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*Long-Term Effects of Dredging
Operations Program*

**Chronic Sublethal Effects of San Francisco
Bay Sediments on *Nereis (Neanthes)*
arenaceodentata; Effect of Storage
Time on Sediment Toxicity**

by David W. Moore, Thomas M. Dillon

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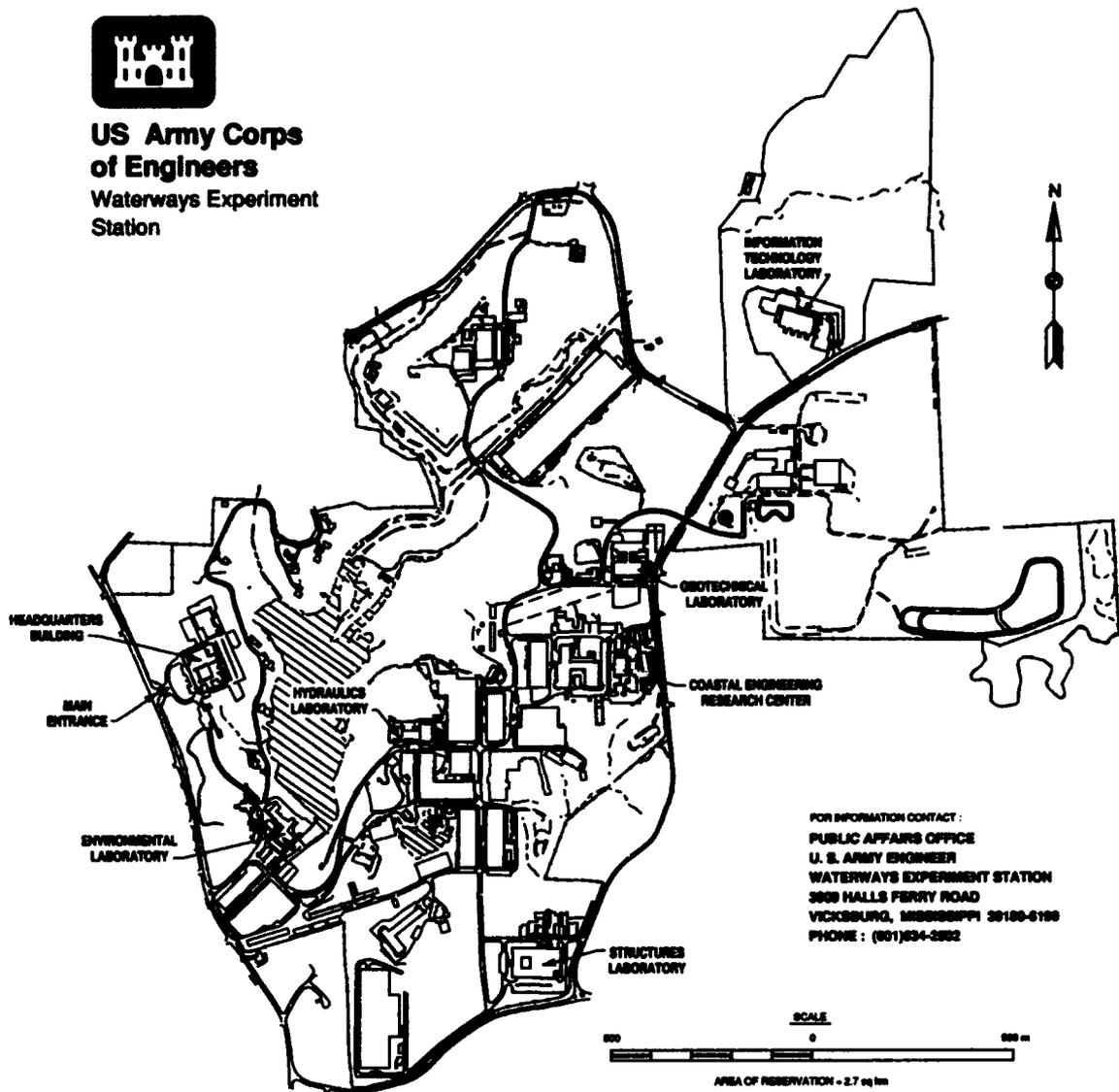
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Preface

The work reported herein was conducted by the U.S. Army Engineer Waterways Experiment Station (WES) for Headquarters, U.S. Army Corps of Engineers (HQUSACE), and the U.S. Army Engineer District (USAED), San Francisco. Financial support was provided by the USAED, San Francisco, through an Intra-Army Order for Reimbursable Services. Additional funding was provided by HQUSACE through the Long-Term Effects of Dredging Operations (LEDO) Program, Work Unit 374-9 "Chronic Sublethal Effects." The LEDO Program is managed through the Environmental Effects of Dredging Programs, Dr. R. M. Engler, Manager.

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1 Introduction

Background

San Francisco Bay is a highly altered estuary. Two major reasons are the diversion of freshwater inflow from the Sacramento-San Joaquin River systems and the loss of wetlands. As of 1980, nearly 60 percent of the historic freshwater inflow to San Francisco Bay estuary had been diverted, mostly for agricultural irrigation. This reduction is projected to increase an additional 10 percent by the year 2000. About 95 percent of all freshwater/estuarine marshlands have been lost to land reclamation since 1850. It is not surprising, therefore, that the estuary has experienced a general decline in health and viability. One of the more noticeable symptoms of this decline has been the gradual loss of biological resources such as the striped bass and Pacific herring fisheries (Nichols et al. 1986).

An increase in the input of environmental contaminants has accompanied the physical alterations to San Francisco Bay. Major pollutant sources include metals associated with mining tailings located in Sacramento-San Joaquin River drainage basins. Additionally, over 50 waste treatment plants and about 200 industries are permitted to discharge directly into the Bay (Luoma and Phillips 1988). Environmental contaminants discharged into aqueous systems tend to associate with particulate material in the water column and with bedded sediments. Periodically, bedded sediments must be removed to maintain navigable waterways. There is a concern that the relocation of these dredged materials may be having unacceptable adverse impacts on aquatic biota within San Francisco Bay.

A large amount of sediment is dredged each year in San Francisco Bay. Approximately 5.5 million cubic meters (mcm) of sediment from Federal projects and permit actions are relocated annually. This value approximates the estimated average annual sediment inflow from natural sources of 6 to 8 mcm (U.S. Army Corps of Engineers (USACE) 1979). It has been estimated that 3.0 to 4.0 mcm of material leaves the Bay annually, while Central and North Bays experience a combined net accumulation of 4.2 mcm (USACE 1979). South Bay shows a net loss of nearly 0.8 mcm per year (Krone 1979). Despite these large numbers, the greatest yearly source of suspended sediment in San Francisco Bay is the resuspension of existing bottom material. Approximately

120 to 130 mcm of sediment are resuspended each year by wind waves and currents (USACE 1979). The effect of these resuspended sediments on fish and aquatic invertebrates is unknown.

To examine whether San Francisco Bay dredged material was causing adverse biological effects, the Planning and Engineering Division of the USACE District, San Francisco, contracted with the Environmental Laboratory of the U.S. Army Engineer Waterways Experiment Station to develop and conduct a series of chronic sublethal sediment bioassays using material from selected sites within the Bay.

Regulatory History of Dredged Material Management in San Francisco Bay

To help define what is known regarding the potential toxicity of San Francisco Bay sediments, it is useful to first examine how dredged material has been regulated in the past. Important milestones in that process are shown in Table 1. It was recognized very early that San Francisco Bay is a physically dynamic system and that most dredged material disposal sites were dispersive. Consequently, initial management concerns were mostly operational. That is, efforts were directed towards optimizing dredging and disposal operations to minimize transportation costs and redredging.

Passage of the National Environmental Policy Act in 1970 outlined the Federal Government's policy toward the environment and signaled an increasing awareness for environmental protection in this country. That same year the San Francisco District initiated the Dredge Disposal Study (DDS) (USACE 1977). The DDS was a multifaceted interdisciplinary study designed, in part, to address some of the environmental concerns regarding potential impacts of dredge disposal operations. Although sediment toxicity was not examined directly, the physical impacts on biota (USACE 1975a) and the bioaccumulation of contaminants from dredged material were evaluated in laboratory and field studies (USACE 1975a; USACE 1975b). Those studies demonstrated the following:

- a. Estuarine animals can survive suspended sediment loads in excess of those normally encountered during dredging and disposal.
- b. In laboratory exposures to San Francisco Bay sediments, estuarine animals can bioaccumulate trace contaminants.
- c. In field studies, contaminant tissue concentrations in animals near the disposal operations were not different from those far removed. The one exception was slightly elevated p,p'-DDE concentrations in mussels, *Mytilus edulis*, during disposal. These differences were not detected 1 month postdisposal.

**Table 1
Milestones in the Regulation of Dredged Material in San Francisco Bay**

1965	Committee on Tidal Hydraulics suggests San Francisco District (CESPN) may be dredging a significant amount of material.
1970	Passage of National Environmental Policy Act.
1970	CESPN initiates Dredge Disposal Study. Terminated in 1975.
1972	CESPN reduces the number of in-bay disposal sites from 11 to 5.
1972	California RWQCB adopts USEPA's Jensen bulk sediment criteria. Material classified as "polluted" by these criteria was either placed upland or taken offshore to the 180-m ocean disposal site.
1973	USACE initiates Dredged Material Research Program.
1976	USACE publishes interim guidance manual for implementation of Section 404 (b) of Public Law 92-500.
1977	Publication of USEPA/USACE Ocean Disposal Implementation Manual.
1978	Public Notice 78-1 (PN 78-1) was drafted by the CESPN. Elutriate test procedures adopted from the Ocean Disposal Implementation Manual and in-bay disposal limited to three dispersive sites (Alcatraz, San Pablo Bay, and Carquinez Strait).
1980	California RWQCB adopts PN 78-1.
1980	100-fathom ocean disposal site becomes part of the Point Reyes-Farallon Islands Marine Sanctuary and is subsequently removed from the final designation process by USEPA.
1982	Mounding at the Alcatraz site noted in November.
1984	CESPN implements slurry policy to enhance dispersion during disposal.
1985	CESPN establishes the Disposal Management Program to find operational, environmentally acceptable solutions to disposal problems.
1985	San Francisco Bar Channel ocean disposal site receives final designation by USEPA. It can receive only coarse-grained material.
1988	Bioassay procedures used to evaluate Oakland Inner Harbor sediments under Section 401 of the Clean Water Act.
1989	The Long-Term Management Strategy was initiated to reflect increasing regulatory and environmental concerns related to dredged material disposal in San Francisco Bay.
1991	Final revision of USEPA/USACE Ocean Disposal Implementation Manual.

