17U	15U
Di-n-butyl phthalate	1400		1400	67J	21.00	10M	23U	17U	15U
Butyl Benzyl phthalate	470		>470	110M	59.00	54.00	23U	17U	15U
Bis(2-ethylhexyl)phthalate	3100		1900	860.00	410.00	190.00	400.00	160M	75.00
Di-n-octyl phthalate	6200		>420	72U	14U	13U	23U	17U	15U
PHENOLS									
Phenol	120	1200	420	50J	130.00	160.00	180.00	97.00	10M
2 Methylphenol	20	72	63	52J	42.00	13J	23U	7.9M	15U
4 Methylphenol	120	1200	670	72U	30.00	23.00	23U	28.00	11M
2,4-Dimethylphenol	29	50	29	72U	16M	13U	23U	18M	15U
Pentachlorophenol	100	690	>140	280J	230.00	98.00	110U	84U	75U

Boxes around the values indicates SL exeedences.
Shading of the values indicates ML exceedences

Table IIIB-2. Concentrations of PSDDA Chemicals of Concern Found in Test Sediments.

		PSDDA		STATION					
PSDDA PARAMETER	SL	ML	1988 OYSTER AET	M1	M1C2 50/50	M1C2 25/75	D1	D1C4 50/50	D1C4 25/75
MISCELLANEOUS EXTRACTABLES									
Benzyl Alcohol	25	73	73	72U	13M	13U	23U	17U	15U
Benzoic acid	400	690	650	72U	61J	130U	220U	170U	150U
Dibenzofuran	54	540	540	240.00	110.00	47.00	1200.00	1200.00	530.00
Hexachloroethane	1400	14000		72U	14U	13U	23U	17U	15U
Hexachlorobutadiene	29	290	270	72U	14U	13U	23U	17U	15U
N-Nitrosodiphenylamine	28	220	130	72U	14U	13U	23U	17U	15U
VOLATILE ORGANICS									
Trichloroethene	160	1600		1.7U	1.3U	NO DATA	2.4U	1.4U	NO DATA
Tetrachloroethene	14	210	140	1.7U	1.3U	NO DATA	2.4U	1.4U	NO DATA
Ethylbenzene	10	50	37	1.7U	1.3U	NO DATA	2.4U	1.4U	NO DATA
Total Xylene	12	160	120	3.4U	2.5U	NO DATA	4.7U	2.1M	NO DATA
PESTICIDES and PCBs									
Total DDT	6.9	69							
4,4'-DDD				29U	9.90	4.7U	13U	11.00	1.6U
4,4'-DDE				11U	6.3U	2.1U	6.2U	3U	1.6U
4,4'-DDT				2.4U	12U	5.0U	2.4U	2.4U	1.6U
Aldrin	10			13U	2.5U	0.8U	3.2U	1.4U	0.8U
Chlordane	10			1.2U	1.2U	0.8U	1.2U	1.2U	0.8U
Dieldrin	10			5.0U	2.9U	1.6U	6.4U	2.4U	1.6U
Heptachlor	10			1.2U	1.2U	0.8U	2.5U	1.2U	0.8U
Lindane	10			1.2U	1.2U	0.8U	1.8U	1.2U	0.8U
PCB-1016/1242				72U	38U	40U	96U	43U	48U
PCB-1248				72U	120U	50U	200U	85U	25.00
PCB-1254				270.00	190.00	71.00	180.00	71.00	28.00
PCB-1260				280U	150U	68C	450U	150U	65U
Total PCBs	130	2500	1100	270.00	190.00	139.00	180.00	71.00	53.00

Data Qualifiers

U	Indicates compound was analyzed for, but not detected at the given detection limit
J	Indicates an estimated value when result is less than specified detection limit.
M	Indicates an estimated value of analyte found and cofirmed by analyst, but with low spectral matach parameters
C	This flag is used when the analyte is detected in both the sample and blank. Indicates possible/probable blank contamination

Boxes around the values indicates SL exeedences.
Shading of the values indicates ML exceedences

General Results By Station and Species

In general, the echinoderms showed a greater magnitude of response in all aerated treatments, than did the oysters for the M1 series, but neither species showed a great deal of response to any of the D1 series (Table IIIB-3). For the unaerated treatments, there was virtual similarity in the percent mortality for both species. For the M1 series, both oysters and echinoderms showed an decreased response with decreased percent of the contaminated material for all treatments, except the 24 hour settlement. In the latter treatments, the dose/response was flat.

Virtually all of the oyster response to exposure was in mortality (Figure IIIB-1); the percent abnormality was less than 10% for all sediments and treatments. By contrast, the echinoderms showed a much higher level of response to the M1 series (Figure IIIB-2), with abnormality comprising a large part of the combined endpoint (Figure IIIB-3).

Oyster larval mortality responses to the D1 series are shown in Figure IIIB-4. A significantly elevated response was observed only at the whole-D1 sediment for the Green Book/PSDDA. The standard Green Book method for the D1 series did not exhibit a dose/response relationship. Echinoderm larvae showed very little mortality or abnormality response to exposure to D1, with the exception of the 4-hour unaerated treatment (Figure IIIB-5). While overall the mortality was generally low, the pattern of highest echinoderm response in the 4-hour unaerated was observed in the D1 treatments. The abnormality response to D1 for the PSDDA elutriate preparations was low; $\leq$ 15%. Both Green Book preparations showed abnormalities ranging from 15 - 35%.

Results of PSDDA *t*-Test Comparisons

The results of PSDDA *t*-test and trigger levels comparisons show that both species' response to the test sediment/treatments show good correspondence when compared to PSDDA guideline values. Table IIIB-4 shows that for the M1 series, both species were in agreement on eight of nine treatments that exceeded the 30% over the reference sediment guideline. Some differences were noted in comparisons with the 20% guideline value. The oysters exceeded the 20% trigger value for the 24-hour unaerated treatment of M1C2 50/50, whereas the echinoderm mortality was only 4.5%. Conversely, the echinoderms exceeded the 20% mark for the Green Book/PSDDA treatment of M1C2 25/75, but the oyster mortality was only 8.5%.

For the D1 series, both species were in agreement on identifying the two 30% trigger exceedances, and one 20% exceedance (Table IIIB-5). The oysters exceeded the 20% triggers for two of the D1C4 series, but there were no exceedences for the echinoderms.

Results of Species Responses to M1 Treatments

Relatively little response that occurred with exposure to D1, thus between species comparisons were limited to the M1 treatments. The results of *t*-test comparisons between species for mortality responses to the M1 dilution series is given in Table IIIB-6. These comparisons reinforce the earlier observation that in the aerated treatments, echinoderms were more sensitive to the exposures than were the oysters. In the 4-hour settling/unaerated treatments, there were no significant differences between the two species. For the 24-hour settling/unaerated treatments, the oysters exhibited significantly greater mortality response.