In 1972, the California Regional Water Quality Control Board (RWQCB) adopted the Jensen criteria (Bowden 1977). These numerical criteria were developed by the U.S. Environmental Protection Agency (USEPA) for freshwater sediment in the Great Lakes and classified sediment as highly polluted, moderately polluted, or slightly polluted based on bulk sediment chemistry. As research on dredged material progressed, it became clear that these and other chemically based numerical criteria were technically inadequate because they did not assess either bioaccumulation potential or toxicity.

The San Francisco District adopted the use of bioassays for evaluating dredged material in 1980. Regulatory procedures were outlined in Public Notice (PN) 78-1. Elutriate procedures were emphasized since disposal sites in San Francisco Bay were generally dispersive. PN 78-1 also reduced the number of disposal sites from five to three. These were located in the Carquinez Strait, San Pablo Bay, and near Alcatraz Island. To facilitate net export out of the Bay, most dredged material was taken to the Alcatraz disposal site.

In 1982, shoaling was noted at the Alcatraz site. As a result of this important development, the San Francisco District took several steps. The District instituted a slurry policy to enhance dispersion during disposal. It greatly reduced the amount of new dredged material taken to the Alcatraz site and even removed 30 tons (27,200 kg) of construction debris from the site. It monitored the physical configuration of the mound at Alcatraz and found it to be stable after two winter seasons. All of these actions led to the conclusion that the Alcatraz site could not be considered fully dispersive. Since the majority of dredged material in San Francisco Bay was taken to Alcatraz, a reduction in the capacity of that site represented a major impediment to maintenance dredging and to anticipated new work activities. The San Francisco District formed the Disposal Management Program (DMP) in 1985 and charged it with finding solutions to the disposal problem.

The Long-Term Management Strategy (LTMS) was initiated in 1989 to address increasing environmental concerns and to reflect the San Francisco District's commitment to a long-term management strategy for dredged material. In 1991, the Ocean Disposal Implementation Manual was revised to reflect 14 years of regulatory experience and the many scientific advances that had occurred since 1977 (USEPA/USACE 1991).

Overview of Sediment Toxicity Test Development in the United States

As indicated in the previous discussion, the regulation of dredged material disposal in San Francisco Bay has taken advantage of scientific advancements that have occurred elsewhere in the United States. To address concerns specific to the potential toxicity of San Francisco Bay sediments, it is important to have some general knowledge of advances in the field of sediment ecotoxicology. The following is not intended to be a comprehensive review per se;

rather, it is meant to provide the reader with an overview of the advances that have occurred over the past 20 years.

The first peer-reviewed journal article that reported assessment of sediment toxicity was published in 1971 by Gannon and Beeton (1971) (Table 2). The laboratory procedure involved exposing amphipods to freshwater dredged material that had been placed in modified milk cartons. In 1973, recognizing the need for a strong technical base in its regulatory program, USACE initiated the Dredged Material Research Program (DMRP). Included in the scope of this large program was the development of elutriate and solid phase bioassays to assess potential water column and benthic impacts, respectively (Saucier, Calhoun, and Engler 1978). The bioassays developed during the DMRP were subsequently incorporated into both the Ocean Disposal Implementation Manual (USEPA/USACE 1977) and the interim guidance manual for discharge of dredged or fill material into navigable waters (i.e., the 404 Manual) (USACE 1976). These sediment bioassays represented a balance between the state of the art and what could be routinely conducted in a regulatory program.

Table 2 Milestones in Scientific Development of Sediment Toxicity Tests	
1971	Gannon and Beeton published first journal article on sediment bioassays.
1973	USACE initiates Dredged Material Research Program (DMRP).
1976	Publication of Priority Pollutant List of USEPA.
1976	Publication of USACE 404 Manual
1977	Publication of USEPA/USACE Ocean Disposal Implementation Manual.
1978	DMRP completed.
1984	Pellston Conference on Fate and Effect of Sediment-Bound Chemicals.
1987	Formation of ASTM Subcommittee E47.03 on Sediment Toxicology.
1991	Final revision of USEPA/USACE Ocean Disposal Implementation Manual.

Prior to the mid-1970s, the scientific community expressed relatively little interest in sediment toxicity. Most of their energies were focused on the fate and effects of environmental contaminants dissolved in aqueous solutions. After the Priority Pollutant List was published in 1976, that emphasis shifted for two reasons. First, it was discovered that many of the chemicals on the Priority Pollutant List were not very soluble in water and, hence, were not bioavailable. Second, as more field data were gathered, it became apparent that concentrations of many contaminants on the Priority Pollutant List were much higher in the sediment than in the overlying water. Those findings led to initial speculation that sediments might be extremely toxic. However, subsequent research showed that the same forces causing chemicals to partition into the sediments also restricted their bioavailability to aquatic organisms.

A major milestone marking these scientific advances was the sixth Pellston Conference held in 1984 (Dickson, Maki, and Brungs 1984). This was the first time leaders in the scientific community formally met to discuss the fate and effects of sediment-associated contaminants. Bioassay procedures contained in the 1977 USEPA/USACE Ocean Disposal Implementation Manual formed the basis for initial discussion. The researchers reached consensus regarding sediment toxicity (Anderson et al. 1984). They recognized that species sensitivity was related, in part, to the degree of contact between sediment and organism. They recommended amphipods and mysid shrimp for lethal tests and polychaetes, bivalves, oligochaetes, and fish for behavioral or sublethal tests. There was also a strong endorsement of the Tiered Testing Approach for evaluating contaminated sediments (USEPA/USACE 1991). This approach eliminates unnecessary testing and directs limited resources to solving more urgent problems.

Another important milestone in the evolution of sediment toxicity methods occurred in 1987. Members of the American Society for Testing and Materials (ASTM) created a new subcommittee, E47.01 Sediment Toxicology. This subcommittee was charged with identifying technically sound procedures for evaluating sediment toxicity and with drafting appropriate standardized guideline documents. Guidelines, which are in various states of preparation, include the following:

- a. Solid Phase Toxicity Tests with Freshwater Invertebrates.
- b. Solid Phase Toxicity Tests with Marine Amphipods.
- c. Solid Phase Toxicity Tests with Marine Polychaetes.
- d. Solid Phase Bioaccumulation Tests with Invertebrates.
- e. Solid Phase Bioaccumulation Tests with Fish.
- f. Guidance for Designing Sediment Toxicity Tests.
- g. Guidance for Collection, Storage, Characterization, and Manipulation of Sediment prior to Toxicity Testing.

When the USEPA/USACE Ocean Disposal Implementation Manual was first published in 1977, the procedures represented a balance between the state of the art and what could be achieved in the regulatory testing environment. It was realized at that time that revisions would have to be made to reflect scientific and regulatory advances. The manual has recently (1991) been revised. Significant improvements to the current manual as they relate to sediment toxicity evaluations include the following:

- a. Formalizing the Tiered Testing Approach.
- b. Refinements to the species selection process.

c. Provisions for evaluating chronic sublethal effects.

The assessment of chronic sublethal effects is treated as a Tier IV assessment and would be carried out only if there is a reason to believe chronic impacts may be occurring and if technically sound test protocols are available.

Scope

The objective of this report is to assess the effect of sediment storage on the chronic sublethal toxicity of San Francisco Bay sediment. This report is not designed to be used in a regulatory decision-making process (i.e., 404 or 103); rather, it is intended to provide input to the District's DMP and LTMS for dredged material disposal in the San Francisco Bay area.

The current recommendation (USEPA/USACE 1991) for storage of dredged material prior to testing is 10 days and no longer than 6 weeks at 4 °C. Unfortunately, the logistics of conducting a complete tiered assessment can result in an exceedance of the prescribed holding time. To assess potential changes in sediment toxicity with increasing holding time, 21-day chronic sublethal tests were conducted with juvenile *N. arenaceodentata*. Tests were conducted shortly after sediment collection (i.e., within 30 days) and at points in time beyond the prescribed holding limits up to 2 years after collection.

2 Material and Methods

Test Species

Nereis (Neanthes) arenaceodentata is a benthic infaunal polychaete widely distributed in shallow marine and estuarine benthic habitats of Europe, all three coasts of North America, and Hawaii (Reish 1957; Sanders et al. 1962; Reish 1963; Pettibone 1963; Reish and Alosi 1968; Day 1973; Gardiner 1975; Whitlatch 1977; Taylor 1984). This subsurface deposit-feeder constructs one or more mucoid tubes in the upper 2 to 3 cm of sediment and ingests sediment particles up to 70 μm in diameter with a preference for particles around 12 μm (Whitlatch 1980). *Nereis (neanthes) arenaceodentata* has been accepted by the regulatory community as an appropriate test species for evaluating sediment (USEPA/USACE 1977, 1991; Johns, Gutjahr-Gobell, and Schauer 1985). A considerable amount of toxicological information on a wide variety of environmental contaminants already exists for this species (Reish 1985; Jenkins and Mason 1988; Anderson et al. 1990).