Table IIIB-3. Results of Phase IIIB Larval Exposures.

PHASE IIIB OYSTER MORTALITY AND ABNORMALITY

STATION	PSDDA 4-HOUR SETTLING (AERATED)		PSDDA 4-HOUR SETTLING (UNAERATED)		PSDDA 24-HOUR SETTLING (UNAERATED)		GREEN BOOK		GREEN BOOK WITH PSDDA COUNTS	
	Mortality	Abnormality	Mortality	Abnormality	Mortality	Abnormality	Mortality	Abnormality	Mortality	Abnormality
M1	61.03	3.70	41.47	7.03	15.51	2.65	59.88	5.98	45.34	4.84
M1C2-50%	44.71	1.66	35.81	3.70	25.04	3.05	32.55	6.49	55.13	6.35
M1C2-25%	35.88	0.57	18.29	1.54	18.26	4.31	18.96	1.53	8.50	0.50
C2	11.00	2.10	9.80	1.00	0.20	0.50	**	**	**	**
D1	24.63	1.50	29.30	5.06	15.88	3.14	11.87	1.69	43.54	1.70
D1C4 - 50/50	17.43	0.94	12.88	4.91	14.27	5.22	7.07	1.57	34.70	0.88
D1C4 - 25/75	21.32	0.63	13.41	3.10	15.88	4.25	4.58	2.36	13.46	1.61
C4	17.10	1.40	20.50	0.70	0.10	0.30	**	**	**	**

PHASE IIIB ECHINODERM MORTALITY AND ABNORMALITY

STATION	PSDDA 4-HOUR SETTLING (AERATED)		PSDDA 4-HOUR SETTLING (UNAERATED)		PSDDA 24-HOUR SETTLING (UNAERATED)		GREEN BOOK		GREEN BOOK WITH PSDDA COUNTS	
	Mortality	Abnormality	Mortality	Abnormality	Mortality	Abnormality	Mortality	Abnormality	Mortality	Abnormality
M1	99.02	98.80	46.89	21.91	12.20	9.15	98.39	98.63	99.63	98.27
M1C2-50%	84.06	79.21	28.71	11.86	4.46	8.73	67.02	75.55	56.07	49.63
M1C2-25%	49.87	45.75	19.08	8.83	1.65	7.96	15.11	36.88	34.98	31.23
C2	13.30	17.80	31.60	13.70	7.10	-10.40	**	**	**	**
D1	17.48	13.57	39.53	7.81	11.77	10.46	10.83	25.38	25.16	14.51
D1C4 - 50/50	19.33	13.05	24.96	7.02	8.62	7.48	11.22	31.55	62.10	15.04
D1C4 - 25/75	9.87	10.81	21.83	7.70	3.24	8.31	15.99	29.22	67.21	22.03
C4 *	-2.30	8.30	47.2	25.2	-2.1	7.80	**	**	**	**

* Values for C2 and C4 included from IIIA summary table

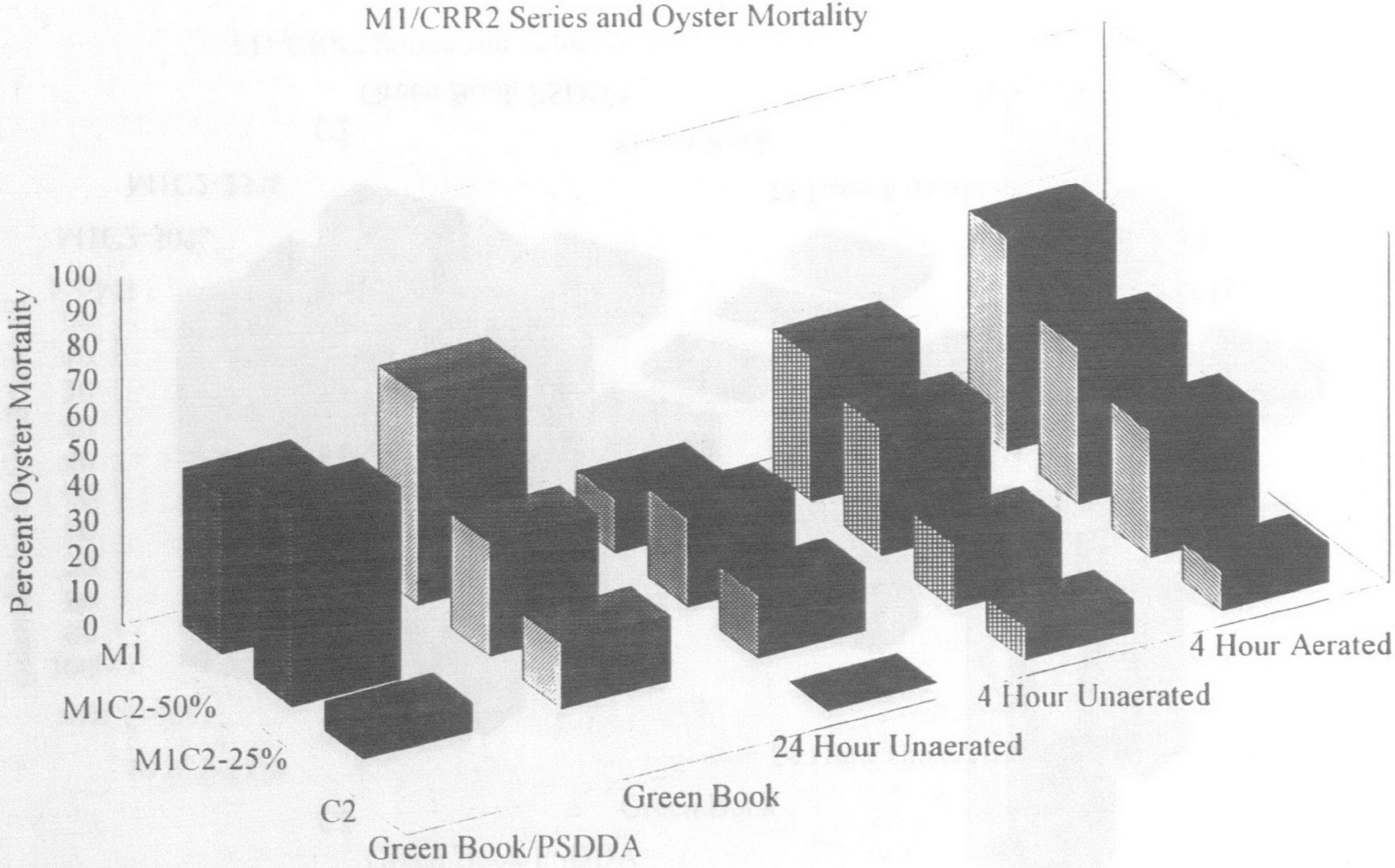
** Green Book treatment data from for C2 and C4 not included due to high mortality and abnormality in Phase IIIA.

Phase IIIB. Contaminated Sediments Effects

NOTE: Bioassay results for reference sediments C2 and C4 were included from Phase IIIA testing. Sediments C2 and C4 were not run synoptically with the IIIB treatments due to logistical constraints.

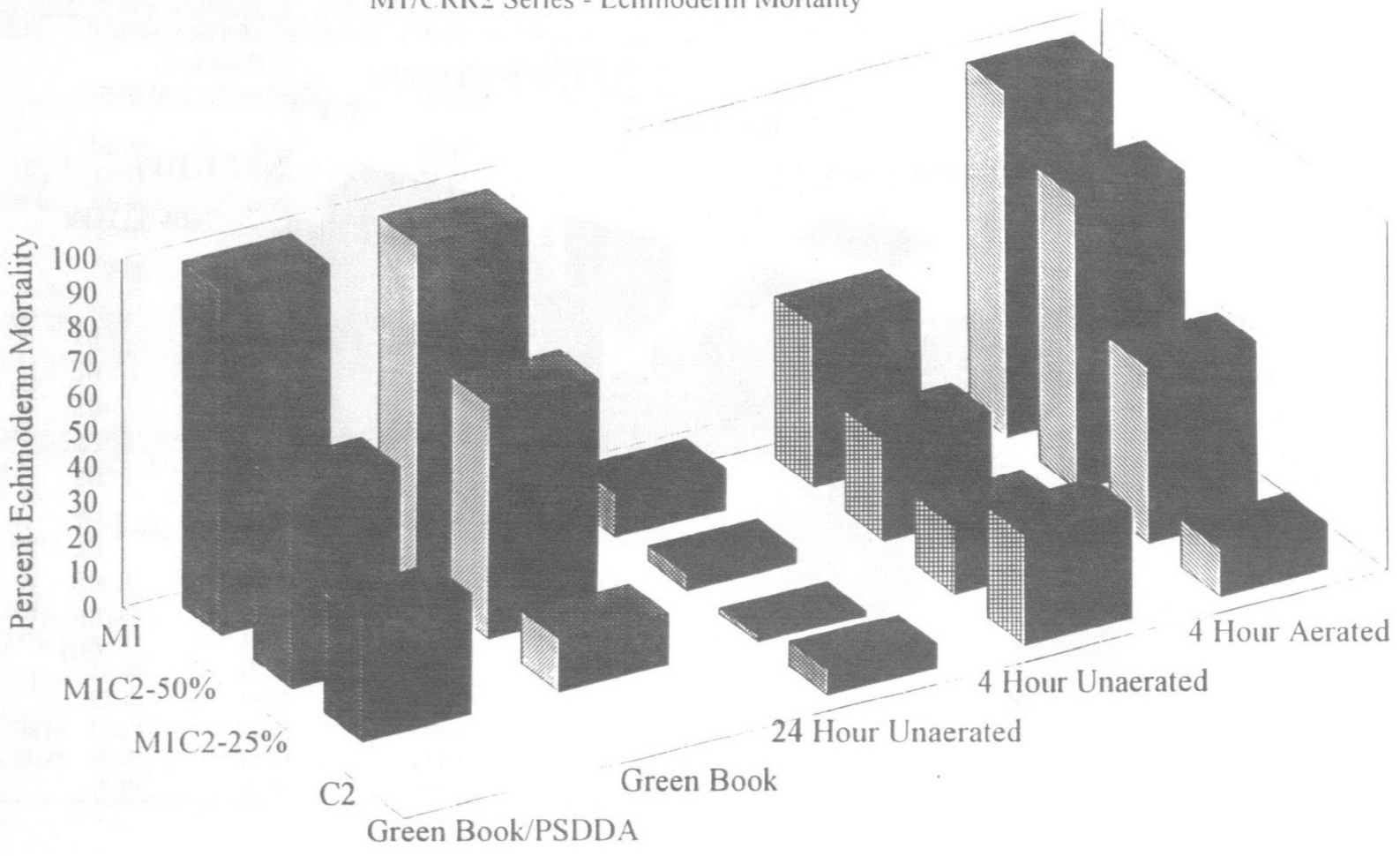
Phase IIIB, Figure 1

M1/CRR2 Series and Oyster Mortality

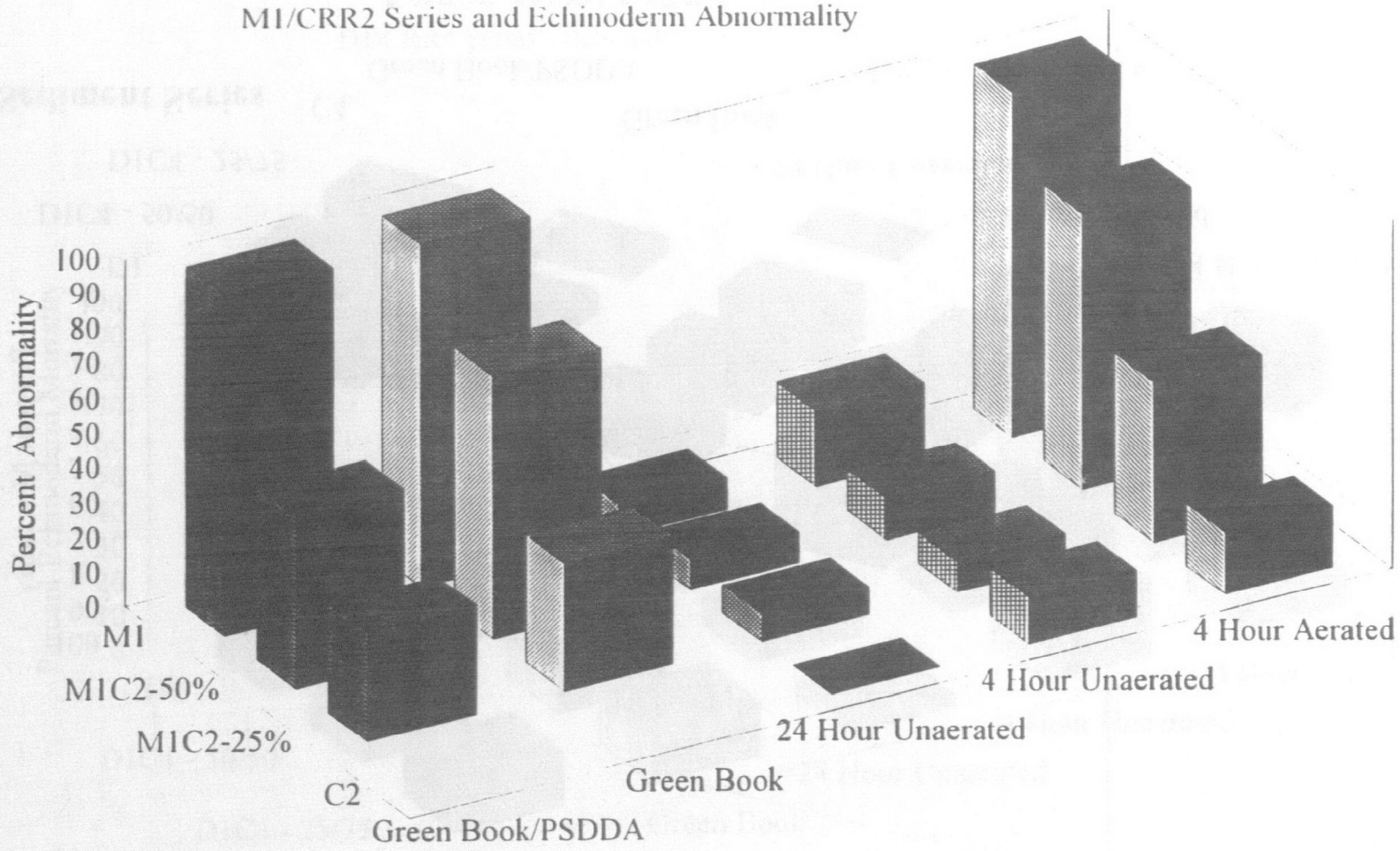


Phase IIIB, Figure 2

M1/CRR2 Series - Echinoderm Mortality

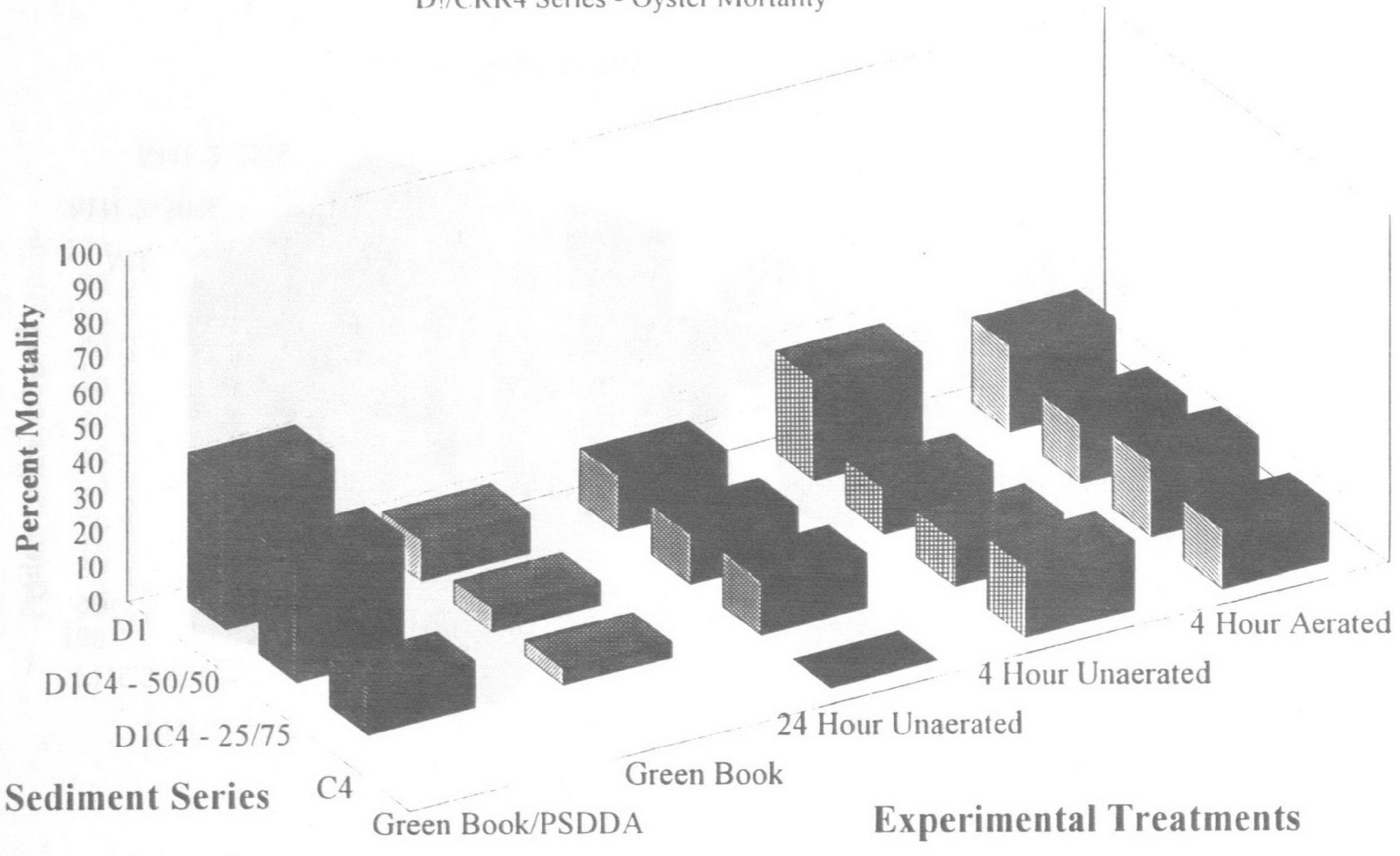


Phase IIIB, Figure 3



Phase IIIB, Figure 4

D!/CRR4 Series - Oyster Mortality



Phase IIIB, Figure 5

D1\CRR4 Series - Echinoderm Mortality

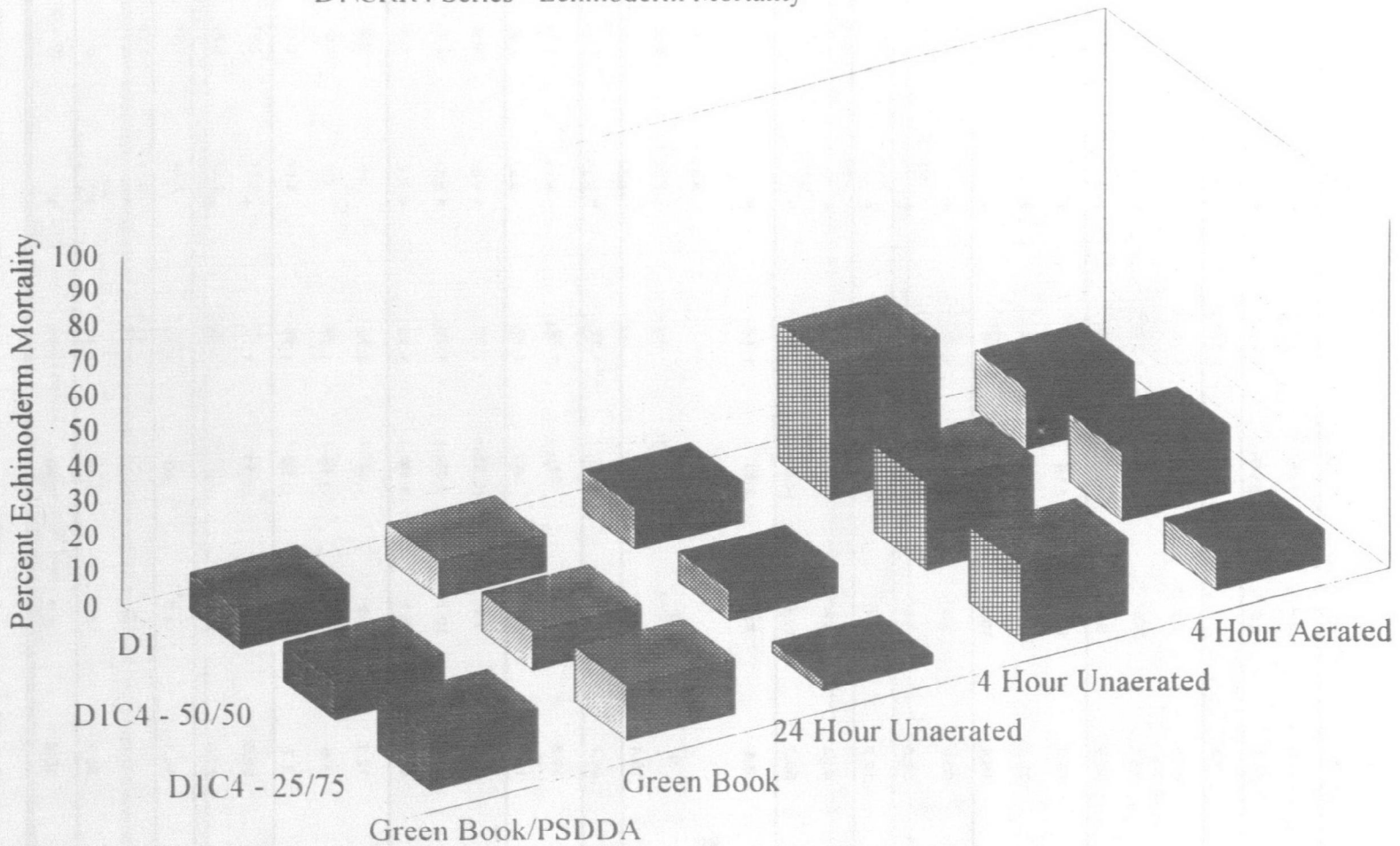


Table IIIB-4. Application of PSDDA bioassay criteria to Oyster as Echinoderm responses to the (M1) dilution series and treatments.

STATION	MEAN	STANDARD DEVIATION	COMPUTED t	CRITICAL t	STATISTICAL SIGNIFICANCE	20% OVER CONTROL	30% OVER REF
			PERCENT MORTALITY				
OYSTERS							
SW	0	0					
CARR2	0	0					
OM14UA	41.5	10.4	13.95	1.77	•		•
OM54UA	34.4	8.8	15.06	1.78	•		•
OM24UA	18.3	8.8	7.83	1.77	•		
OM14A	61.1	7.2	26.48	1.77	•		•