Taxonomists are still debating the appropriate nomenclature for this species. Pettibone (1963), who suggested *Nereis (Neanthes) arenaceodentata*, lists five other names for this species: *Spio caudatus*, *Nereis (Neanthes) caudata*, *Nereis arenaceodentata*, *Neanthes cricognatha*, and *Neanthes caudata*. Day (1973) dismissed *arenaceodentata* in favor of *acuminata*, which was subsequently used by Gardiner (1975), Taylor (1984), and Weinberg et al. (1990). *Neanthes arenaceodentata* is most commonly used in the toxicological literature. Recent evidence suggests that the North American Atlantic and Pacific populations are genetically dissimilar, reproductively isolated, and probably of different species (Weinberg et al. 1990). Until the taxonomic status of this species is resolved, the name most familiar to toxicologists will be used and the original source of worms will be reported.

The life cycle of *N. arenaceodentata* is well documented as are culture methods (Reish 1980). As worms approach sexual maturity, males and females establish pairs and occupy a common tube. Eggs are deposited by the female within the tube, and the male presumably fertilizes the eggs at this time. The spent female either exits the tube and dies within 1 to 2 days or is eaten by the male. The male remains in the tube to incubate and guard the developing eggs. He creates a current of water via rhythmic undulations to

remove metabolic waste and prevent hypoxic conditions. Larval development is direct via nonplanktonic metatrochophore larvae and occurs entirely within the parental tube. Emergent juveniles (EJs) exit the parental tube about 3 weeks after egg deposition. They begin to feed and establish tubes of their own. Juvenile worms grow, and eggs become visible in the coelom of females about 6 weeks postemergence. Egg deposition follows 3 to 7 weeks later. The entire life cycle can be completed in the laboratory in 12 to 16 weeks at 20 to 22 °C. Nonplanktonic benthic larvae and paternal care is unusual among the Nereidae. These features also facilitate laboratory culture and the experimental investigation of sublethal effects on growth and reproduction.

Laboratory Cultures

Stock populations of *Nereis (Neanthes) arenaceodentata* were obtained in March 1988 from Dr. D. J. Reish, California State University at Long Beach. Laboratory cultures were maintained using methods adapted from those described by Reish (1980) and Pesch and Schauer (1988). Briefly, EJs were raised to sexual maturity in 38-L aquaria containing 30 L of 30-ppt seawater (Instant Ocean) maintained at a temperature of 20 °C. The photoperiod was 12 hr light. Animals were fed a combination of ground Tetramarin flakes (2 mg/worm) and alfalfa (1 mg/worm) twice weekly. This feeding regime is sufficient to maintain adequate water quality in a static-renewal system and has been found to produce survival and reproduction consistent with what has been reported for other laboratory populations of *N. arenaceodentata* (i.e., survival >80 percent; fecundity, ca. 100 to 1,000 eggs/brood; EJ production, ca. 50 to 500 EJs/brood) (Reish 1980; Pesch et al. 1987; Anderson et al. 1990).

Seawater was renewed (80 percent of volume) every 3 weeks. This renewal schedule, based on water quality monitoring data, is sufficient to maintain good water quality. After 10 weeks, worms were paired using the fighting response (Reish and Alosi 1968) and the presence or absence of eggs in the coelom. Unpaired worms were discarded. Pairs were placed in 600-ml beakers with 500 ml seawater. Gentle aeration was provided via Pasteur pipettes, and the beakers were covered with watch glasses to reduce evaporation. Water was carefully renewed weekly in a manner to avoid disturbing worm pairs.

Beakers were monitored daily for the presence of eggs and EJs. When discovered, EJs were mixed with other broods and returned to the 37 L aquaria to complete the culture cycle. These culture conditions and feeding rations were used in all experiments described below unless otherwise noted.

Test Sediments

Test sediments were collected from seven sites in the San Francisco Bay area. Test sediments fell into two categories: project sediments (collected

from areas of proposed dredging) and reference sediments (selected to represent potential disposal areas). All test sediments were composites of several cores taken to project depth (38 ft (11.6 m) below mean low water mark) from a specific area. Reference sediments were collected from three potential in-bay disposal areas: on the mound at the Alcatraz disposal site (AMR), surrounding areas adjacent to the mound (AER), and the Bay Farm Borrow Pit in South Bay (BFR). An additional reference sediment was collected from an area outside the bay, Point Reyes (PRR), to represent a potential ocean disposal site. Project sediments were collected from three areas in Oakland Harbor: Oakland Inner Harbor (OI); Oakland Outer Harbor (OO); and from areas of Oakland Inner Harbor known to be contaminated with metals and polyaromatic hydrocarbons (PAHs), Oakland Contaminated (OC). In addition to the three project and four reference sediments, a control sediment from Sequim, WA (SC), was also tested. This control sediment was essentially free of contamination and used to validate experimental results. Sediment collection was performed under the direction of Battelle Pacific Northwest Laboratory (for a complete description of sampling methods and protocols, see Mayhew et al., In Preparation). For a summary of sampling locations, sediment collection, handling, and results of chemical analysis, see Moore and Dillon (1993).

Experimental Approach

Sediments were evaluated in 21-day juvenile growth assays with the marine polychaete *Nereis (Neanthes) arenaceodentata*. Sediments were stored at 4° C, and tests were conducted with sediment beginning on Days 30, 65, 113, 194, 286, 334, 427, 469, 621, and 740 postcollection. Sediments were added to 1-L beakers to a depth of 2.5 cm. Approximately 600 ml of 30-ppt salinity seawater was gently added to each beaker, carefully avoiding resuspension of the bedded sediment. Beakers were then equilibrated to test conditions for 24 hr prior to addition of test animals. To initiate the test, 2- to 3-week-old juvenile worms (n = 225) were taken from laboratory culture and randomly distributed among 40 beakers (eight sediments, five replicates/sediment, five animals/replicate). Except for tests conducted on Days 30, 65, 113, a subset of worms (n = 25) was retained for initial estimated individual dry weight determinations (see below). The test was conducted under static-renewal conditions (weekly renewal 80 percent overlying water) at a temperature of 20 °C and a 12-hr photoperiod. Gentle aeration was provided to each beaker. Worms were fed twice weekly a combination of finely ground Tetramarin and alfalfa prepared in a seawater slurry. Dissolved oxygen, salinity, temperature, and pH were monitored weekly. In addition, a 30-ml sample was collected from each beaker, fixed with 50 µL of 1 N HCL, refrigerated, and subsequently analyzed for total ammonia. Total ammonia (milligrams/liter) was determined with an Orion, ammonia-specific electrode after adjusting sample pH to 12 with 5 N NaOH.

After 21 days, worms were removed from all beakers and counted. Estimated individual dry weights were determined by placing all surviving worms

within a replicate on a preweighed aluminum pan, drying for 24 hr at 60 °C, weighing to the nearest 0.01 mg on an electrobalance, subtracting the pan weight, and dividing the resultant mass by the number of animals recovered within the replicate.

Growth was expressed as a rate for each sediment using the following equation:

$$G = (WT_{t_2} - WT_{t_1}) / (t_2 - t_1) \quad (1)$$

where

G = growth rate (mg/day)

WT_{t_2} = mean estimated individual dry weight of worms at test termination

WT_{t_1} = mean estimated individual dry weight at test initiation

$t_2 - t_1$ = duration of exposure (21 days)

In tests conducted on Days 30, 65, and 113 (where initial weights were not collected), WT_{t_1} = the mean initial estimated individual dry weight of all subsequent tests.

Reference Toxicant Tests

The general viability of juvenile worms taken from cultures and used in these experiments was assessed by conducting reference toxicant tests with the heavy metal cadmium (as $CdCl_2$). Tests were conducted as described in Dillon, Moore, and Gibson (1993). Nominal exposure concentrations were analytically confirmed in each test with an Orion specific-ion cadmium electrode. Generally, reference toxicant tests were conducted concurrently with sediment storage tests.

Data Analysis

All statistical analysis and data transformation were conducted using SYSTAT statistical software (Wilkinson 1988). All data was screened for normality and homogeneity of variance via residual plots and Bartlett's test, respectively. Effects of storage time on sediment toxicity (survival and growth) were evaluated using analysis of variance (ANOVA). If the F-statistic was significant, mean separation was performed using a Tukey's HSD (Honest Significant Difference) test. If significant differences were observed and there

appeared to be a relationship between toxicity and storage time (i.e., increasing/decreasing toxicity with increasing storage time), regression analysis was performed. All tests for significance were analyzed at a significance level of $\alpha = 0.05$.

3 Results

Survival and Growth

Mean worm survival was high (80 to 100 percent) for each of the treatments tested. Significant mortality was observed in only a single instance (i.e., 0-percent survival in OC sediment on Day 427) (Figures 1-8 and Appendix A). This instance of low survival may have been related to high levels of ammonia (mean total ammonia = 11 mg/L) measured in the overlying water (see below). Growth was more variable than survival (mean coefficient of variation of 50 and 13 percent, respectively). Only OI sediment showed statistically significant differences in growth with sediment storage time. Growth was significantly lower during the Day 113 test (0.13 mg dry weight/day) relative to growth during the test initiated on Day 194 (0.30 mg dry weight/day) (Appendix A). While OC sediments showed a trend towards increasing growth with increasing storage time, the ANOVA was not significant.

Water Quality

With the exception of total ammonia, water quality was acceptable (mean dissolved oxygen = 6.95 mg/L, pH = 8.10, salinity = 30.4 ppt, temperature = 20.1 °C) in all sediment treatments (Appendix B). Ammonia, however, was elevated in several instances. These peaks appeared to be cyclical (Days 65, 427, and 740) and occurred only in certain sediments (SC, PRR, OO, and OC) (Figure 9). High ammonia concentrations were never observed in four sediments (AER, AMR, BFR, and OI). Peaks in mean total ammonia concentrations occurred during tests conducted on Days 65, 427, and 740. The highest values (mean = 11.9 mg/L) correspond to the single instance of low survival (i.e., OC sediment on Day 427).

Reference Toxicant Tests

Response of juvenile *N. arenaceodentata* to the reference toxicant cadmium chloride was consistent (within 2 standard deviations of the mean) over time (Figure 10). Measured concentrations deviated beyond two standard deviations

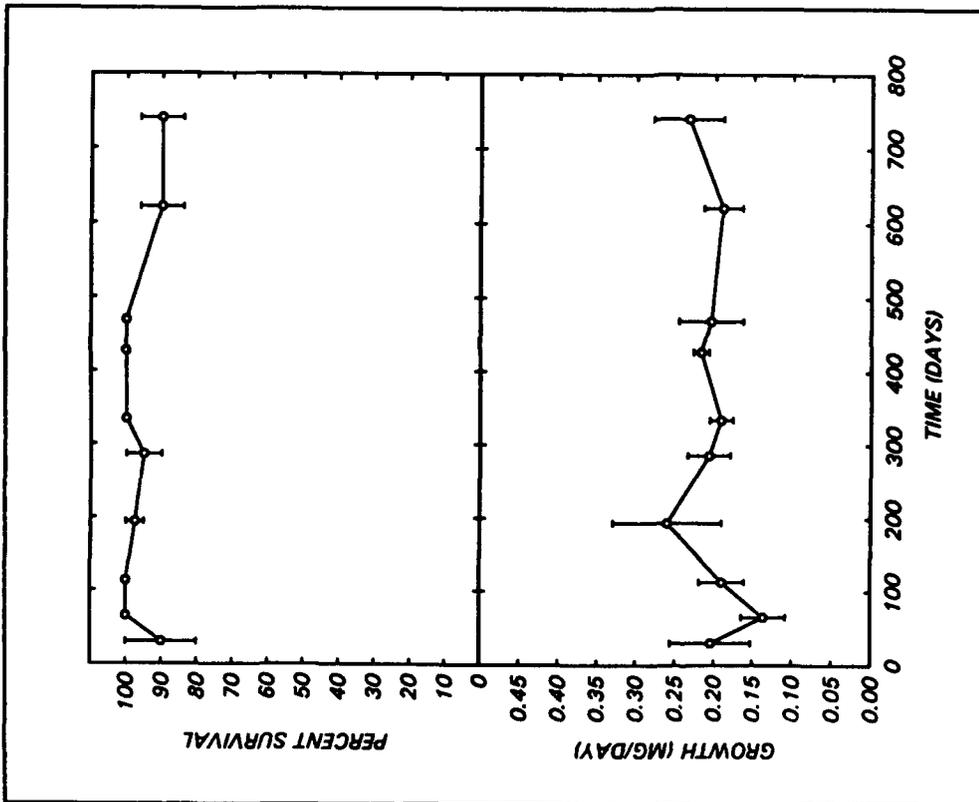


Figure 1. Effect of sediment storage time on mean percent survival and growth (mg/day) of juvenile *N. arenaceodentata* exposed to Sequim Control (SC) sediment

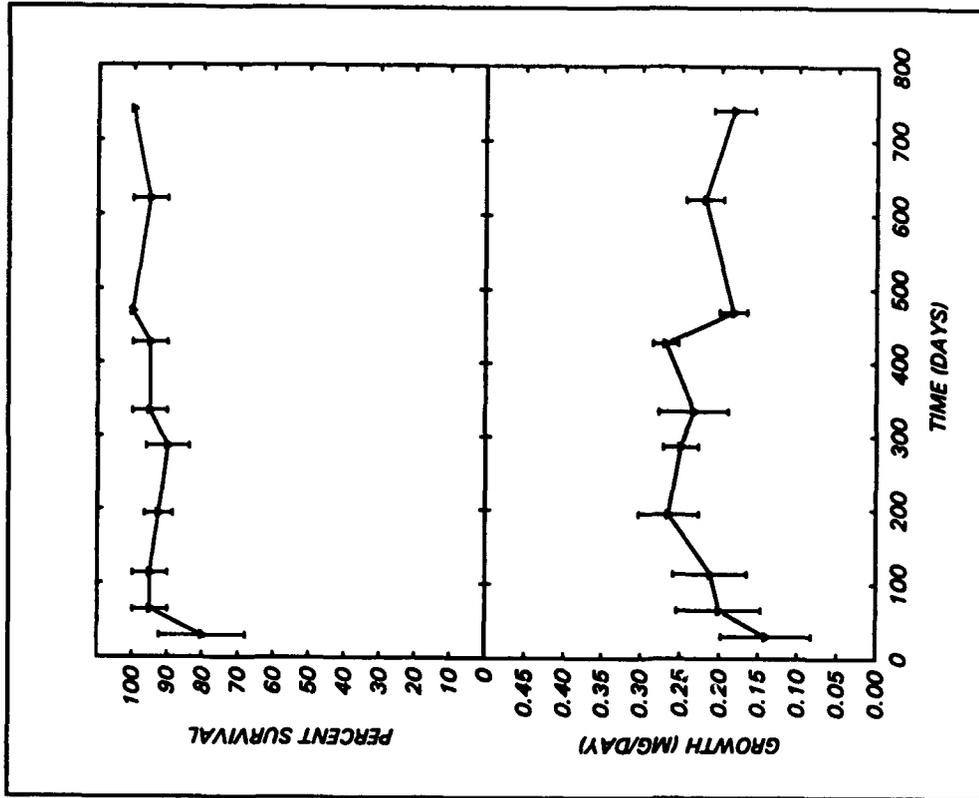


Figure 2. Effect of sediment storage time on percent survival and growth (mg/day) of juvenile *N. arenaceodentata* exposed to Alcatraz Environs Reference (AER) sediment

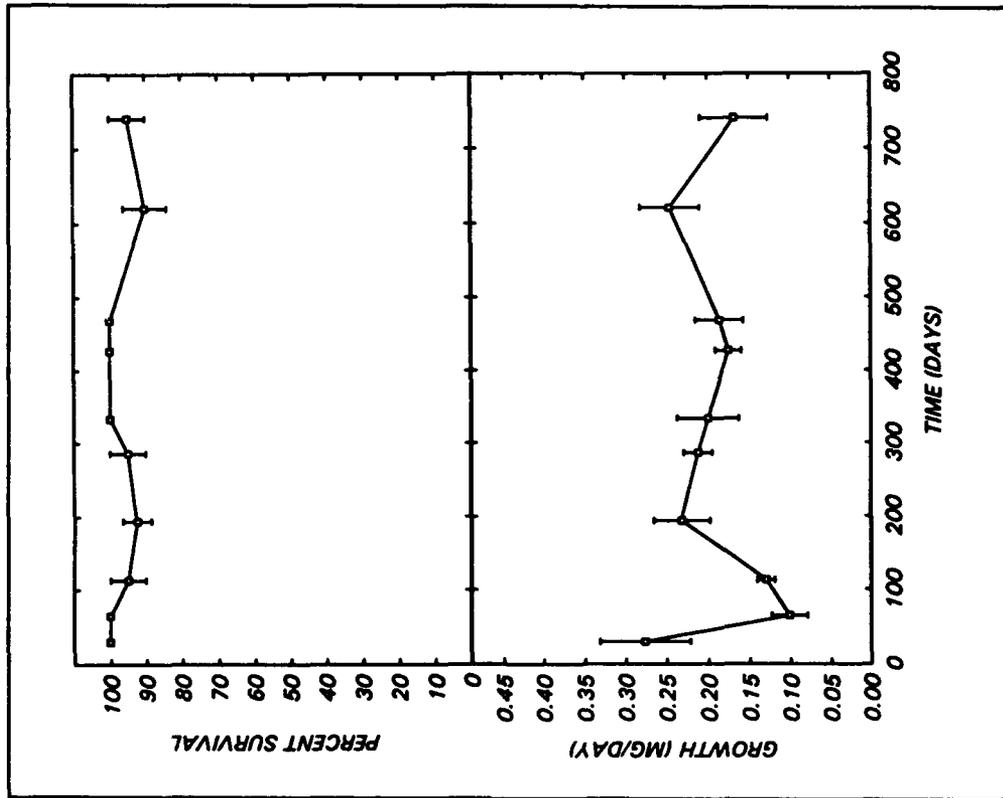


Figure 3. Effect of sediment storage time on percent survival and growth (mg/day) of juvenile *N. arenaceodentata* exposed to Alcatraz Mound Reference (AMR) sediment

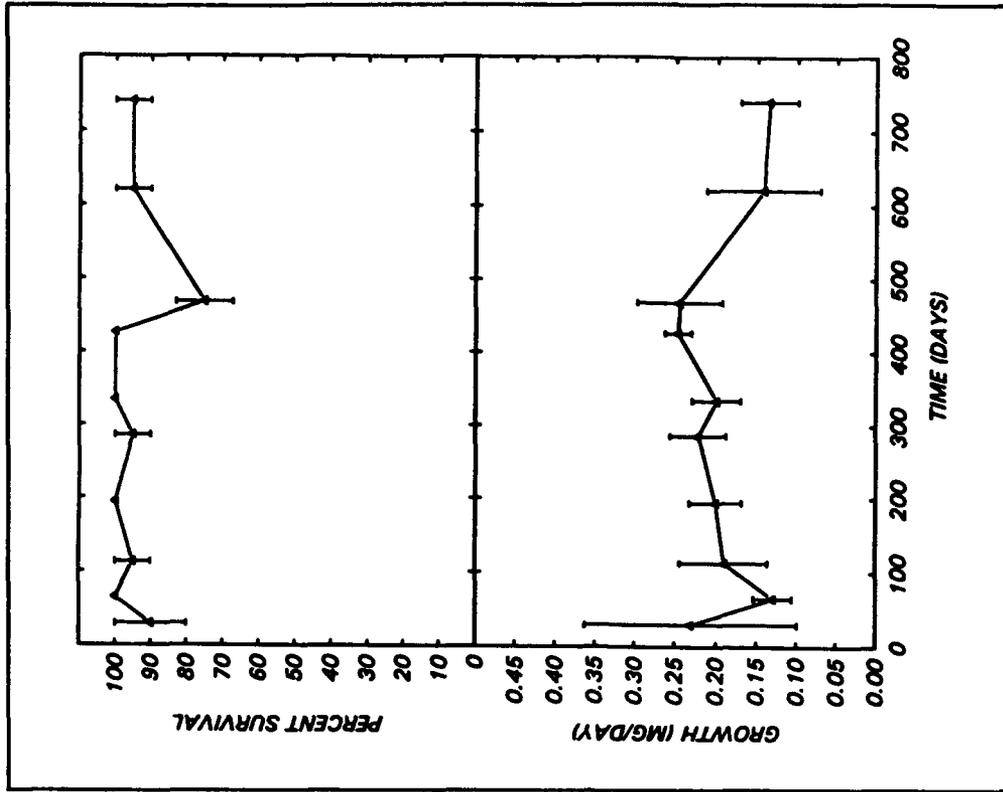


Figure 4. Effect of sediment time on survival and growth (mg/day) of juvenile *N. arenaceodentata* exposed to Bay Farm Reference (BFR) sediment