OM54A	44.7	8.8	17.60	1.77	•		•
OM24A	35.9	8.8	14.15	1.77	•		•
OM124UA	15.5	18.3	2.18	1.77	•		
OM524UA	25.1	13.0	7.45	1.77	•		
OM224UA	18.4	10.3	6.40	1.80	•		
OM1G	80.0	3.5	53.17	1.77	•		•
OM5G	32.6	7.3	16.93	1.77	•		•
OM2G	17.9	15.0	3.60	1.78	•		
OM1GP	46.3	8.8	17.97	1.77	•		•
OM5GP	55.2	12.6	14.09	1.77	•		•
OM2GP	8.4	8.4	2.63	1.77	•		
ECHINODERMS							
SW	0	0					
CARR2	15.4	9.6					
EM14UA	46.7	7.8	5.35	1.77	•		•
EM54UA	28.6	22.1	99	1.77			
EM24UA	19.1	12.6	36	1.77			
EM14A	99.0	1.8	15.32	1.77	•		•
EM54A	84.0	10.2	8.01	1.77	•		•
EM24A	49.9	15.1	4.44	1.77	•		•
EM124UA	12.2	8.9	44	1.77			
EM524UA	4.5	7.9	2.12	1.77			
EM224UA	1.7	5.4	-3.56	1.77			
EM1G	98.5	1.7	14.73	1.77	•		•
EM5G	67.0	14.5	6.31	1.77	•		•
EM2G	15.1	18.6	.40	1.77			
EM1GP	99.8	.7	17.18	1.77	•		•
EM5GP	56.1	12.2	8.69	1.77	•		•
EM2GP	35.0	14.0	2.90	1.77	•		

O = oyster
E = echinoderm
M = M1
D = D1
1 = 100%
5 = 50%
2 = 25%
4UA = 4 hour unaerated
4A = 4 hour aerated
24UA = 24 hour unaerated
G = Green Book
GP = Green Book/PSDDA

Table IIIB-5. Application of PSDDA bioassay criteria to Oyster as Echinoderm responses to the (D1) dilution series and treatments.