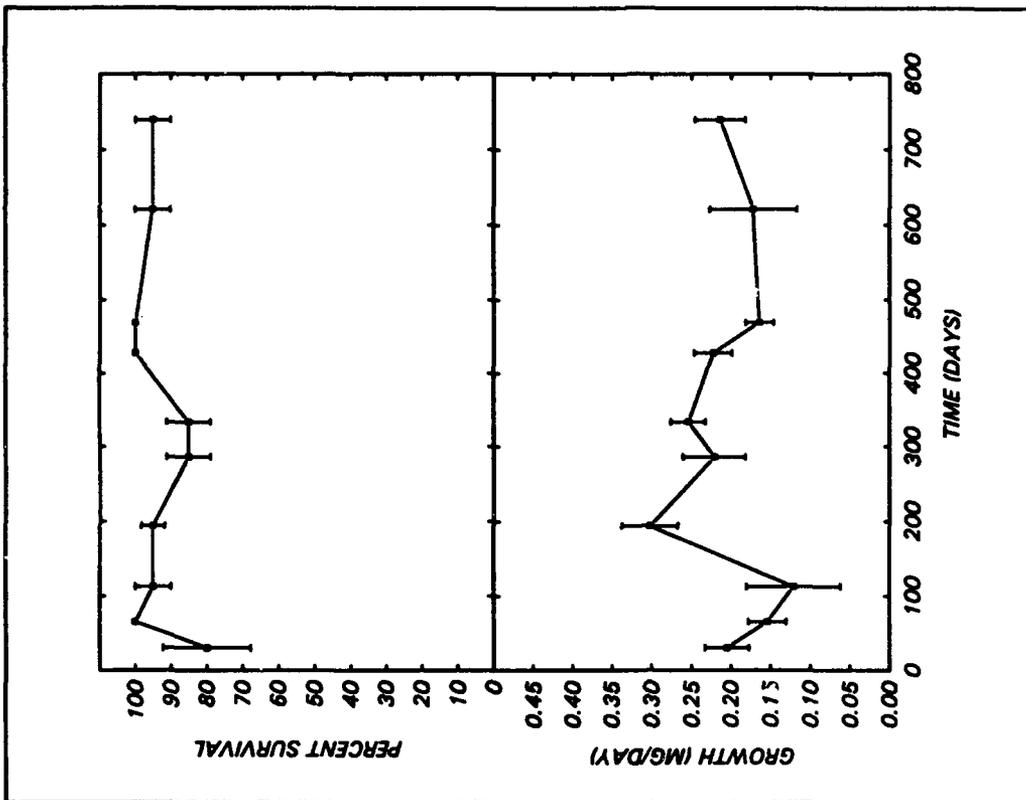


Figure 6. Effect of sediment storage time on survival and growth (mg/day) of juvenile *N. arenaceodentata* exposed to Oakland Inner Harbor (OI) sediment

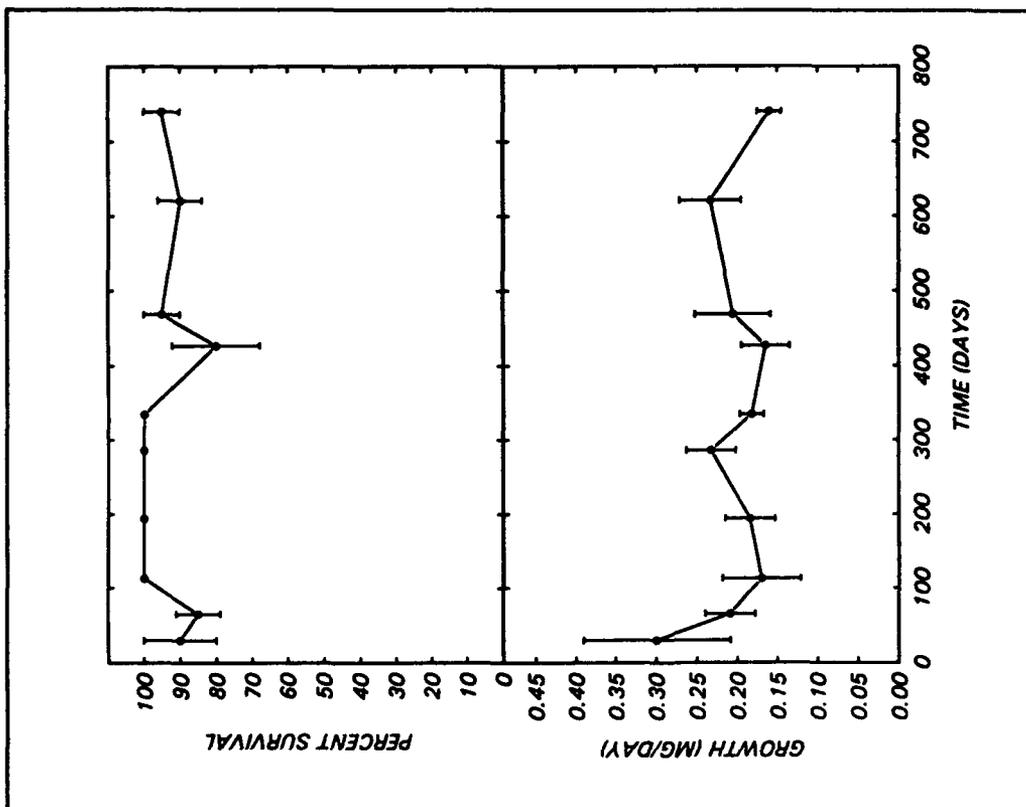


Figure 5. Effect of sediment storage time on survival and growth (mg/day) of juvenile *N. arenaceodentata* exposed to Point Reyes Reference (PRR) sediment

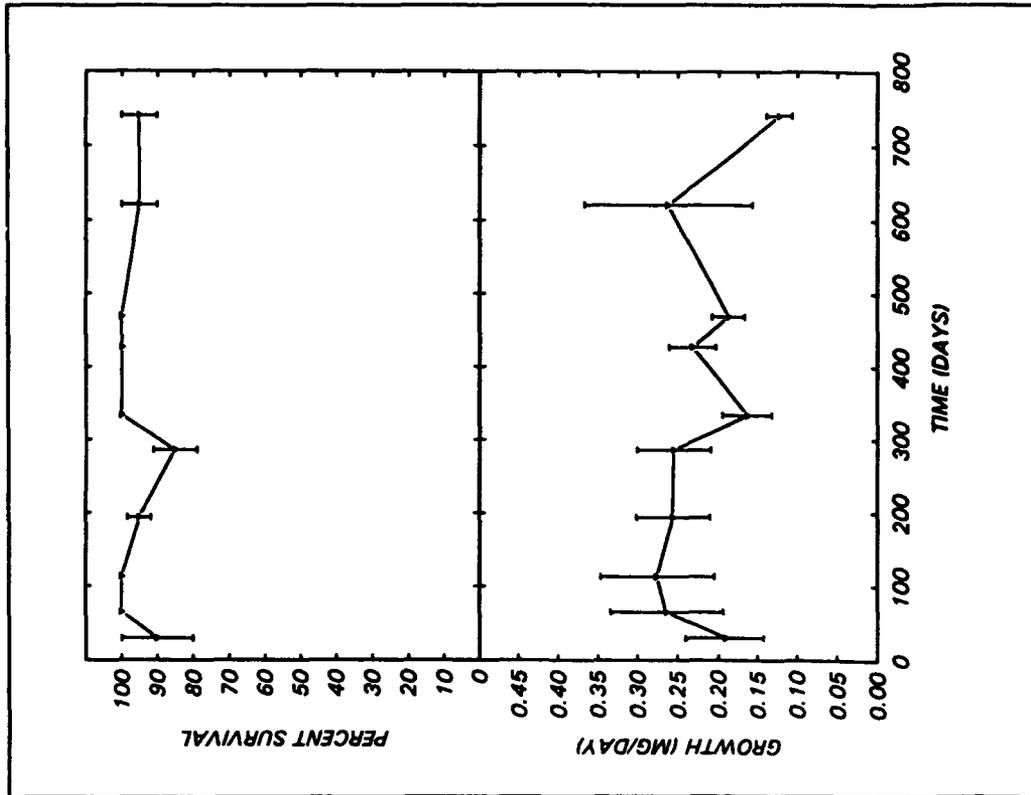


Figure 7. Effect of sediment storage time on survival and growth (mg/day) of juvenile *N. arenaceodentata* exposed to Oakland Outer Harbor (OO) sediment

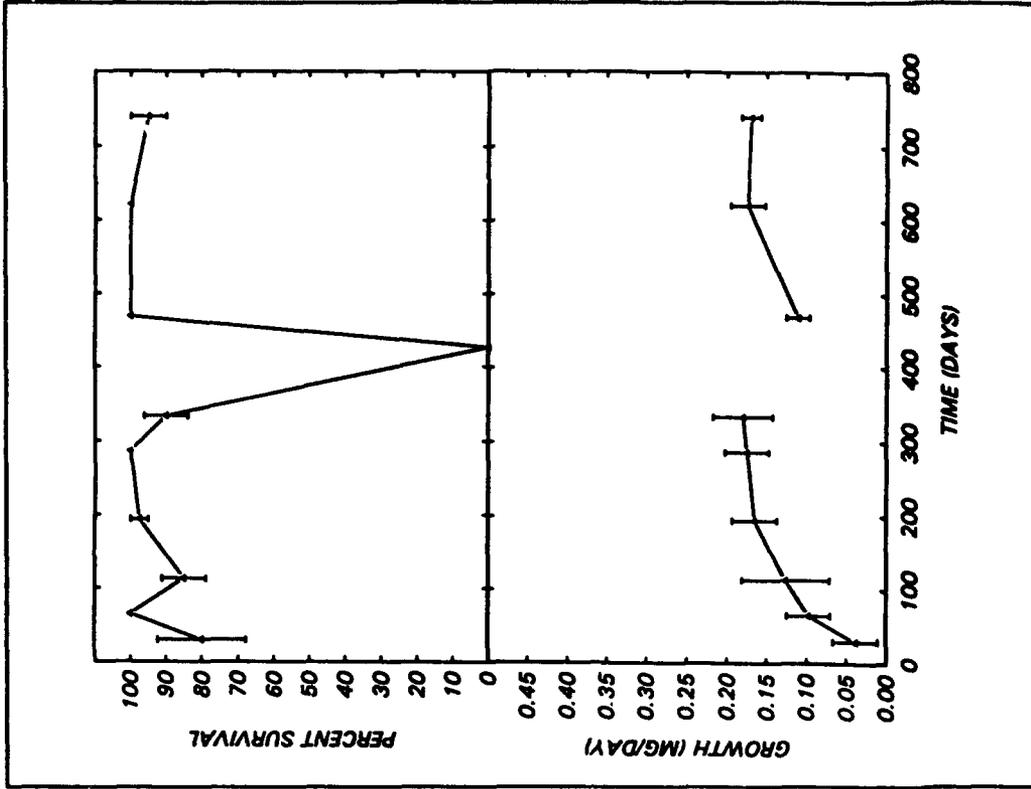


Figure 8. Effect of sediment storage time on survival and growth (mg/day) of juvenile *N. arenaceodentata* exposed to Oakland Contaminated (OC) sediment

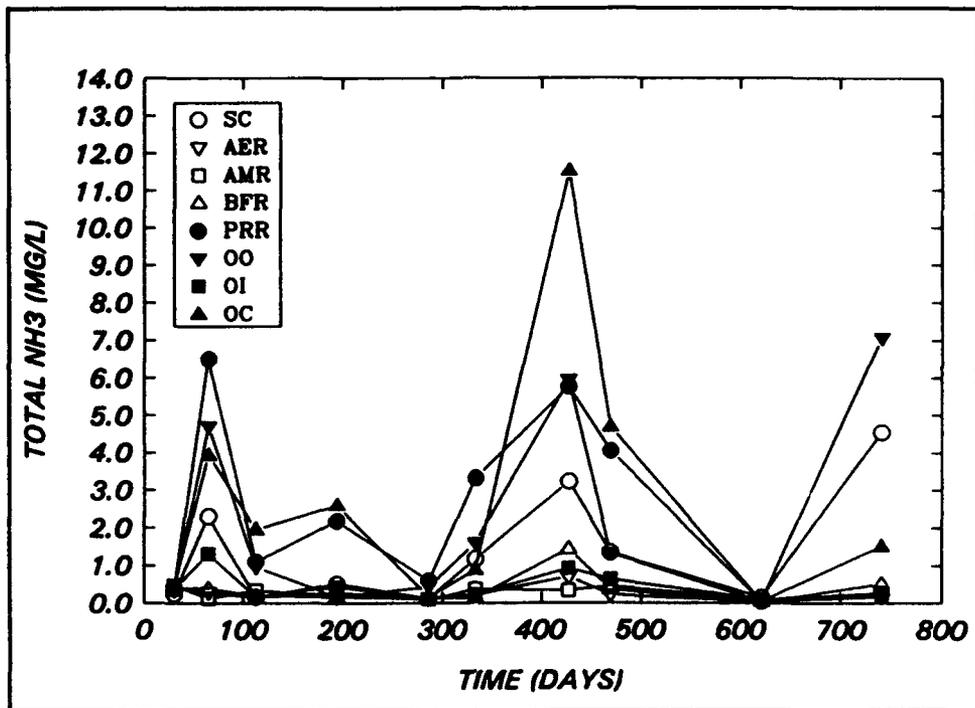


Figure 9. Mean total ammonia concentrations (mg/L) measured in the overlying water during each 21-day chronic sublethal test with San Francisco Bay sediments

in three of the early test (i.e., Days 65, 149, and 192) but stabilized after Day 200 (Figure 10).

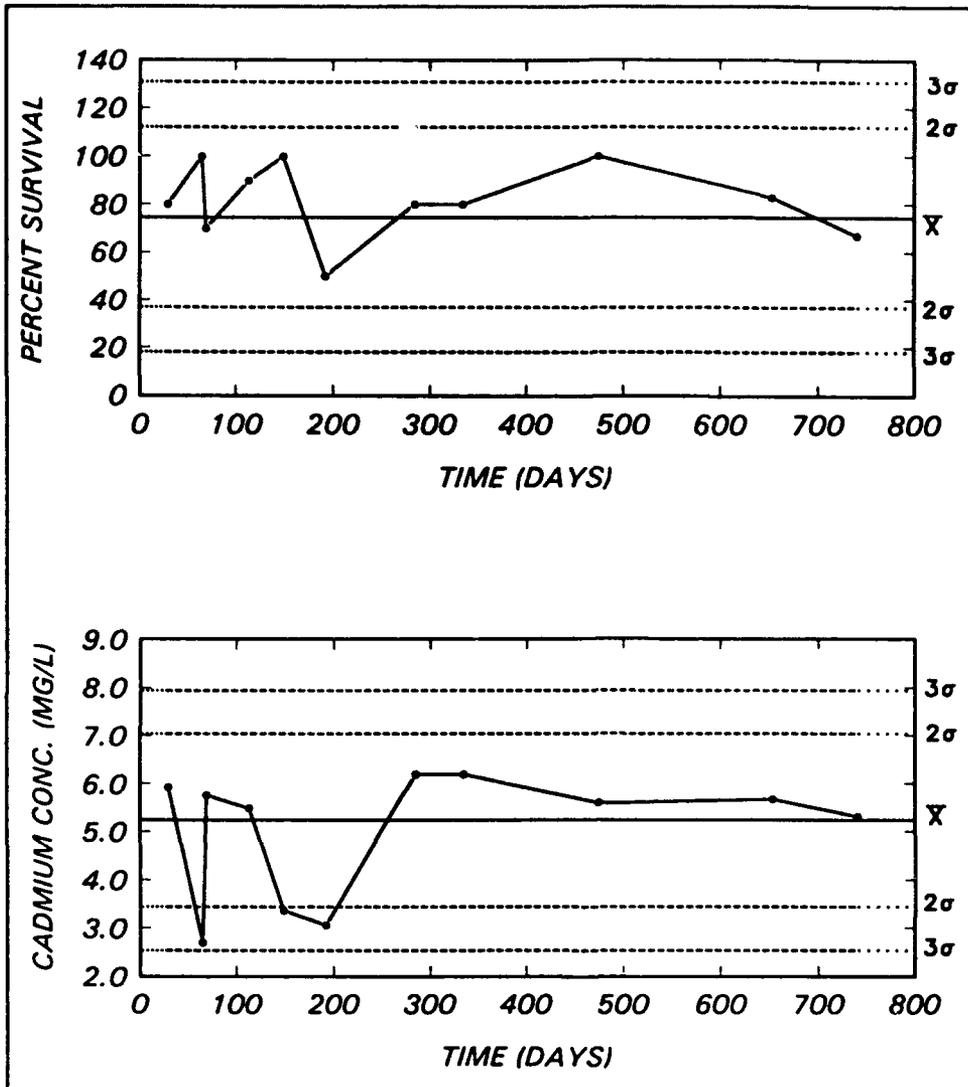


Figure 10. Control charts of survival (in nominal concentration of 6.0 mg/L) and measured concentrations of cadmium chloride in 96h reference toxicant tests

4 Discussion

In general, increasing sediment storage time had little effect on survival and growth of juvenile *N. arenaceodentata* exposed to San Francisco Bay area sediments. Survival was significantly reduced (100-percent mortality) in a single sediment (i.e., OC) at a single point in time (i.e., the test initiated on Day 427). However, measured levels of total ammonia in the overlying water of the OC sediment treatment during this test were the highest recorded for this study. Previous studies have shown reduced survival and growth in juvenile *N. arenaceodentata* exposed for 3 weeks to total ammonia concentrations ≥ 20 mg/L (Moore and Dillon 1992; Dillon, Moore, and Gibson 1993), indicating ammonia may have been responsible for the observed toxicity.

In evaluating the growth end point only, the OI sediment treatment resulted in a significant F-statistic ($p = 0.024$) for ANOVA. Mean separation via Tukey's HSD test indicated that growth was significantly reduced in animals exposed to OI sediment stored for 113 days (0.12 mg/day) compared with those animals exposed to sediment stored for 194 days (0.30 mg/day). No other significant differences were observed.

There did appear to be a trend (albeit not statistically significant) toward increased growth with increasing storage time in juvenile worms exposed to the OC sediment treatment. The reasons for this apparent change in toxicity are not known. Results of the reference toxicant tests indicated that the response of juvenile *N. arenaceodentata* exposed to cadmium chloride was consistent over the duration of the study (Appendix C). Consequently, it is improbable that the reduction in toxicity of OC sediment was due to changing sensitivity of the test organism. Results of bulk chemistry indicated that the OC sediment treatment was contaminated with PAHs and metals (Moore and Dillon 1993). Studies of metal-contaminated freshwater sediments have shown both increased (Malueg, Schuytema, and Krawczyk 1986) and decreased toxicity with increasing storage time (Wiederholm and Dave 1989; Stemmer, Burton, and Liebfritz-Frederick 1990). Landrum (1989) showed that bioavailability of selected PAHs in freshwater sediments decreased with increased storage time. While the reasons for this apparent reduction in toxicity are not known, it is speculated that it may have been due to contaminant loss and/or a reduction in bioavailability. Microbial communities associated with sediments can have a significant effect on the degradation of a variety of chemical structures (Pritchard 1984). Additionally, such factors as changes in the sulfide

pool (Ankley et al. 1991) and the sorption/desorption kinetics of sediment-associated contaminants (Karickhoff and Morris 1984) can affect bioavailability.