STATION	MEAN	STANDARD DEVIATION	COMPUTED t	CRITICAL t	STATISTICAL SIGNIFICANCE	20% OVER CONTROL	30% OVER REF.
			PERCENT MORTALITY				
OYSTERS							
SW	0	0					
CARR4	2.2	4.9					
OD14UA	29.3	6.8	6.63	1.77	•	○	
OD64UA	12.9	7.9	3.22	1.77	•		
OD24UA	13.6	9.4	3.06	1.77	•		
OD14A	24.6	12.6	4.13	1.77	•	○	
OD64A	17.5	9.2	3.82	1.77	•		
OD24A	21.6	7.0	6.67	1.77	•	○	
OD124UA	16.0	10.2	3.35	1.77	•		
OD624UA	14.3	9.1	3.29	1.77	•		
OD224UA	16.8	9.4	3.66	1.77	•		
OD1G	11.8	7.0	3.60	1.77	•		
OD6G	7.2	11.1	1.13	1.77			
OD2G	4.6	6.0	1.30	1.77			
OD1GP	43.4	20.3	5.87	1.77	•	○	•
OD6GP	34.7	12.6	7.15	1.77	•	○	•
OD2GP	13.5	16.8	1.97	1.77	•		
ECHINODERMS							
SW	0	0					
CARR4	12.6	22.8					
ED14UA	39.6	12.8	3.57	1.77	•	○	
ED64UA	26.0	16.2	1.68	1.77			
ED24UA	21.6	16.0	1.64	1.77			
ED14A	17.5	13.6	68	1.89			
ED64A	19.3	18.7	73	1.77			
ED24A	9.9	10.7	-.12	1.77			
ED124UA	11.8	12.0	23	1.77			
ED624UA	8.8	9.6	-.16	1.77			
ED224UA	3.2	4.2	-1.20	1.77			
ED1G	10.7	9.7	17	1.77			
ED6G	11.3	13.2	-.10	1.77			
ED2G	16.1	11.1	.91	1.77			
ED1GP	26.2	12.2	1.76	1.77			
ED6GP	62.1	14.2	5.44	1.77	•	○	•
ED2GP	67.1	13.8	5.88	1.77	•	○	•