One of the more significant observations of this study was the cyclical nature of total ammonia levels in the overlying water in several of the test sediments. Peaks in mean total ammonia in the overlying water of SC, PRR, OO, and OC sediment occurred on nearly an annual basis (test initiated on Days 65, 427, and 740). Results of physico-chemical analysis indicated little in common among these four sediments (Moore and Dillon 1993). The SC, OO, and OC sediments were fine grained (mostly silt and clay), while the PRR sediment was mostly sand (i.e., >50-percent sand). Percent total organic carbon ranged from 0.2 in the OC sediment to 0.8 in the OO sediment. Total Kjeldahl nitrogen averaged 450 to 550 mg/kg for the PRR, OO, and OC sediments, but was markedly higher in the SC sediment (approximately 3,500 mg/kg). Analysis of interstitial water from these sediments shortly after collection indicated high levels of total ammonia ranging from 15 mg/L in the SC sediment to 40 mg/L in the OC sediment. However, two of the sediments that did not show this cyclical pattern (BFR and OI) also had high ammonia levels (17 and 11 mg/L, respectively).

While the mechanism behind this cyclical phenomenon is not known, it is speculated that succession in sediment-associated microbial communities during storage may have played a role. Under anaerobic conditions (conditions of storage), nitrification (the oxidation of ammonia and nitrite to nitrate) does not occur; consequently, if there were no microorganisms to assimilate the ammonia, it would accumulate (Brock 1970). If the microbial populations associated with these stored sediments followed a "boom and bust" pattern of population growth, then one would expect ammonia levels to peak when the microbial population was small and decrease as the population grew. Once sediments are removed from storage and placed in a test beaker, the high concentrations of interstitial ammonia would then diffuse to the overlying water until equilibrium is established.

Based on the findings of this study, storage time had no significant effect on sediment toxicity with the marine polychaete *N. arenaceodentata*. However, cyclical patterns in total ammonia concentrations suggest that there is a potential for effects because of ammonia toxicity.

5 Conclusions

Conclusions based on this study are summarized below.

- Storage time had no significant effect on sediment toxicity.
- Measured levels of total ammonia (overlying water) in the SC, PRR, OO, and OC sediment followed a cyclical pattern with increasing storage time.
- High total ammonia levels (11 mg/L) during the test with OC sediment stored for 427 days resulted in 100-percent mortality of juvenile *N. arenaceodentata*.

6 Recommendations

Based on the findings of this study, the following recommendations are made:

- **Interstitial ammonia levels should be measured prior to conducting toxicity tests. In addition, ammonia levels in the overlying water should be routinely monitored during a test.**
- **The effect of microbial activity on contaminant concentrations and ammonia production in stored sediments should be addressed through additional research.**

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Appendix A Mean Survival, Dry Weight, and Growth for 21-Day Storage Assays

Mean Percent Survival, Estimated Individual Dry Weight (mg), and Growth (mg/day) During 21-Day Exposures to Bedded Sequim Control Sediments, Mean (SE) (N = 5)

Storage Time days	Survival	Initial Estimated Individual Dry Weight	Final Estimated Individual Dry Weight	Growth
30	90 (10)	1.51 ¹ (0.940)	5.79 (1.085)	0.20 (0.051)
65	100 (0)	1.51 ¹ (0.940)	4.37 (0.594)	0.14 (0.028)
113	100 (0)	1.51 ¹ (0.940)	5.51 (0.610)	0.19 (0.029)
194	98 (3)	1.18 (0.297)	4.93 (0.579)	0.21 (0.028)
286	95 (5)	0.57 (0.137)	4.92 (0.579)	0.21 (0.028)
334	100 (0)	1.64 (0.135)	5.65 (0.317)	0.19 (0.015)
427	100 (0)	0.94 (0.122)	5.50 (0.211)	0.22 (0.010)
469	100 (0)	1.20 (0.146)	5.49 (0.871)	0.20 (0.041)
621	90 (6)	2.94 (0.220)	6.95 (0.530)	0.19 (0.025)
740	90 (6)	2.07 (0.550)	6.43 (0.939)	0.23 (0.045)

¹ Grand mean of initial estimated individual weighs from all tests conducted after Day 113.

Mean Percent Survival, Estimated Individual Dry Weight (mg), and Growth (mg/day) During 21-Day Exposures to Bedded Alcatraz Environs Reference Sediments, Mean (SE) (N = 5)

Storage Time days	Survival	Initial Estimated Individual Dry Weight	Final Estimated Individual Dry Weight	Growth
30	85 (12)	1.51 ¹ (0.940)	4.44 (1.226)	0.14 (0.058)
65	95 (5)	1.51 ¹ (0.940)	5.72 (1.141)	0.20 (0.054)
113	95 (5)	1.51 ¹ (0.940)	5.96 (0.978)	0.21 (0.047)
194	93 (4)	1.18 (0.297)	6.44 (0.453)	0.25 (0.022)
286	90 (6)	0.57 (0.137)	5.96 (0.651)	0.26 (0.031)
334	95 (5)	1.63 (0.135)	6.53 (0.935)	0.23 (0.045)
427	95 (5)	0.94 (0.122)	6.60 (0.340)	0.27 (0.016)
469	100 (0)	1.20 (0.146)	5.05 (0.372)	0.19 (0.018)
621	95 (5)	2.97 (0.220)	7.59 (0.515)	0.22 (0.025)
740	100 (0)	2.07 (0.550)	5.34 (0.564)	0.18 (0.027)

¹ Grand mean of initial estimated individual weights from all tests conducted after Day 113.

Mean Percent Survival Estimated Individual Dry Weight (mg), and Growth (mg/day) During 21-Day Exposures to Bedded Alcatraz Mound Reference Sediments, Mean (SE) (N = 5)

Storage Time days	Survival	Initial Estimated Individual Dry Weight	Final Estimated Individual Dry Weight	Growth
30	100 (0)	1.51 ¹ (0.940)	7.31 (1.165)	.028 (0.055)
65	100 (0)	1.51 ¹ (0.940)	3.63 (0.469)	0.10 (0.022)
113	95 (5)	1.51 ¹ (0.940)	4.24 (0.231)	0.13 (0.011)
194	93 (4)	1.18 (0.297)	6.05 (0.724)	0.23 (0.034)
286	95 (5)	0.57 (0.137)	5.02 (0.360)	0.21 (0.017)
334	100 (0)	1.63 (0.135)	5.84 (0.774)	0.20 (0.037)
427	100 (0)	0.94 (0.122)	4.61 (0.332)	0.18 (0.016)
469	100 (0)	1.20 (0.146)	5.10 (0.615)	0.19 (0.030)
621	90 (6)	2.97 (0.220)	8.11 (0.768)	0.24 (0.037)
740	95 (5)	2.07 (0.550)	5.04 (0.873)	0.17 (0.041)

¹ Grand mean of initial estimated individual weights from all tests conducted after Day 113.

Mean Percent Survival, Estimated Individual Dry Weight (mg), and Growth (mg/day) During 21-Day Exposures to Bay Farm Reference Sediments, Mean (SE) (N = 5)

Storage Time days	Survival	Initial Estimated Individual Dry Weight	Final Estimated Individual Dry Weight	Growth
30	90 (6)	1.51 ¹ (0.940)	6.37 (2.782)	0.23 (0.132)
65	100 (0)	1.51 ¹ (0.940)	4.24 (0.507)	0.13 (0.024)
113	95 (5)	1.51 ¹ (0.940)	5.52 (1.131)	0.19 (0.054)
194	100 (0)	1.18 (0.297)	5.41 (0.637)	0.21 (0.030)
286	95 (5)	0.57 (0.137)	5.24 (0.709)	0.22 (0.033)
334	100 (0)	1.63 (0.135)	5.83 (0.624)	0.20 (0.030)
427	100 (0)	0.94 (0.122)	6.13 (0.340)	0.25 (0.016)
469	75 (8)	1.20 (0.146)	6.35 (1.092)	0.25 (0.052)
621	95 (5)	2.97 (0.0220)	5.95 (1.488)	0.14 (0.071)
740	95 (5)	2.07 (0.550)	4.34 (0.760)	0.13 (0.036)

¹ Grand mean of initial estimated individual weights from all tests conducted after Day 113.

**Mean Percent Survival, Estimated Individual Dry Weight (mg),
and Growth (mg/day) During 21-Day Exposures to Bedded Point
Reyes Reference Sediments, Mean (SE) (N = 5)**

Storage Time days	Survival	Initial Estimated Individual Dry Weight	Final Estimated Individual Dry Weight	Growth
30	90 (10)	1.51 ¹ (0.940)	7.80 (1.914)	0.30 (0.091)
65	85 (6)	1.51 ¹ (0.940)	5.89 (0.660)	0.21 (0.031)
113	100 (0)	1.51 ¹ (0.940)	5.09 (1.021)	0.17 (0.048)
194	100 (0)	1.18 (0.297)	5.04 (0.656)	0.18 (0.031)
286	100 (0)	0.57 (0.137)	5.44 (0.639)	0.23 (0.030)
334	100 (0)	1.63 (0.135)	5.46 (0.316)	0.18 (0.015)
427	80 (12)	0.94 (0.122)	4.40 (0.629)	0.17 (0.030)
469	95 (5)	1.20 (0.146)	5.50 (0.991)	0.21 (0.047)
621	90 (6)	2.97 (0.220)	7.87 (0.794)	0.23 (0.038)
740	95 (5)	2.07 (0.550)	4.87 (0.115)	0.16 (0.006)

¹ Grand mean of initial estimated individual weights from all tests conducted after Day 113.