O = oyster M = M1
E = echinoderm D = D1

1 = 100% 4UA = 4 hour unaerated
6 = 60% 4A = 4 hour aerated
2 = 26%

24UA = 24 hour unaerated
G = Green Book
GP = Green Book/PSDDA

Table IIIB-6. Two-tailed *t*-test comparisons between echinoderm vs. oysters responses for the M1 dilution series by treatment. Positive values indicate greater echinoderm response; negative values greater oyster response. Shaded areas indicate statistical significance at $\alpha = 0.05$.

	4-HOUR UA	4-HOUR A	24-HOUR UA	GREEN BOOK	GREEN BOOK/PSDDA	
M1	1.33	16.91	-0.24	18.33	24.52	Calculated t
	2.10	2.10	2.11	2.10	2.10	Critical t
M1C2 50/50	1.06	7.20	-5.15	6.56	0.18	
	2.11	2.10	2.10	2.10	2.10	
M1C2 25/75	-0.34	2.48	-5.84	0.72	5.08	
	2.10	2.10	2.12	2.11	2.10	

Results of Differences by Species Between Treatments

The results of the Tukey's Wholly Significant Differences test on arcsin/square root transformed data for oysters and echinoderms are presented in Tables IIIB-7 and IIIB-8. In general for both species, mortality was greatest in the 4-Hour Aerated and Green Book, and least for the 24-Hour treatments. The 4-Hour Unaerated treatments for both species had significantly less mortality than the 4-Hour Aerated treatments.

Results of Species as Predictors of Apparent Sediment Toxicity

In general, the PSDDA SL/ML's and the 1988 Oyster AET values were adequate predictors of sediment toxicity to both species for the M1 dilution series. This was true for all treatments, with the exception of those vessels where the sediment was allowed to sit for 24 hours prior to testing.

In contrast, the responses for D1 did not match the expectations of either the ML or AETs. For both species, the mortality and abnormality responses for D1 were relatively negligible.

Comparison of Species Reference Toxicant Responses

The data collected for the two species for the two reference toxicants is consistent with the observation that oyster response to the toxicant over the range tested was principally mortality, while sand dollars exhibited abnormal development. For the oysters, low numbers of total larvae were recovered at increasing concentrations. Abnormality did occur with increasing frequency, but in a decreasing number of larvae. In contrast, the total number of echinoderm larvae recovered for both reference toxicants were at or near the number of embryos inoculated into the test vessels. However, with increasing dosage, there were increasing numbers of abnormal larvae.

Table IIIB - 7 Results of Tukey's Multiple Comparison Test for Oyster Mortality by Test Protocol
Shaded areas indicate statistically significant differences between treatments.

Oyster Mortality for M1 by Treatment

Calculation of Tukey Q statistic		Pooled	Calculated q value			
Level	Average	Stnd. Error	OM14A	OM1G	OM1GP	OM14UA
OM14A	0.898	0.049				
OM1G	0.885	0.049	0.27			
OM1GP	0.738	0.049	3.27	3.00		
OM14UA	0.698	0.049	4.08	3.82	0.82	
OM124UA	0.306	0.049	12.08	11.82	8.82	8.00
k = 4	D.F. = 45		Critical q 0.05, 4,45 = 3.81			

Oyster Mortality for M1C2 50/50 by Treatment

Calculation of Tukey Q statistic		Pooled	Calculated q value			
Level	Average	Stnd. Error	OM5GP	OM54A	OM54UA	OM5G
OM5GP	0.838	0.035				
OM54A	0.731	0.035	3.06			
OM54UA	0.624	0.035	6.11	3.06		
OM5G	0.605	0.035	6.66	3.60	0.54	
OM524UA	0.513	0.035	9.29	6.23	3.17	2.63
k = 4	D.F. = 44		Critical q 0.05, 4,45 = 3.81			

Oyster Mortality for M1C2 25/75 by Treatment

Calculation of Tukey Q statistic		Pooled	Calculated q value			
Level	Average	Stnd. Error	OM24A	OM24UA	OM224UA	OM2G
OM24A	0.639	0.052				
OM24UA	0.431	0.052	4.00			
OM224UA	0.425	0.058	4.12	0.12		
OM2G	0.390	0.055	4.79	0.79	0.60	
OM2GP	0.236	0.052	7.75	3.75	6.43	2.80
k = 4	D.F. = 42		Critical q 0.05, 4,45 = 3.81			

O = Oyster
M = M1 Sediment Series

1 = 100% M1
5 = 50% M1
2 = 25% M1

4A = 4 Hour Aerated
4 UA = 4 Hour Unaerated
24 UA = 24 Hour Unaerated
G = Green Book Procedure
GP = Green Book with PSDDA counts

Table IIIB-8. Results of Tukey's Multiple Comparison Test for Echinoderm Mortality by Test Proto
Shaded areas indicate statistically significant differences between treatments.

Echinoderm Mortality for M1 by Treatment

Calculation of Tukey Q statistic		Pooled	Calculated q value			
Level	Average	Stnd. Error	EM1GP	EM14A	EM1G	EM14U
EM1GP	1.538	0.036				
EM14A	1.520	0.036	0.51			
EM1G	1.476	0.036	1.75	1.24		
EM14U	0.754	0.036	22.08	21.58	20.34	
EM124UA	0.313	0.036	34.51	34.00	32.76	12.42
k = 4	D.F. = 45		Critical q 0.05, 4,45 = 3.81			

Echinoderm Mortality for M1C2 50/50 by Treatment

Calculation of Tukey Q statistic		Pooled	Calculated q value			
Level	Average	Stnd. Error	EM54A	EM5G	EM5GP	EM54UA
EM54A	1.192	0.062				
EM5G	0.966	0.062	3.65			
EM5GP	0.849	0.062	5.53	1.89		
EM54UA	0.512	0.062	10.97	7.32	5.44	
EM524UA	0.148	0.062	16.84	13.19	11.31	5.87
k = 4	D.F. = 45		Critical q 0.05, 4,45 = 3.81			

Echinoderm Mortality for M1C2 25/75 by Treatment

Calculation of Tukey Q statistic		Pooled	Calculated q value			
Level	Average	Stnd. Error	EM24A	EM2GP	EM24UA	EM2G
EM24A	0.782	0.065				
EM2GP	0.628	0.065	2.37			
EM24UA	0.403	0.065	5.83	3.46		
EM2G	0.300	0.065	7.42	5.05	1.58	
EM224UA	0.042	0.065	11.38	9.02	5.55	3.97
k = 4	D.F. = 45		Critical q 0.05, 4,45 = 3.81			

E = Echinoderm
D = D1 Series Sediments

1 = 100% M1
5 = 50% M1
2 = 25% M1

4A = 4 Hour Aerated
4 UA = 4 Hour Unaerated
24 UA = 24 Hour Unaerated
G = Green Book Procedure
GP = Green Book with PSDDA counts

The oyster LC_{50} and the echinoderm EC_{50} are presented in Table IIIB-9. Species were comparable in their responses to phenol, but the EC_{50} response to cadmium was higher for the echinoderms.

Table IIIB-9. Reference Toxicant LC_{50} and EC_{50} Values for Phases IIIA, and IIIB.

PHASE	EC/LC_{50} (mg/L)
OYSTERS	
IIIA - $CdCl_2$	1.65
IIIB - $CdCl_2$	1.24
IIIB - Phenol	112.83
ECHINODERMS	
IIIA - $CdCl_2$	3.90
IIIB - $CdCl_2$	3.54
IIIB - Phenol	96.52

DISCUSSION

SEDIMENT CHEMISTRY

With the exceptions noted above, the "dilution" procedure was successful in producing a range of contaminants for testing. The levels of chemical values observed in these test sediments are such that only two of the six sediments would have ever been considered for biological testing under the PSDDA program: the 50% and 25% dilutions of the M1 test sediment. The other four sediments had ML exceedences for several analytes. As noted above, all of the test sediments exceed the state Sediment Quality Standards. Thus, these six test sediments represented a good range contaminants by which to compare regulatory standards to larval bioassay results. It is interesting to note that if those two sediments were being evaluated for disposal under the PSDDA program, both larval bioassays exceeded the 30% trigger value and would be designated unsuitable for open water disposal.

To determine why there was a major difference between the reported grain size values for CRR4 between Phase IIIA and IIIB, the field collection notes were reviewed. During collection of Phase IIIA sediments, the field crew navigated by use of the GPS, and reported both GPS and Loran TD values. During Phase IIIB, the field crew only had Loran C, and went to the coordinates provided by the Corps. During IIIB sediment collection, the crew reported problems with the Loran unit, which likely resulted in their being on a different site relative to the IIIA - CRR4 site. Field wet sieving indicated that the material was 35% fines; the discrepancy between field and actual is considerable. While the grain sizes of the M1 dilution series did range between the two "parent" sediments (see Table IIIB-2), it is not likely to impact the interpretive results associated with the between species and between test effects. The silt/clay content of sample M1 were below those levels shown to cause an effect to oyster larvae in Phase IIIA. However, the differences associated with the changes in conventional

parameters (grain size, sulfides, total organic carbon) across the M1 series does limit discussions regarding sediment chemistry effects.

BIOASSAYS

The data produced during these exposures suggest that oyster mortality and echinoderm abnormality respond similarly to toxicant exposure, over the 48 hour exposures. When compared to PSDDA guideline values for bioassays, both species showed similar exceedences. However, there also appears to be varying degrees of response to the different treatments and sediments, with the abnormality response showing greater sensitivity.

The reference toxicant data further buttress the observed relationship between larval oyster mortality and sand dollar abnormality. In the presence of both the metal and organic toxicant, oyster larvae showed positive dose response, but fewer larvae were recovered with higher concentration. The echinoderms also showed positive dose response to increasing toxicant concentration, but the total number of larvae recovered remained stable while the frequency of abnormality increased.

These data, along with the following arguments, suggest that *Dendraster excentricus* is a more suited organism for use in larval elutriate testing. In these experiments, the sand dollar showed greater sensitivity over the range of contaminants in the M1 dilution series, and thus was a better predictor of sediment contamination. Data from Phase IIIB show that sand dollar larvae, under current PSDDA testing procedures, do not appear to be sensitive to increasing silt/clay components in bioassay chambers. *D. excentricus* is native to, and is widely distributed throughout Puget Sound, as well as the North-Eastern Pacific coast. Gravid adult sand dollars can be obtained year-round, and the method of spawning (potassium chloride injection) is quicker and simpler than for oysters (thermal shock). Finally, the sand dollar has a better track record in meeting minimum quality assurance guidelines for control survival (Tim Thompson, personal observation).

An unexpected result was the occurrence of the elevated mortality when the M1 series samples were aerated. The 4-hour settlement with aeration is the current standard practice for all PSDDA larval bioassays. Exactly why that phenomenon occurred is not clear, but it does suggest that some caution should be exercised when recommending aeration during regulatory testing.

An additional unexpected result was that the standard PSDDA elutriate test produced results for both species that were similar to the Green Book elutriate method. Given that the latter method has a bulk sediment loading factor in the test that is roughly 10 times the PSDDA method, one would predict greater mortality and/or abnormality. While a conclusion could be inferred that the two methods are equivalent in predicting sediment contamination, it must be cautioned that this is a limited data set. Further careful documentation would be required before a definitive statement comparing the two methods could be made.

The comparison of the two methods for larval recovery from the Green Book elutriate at test termination with the Green Book elutriate were confounding. No conclusions could be drawn from these data as to which method showed greater larval recovery.

Based on the results of these tests, a rough order of protocol sensitivity for both species could be set as follows:

4-hour Aerated = Green Book > 4-Hour Unaerated > 24-Hour Unaerated.

ANALYTICAL VALUES AS PREDICTORS OF BIOASSAY RESULTS

A discussion concerning the levels of chemicals observed and the percent response in the larval species should be restricted to the current accepted PSEP/PSDDA testing scheme (4-hour settlement and aeration). Under that restriction, the responses to the M1 sediment series were as one would predict; generally decreasing response with decreasing contaminant load. In contrast, the lack of response to the D1 series is contrary to the expectations of the ML and 1988 Oyster AET values. With LPAH and HPAH exceeding by several times the ML/AET's, considerable toxicity is predicted in these sediments.

M1 sediments, overall, contained more and higher levels of metals, and fewer and lower concentrations of organic compounds relative to the sediments collected from D1. The high levels of copper alone are probably sufficient to drive all of the toxicity observed in these sediments. Although several organic compounds (especially LPAHs and HPAHs) were measured above the PSDDA SL in M1 samples, only two organic compounds, phenanthrene and 2,4-dimethylphenol, were detected above the PSDDA ML.

In contrast, D1 samples were predominantly contaminated with organic compounds. For example, in Sample D1, most LPAHs and HPAHs and dibenzofuran were detected above the PSDDA ML values. A total of 5 PSDDA metals were detected above the PSDDA SL in Sample D1 compared to 6 PSDDA metals detected above the SL in Sample M1, one of which (copper) was detected above the ML. These data suggest a possible link between high larval mortality and high metals concentrations. This correlation requires additional study and, at this time, is viewed as an observation only.

RECOMMENDATIONS

- *Crassostrea gigas* and *Dendraster excentricus* larval responses to dredged sediment can be considered equivalent predictors of contamination under the PSDDA program.

- *Dendraster excentricus* is recommended as the primary test organism for sediment characterization.

- The continued use of the combined mortality/abnormality endpoint is recommended for all bivalve and echinoderm elutriate larval testing.

- The Four-hour settlement and aeration treatment is a good predictor of sediment contamination, and is recommended for continuous use under the regulatory program.

- The Twenty-four settling time was the least accurate in predicting sediment contamination, and is therefore not recommended for use in the regulatory program.

REFERENCES

Pastorok, R.A., and D.S. Becker. 1989. *Comparison of Bioassays for Assessing Sediment Toxicity in Puget Sound*. U.S. Environmental Protection Agency, Region 10. Office of Puget Sound. Seattle, WA

Plumb, R.H. 1981. Procedures for handling and chemical analysis of sediment and water samples. Technical Report EPA/CE-81-1. U.S. Army Corps of Engineers, Vicksburg, MS.

PSEP, 1991. Puget Sound Estuary Program. *Recommended Guidelines for Conducting Laboratory Bioassays on Puget Sound Sediments*. U.S. EPA, Region 10, Office of Puget Sound, Seattle, WA.

PSEP. 1989b. Puget Sound Estuary Program. *Recommended protocols for measuring metals in Puget Sound sediment and tissue samples*. Prepared for the U.S. Environmental Protection Agency Region X, Office of Puget Sound, and the U.S. Army Corps of Engineers. PTI Environmental Services, Inc., Bellevue, Washington.

PSEP. 1986. Puget Sound Estuary Program. Recommended protocols for measuring selected environmental variables in Puget Sound. Final Report. Prepared for the U.S. Environmental Protection Agency Region X, Office of Puget Sound, and the U.S. Army Corps of Engineers. Tetra Tech Inc., Bellevue, Washington.

PTI Environmental Services 1991. *Reference Area Performance Standards for Puget Sound*. U.S. EPA, Region 10, Office of Coastal Waters, Seattle, WA

PTI Environmental Services, 1988. *1988 Update and Evaluation of Puget Sound AET*. Vol I and II. U.S. EPA, Region 10, Office of Puget Sound, Seattle, WA.

U.S. EPA 1991. *Evaluation of Dredged Material Proposed for Ocean Disposal, Testing Manual*. United States Environmental Protection Agency and the U.S. Army Corps of Engineers. EPA - 503 /8-91 / 001.

WAC 173-204. Sediment Management Standards. Washington State Department of Ecology. April, 1990.

Zar, J.H. 1984. *Biostatistical Analysis*, Second Edition. Prentice-Hall, Inc., Englewood Cliffs, N.J. 07632. *xv* + 718 pp.

REFINEMENTS TO CURRENT PSDDA BIOASSAYS

FINAL REPORT

CONCLUSIONS

SAIC
An Employee-Owned Company

CONCLUSIONS

This program was designed to try and answer the objectives posed in the Program Overview at the beginning of this document. Those objectives, and the conclusions/recommendations suggested by the data are re-summarized below.

The first objective was to identify the effects of ammonia on larval development. This study provided dose/response data for ammonium chloride to both oyster and sand dollar larvae. Those data were the basis for the following conclusions:

- An ammonia testing criterion of 0.04 mg/L unionized ammonia is proposed for the echinoderm test. Data may be qualified as a possible false positive response if un-ionized ammonia values in echinoderm tests are greater than or equal to 0.04 mg/L.The criterion value relates specifically to echinoderm abnormality, not mortality. For an acute criterion to be set for echinoderm larval mortality, additional work is necessary.

- An interim oyster-specific criterion is proposed as 0.13 mg/L unionized ammonia. Some caution is recommended in using this number for interpretation, as it is an estimate. Additional work is recommended to better define that number.

- Aeration appears to have an effect on ammonia toxicity on echinoderm. PSDDA should continue to use aeration in the larval bioassays.

- All laboratories performing PSDDA bioassays should be required to express all ammonia values as the un-ionized form. The SAIC spreadsheet format could be made standard for data submittal.

The second objective was to compare echinoderm and oyster responses to clean and contaminated sediments. In Phase IIIA, the response of both organisms to both a range of grain sizes, and experimental treatments, were tested. While variability in the larval data prevent definitive conclusions from being drawn, the following trends were strongly suggested by the data:

- *Crassostrea gigas* larvae appear to be sensitive to sediments having a high proportion of clays and silts.

- *Dendraster excentricus*, when tested under current PSDDA protocols, do not show an adverse response to increasing silt and clay fractions. Under conditions of expected high silts/clay, the sand dollar test is recommended.

- The current convention of comparing sediments on the basis of percent sands, silts and clays, may not be a useful parameter in attempting to correlate biological observations. For the purposes of selecting suitable reference material for larval bioassay comparisons, the percent grain-size data should be converted to grams/Liter of silts and clays.

Phase IIIA set as objectives the comparison of the two species as predictors of sediment toxicity, and to determine which of the experimental procedures was most accurate in predicting presence of contaminants in marine sediments. To that end, this report finds the following:

- *Crassostrea gigas* and *Dendraster excentricus* larval responses to dredged sediment can be considered equivalent predictors of contamination under the PSDDA program. During

experimental exposures to contaminated, the oyster larval response was high mortality, but low abnormality. Conversely, the echinoderms had high abnormality, but low mortality.

- *Dendraster excentricus* is recommended as the primary test organism for sediment characterization based upon general availability, consistent control performance, and ease of handling.

- These results support the continued use of the combined mortality/abnormality endpoint bivalve and echinoderm elutriate larval testing.

- These data suggest that an order of protocol sensitivity to contaminants in sediments was as follows:l 4-hour Aerated = Green Book > 4-Hour Unaerated > 24-Hour Unaerated. The Four-hour settlement and aeration treatment is a good predictor of sediment contamination, and is recommended for continuous use under the regulatory program. •The Twenty-four settling time was the least accurate in predicting sediment contamination, and is therefore not recommended for use in the regulatory program.