Mean Percent Survival, Estimated Individual Dry Weight (mg), and Growth (mg/day) during 21-Day Exposures to Bedded Oakland Outer Harbor Sediments, Mean (SE) (N = 5)

Storage Time days	Survival	Initial Estimated Individual Dry Weight	Final Estimated Individual Dry Weight	Growth
30	90 (10)	1.51 ¹ (0.970)	5.52 (1.033)	0.19 (0.049)
65	100 (0)	1.51 ¹ (0.970)	7.05 (1.472)	0.26 (0.070)
113	100 (0)	1.51 ¹ (0.970)	7.31 (1.505)	0.28 (0.071)
194	95 (3)	1.18 (0.297)	7.43 (1.002)	0.30 (0.048)
286	85 (6)	0.57 (0.137)	5.94 (0.956)	0.26 (0.045)
334	100 (0)	1.63 (0.135)	5.06 (0.663)	0.16 (0.031)
427	100 (0)	0.94 (0.122)	5.81 (0.599)	0.23 (0.029)
469	100 (0)	1.20 (0.146)	5.14 (0.436)	0.19 (0.021)
621	95 (5)	2.97 (0.220)	8.47 (2.211)	0.26 (0.105)
740	95 (5)	2.07 (0.550)	4.06 (0.342)	.012 (0.016)

¹ Grand mean of initial estimated individual weights from all tests conducted after Day 113.

**Mean Percent Survival, Estimated Individual Dry Weight (mg),
and Growth (mg/day) During 21-Day Exposures to Bedded
Oakland Inner Harbor Sediments, Mean (SE) (N = 5)**

Storage Time days	Survival	Initial Estimated Individual Dry Weight	Final Estimated Individual Dry Weight	Growth
30	80 (12)	1.51 ¹ (0.940)	5.82 (0.598)	0.20 (0.029)
65	100 (0)	1.51 ¹ (0.940)	4.74 (0.501)	0.15 (0.024)
113	95 (5)	1.51 ¹ (0.940)	4.06 (1.233)	0.12 (0.059)
194	95 (3)	1.18 (0.297)	7.52 (0.734)	0.30 (0.035)
286	85 (6)	0.57 (0.137)	5.21 (0.830)	0.22 (0.040)
334	85 (6)	1.63 (0.135)	6.97 (0.457)	0.25 (0.022)
427	100 (0)	0.94 (0.122)	5.62 (0.512)	0.22 (0.024)
469	100 (0)	1.20 (0.146)	4.64 (0.372)	0.16 (0.017)
621	95 (5)	2.97 (0.220)	6.58 (1.164)	0.17 (0.055)
740	95 (5)	2.07 (0.350)	6.01 (0.675)	0.21 (0.032)

¹ Grand mean of initial estimated individual weights from all tests conducted after Day 113.

Mean Percent Survival, Estimated Individual Dry Weight (mg), and Growth (mg/day) During 21-Day Exposures to Bedded Oakland Contaminated Sediments, Mean (SE) (N = 5)

Storage Time days	Survival	Initial Estimated Individual Dry Weight	Final Estimated Individual Dry Weight	Growth
30	80 (12)	1.51 ¹ (0.940)	2.31 (0.581)	0.04 (0.028)
65	100 (0)	1.51 ¹ (0.940)	3.55 (0.567)	0.09 (0.027)
113	85 (6)	1.51 ¹ (0.940)	4.16 (1.154)	0.13 (0.055)
194	98 (3)	1.18 (0.297)	4.64 (0.608)	0.16 (0.029)
286	100 (0)	0.57 (0.137)	4.23 (0.590)	0.18 (0.028)
334	90 (6)	1.63 (0.135)	5.43 (0.792)	0.18 (0.038)
427	0 (0)	0.94 (0.122)	N.A. ²	N.A.
469	100 (0)	1.20 (0.146)	3.54 (0.304)	0.11 (0.14)
621	100 (0)	2.97 (0.220)	6.62 (0.461)	0.18 (0.022)
740	95 (5)	2.07 (0.550)	5.07 (0.261)	0.17 (0.012)

¹ Grand mean of initial estimated individual weights from all tests conducted after Day 113.

² Not applicable, 100-percent mortality.

Appendix B Summary Data for Water Quality Parameter Monitoring

**Water Quality Parameters During 21-Day Exposures to Bedded
Sequim Control Sediments, Mean (SE) (N = 20)**

Storage Time days	Temp °C	Sal. ppt	D. O. mg/L	pH	Total NH₃ mg/L
30	20.0 (0.00)	30.0 (0.00)	6.75 (0.064)	8.09 (0.015)	0.24 (0.035)
65	20.0 (0.00)	30.0 (0.00)	6.54 (0.067)	8.10 (0.012)	2.3 (0.79)
113	19.8 (0.08)	30.7 (0.09)	6.66 (0.054)	8.24 (0.017)	0.16 (0.047)
194	20.0 (0.00)	30.0 (0.00)	6.52 (0.069)	8.14 (0.022)	0.52 (0.136)
286	20.5 (0.17)	31.7 (0.12)	6.48 (0.117)	8.22 (0.019)	0.13 (0.031)
334	21.0 (0.00)	30.3 (0.12)	7.17 (0.038)	8.34 (0.027)	1.2 (0.28)
427	19.7 (0.09)	31.0 (0.18)	7.27 (0.054)	8.28 (0.014)	3.2 (0.60)
469	20.0 (0.00)	30.6 (0.25)	7.77 (0.092)	8.19 (0.018)	1.3 (0.46)
621	20.9 (0.04)	30.2 (0.15)	7.12 (0.085)	7.55 (0.028)	0.15 (0.031)
740	19.8 (0.19)	30.6 (0.17)	6.83 (0.062)	7.86 (0.059)	4.6 (0.94)

Water Quality Parameters During 21-Day Exposures to Bedded Alcatraz Environs Reference Sediments, Mean (SE) (N = 20)

Storage Time days	Temp. °C	Sal. ppt	D. O. mg/L	pH	Total NH₃ mg/L
30	20.0 (0.00)	30.0 (0.00)	6.53 (0.066)	8.12 (0.012)	0.37 (0.061)
65	20.0 (0.00)	30.0 (0.00)	6.66 (0.060)	8.08 (0.011)	0.29 (0.052)
113	19.8 (0.08)	30.7 (0.09)	6.60 (0.073)	8.25 (0.014)	0.15 (0.025)
194	20.0 (0.00)	30.0 (0.00)	6.61 (0.049)	8.14 (0.020)	0.27 (.054)
286	20.5 (0.17)	31.7 (0.12)	6.88 (0.020)	8.27 (0.024)	0.16 (0.058)
334	21.0 (0.00)	30.3 (0.13)	6.89 (0.144)	8.45 (0.020)	0.23 (0.072)
427	19.7 (0.09)	31.0 (0.18)	7.11 (0.034)	8.25 (0.021)	0.70 (0.205)
469	20.0 (0.00)	30.6 (0.20)	7.90 (0.076)	8.20 (0.016)	0.22 (0.079)
621	20.9 (0.04)	30.2 (0.15)	7.21 (0.061)	7.76 (0.027)	0.05 (0.004)
740	19.8 (0.19)	30.7 (0.19)	6.99 (0.055)	7.48 (0.387)	0.18 (0.046)

**Water Quality Parameters During 21-Day Exposures to Bedded
Alcatraz Mound Reference Sediments, Mean (SE) (N = 20)**

Storage Time days	Temp °C	Sal. ppt	D. O. mg/L	pH	Total NH₃ (mg/L)
30	20.0 (0.00)	30.0 (0.00)	6.70 (0.075)	8.11 (0.015)	0.47 (0.058)
65	20.0 (0.00)	30.0 (0.00)	6.69 (0.073)	8.11 (0.013)	0.13 (0.017)
113	19.8 (0.08)	30.7 (0.09)	6.54 (0.059)	8.25 (0.016)	0.35 (0.102)
194	20.0 (0.00)	30.0 (0.00)	6.49 (0.060)	8.14 (0.017)	0.20 (0.032)
286	20.5 (0.17)	31.7 (0.12)	6.95 (0.094)	8.31 (0.017)	0.10 (0.014)
334	21.0 (0.00)	30.3 (0.21)	7.15 (0.074)	8.31 (0.030)	0.37 (0.109)
427	19.7 (0.09)	31.0 (0.18)	7.19 (0.060)	8.28 (0.010)	0.34 (0.073)
469	20.5 (0.53)	30.6 (0.26)	8.09 (0.068)	8.20 (0.015)	0.44 (0.123)
621	20.9 (0.04)	30.2 (0.15)	7.24 (0.099)	7.71 (0.026)	0.09 (0.018)
740	19.8 (0.19)	30.6 (0.15)	6.86 (0.069)	7.81 (0.025)	0.29 (0.100)

Water Quality Parameters During 21-Day Exposures to Bedded Bay Farm Reference Sediments, Mean (SE) (N = 20)

Storage Time days	Temp. °C	Sal. ppt	D. O. mg/L	pH	Total NH ₄ mg/L
30	20.0 (0.00)	30.0 (0.00)	6.68 (0.069)	8.10 (0.017)	0.41 (0.060)
65	20.0 (0.00)	30.0 (0.00)	6.70 (0.105)	8.08 (0.013)	0.39 (0.101)
113	20.0 (0.0)	30.8 (0.1)	6.64 (0.072)	8.25 (0.017)	0.20 (0.047)
194	20.0 (0.00)	30.0 (0.00)	6.51 (0.053)	8.17 (0.022)	0.16 (0.021)
286	20.5 (0.17)	31.7 (0.12)	6.79 (0.103)	8.27 (0.020)	0.14 (0.038)
334	21.0 (0.00)	30.3 (0.13)	7.19 (0.040)	8.48 (0.019)	0.14 (0.040)
427	19.7 (0.09)	31.0 (0.18)	7.24 (0.067)	8.27 (0.015)	1.44 (0.48)
469	20.5 (0.53)	30.90 (0.27)	7.88 (0.069)	8.28 (0.018)	0.36 (0.104)
621	20.9 (0.04)	30.2 (0.15)	7.34 (0.092)	7.73 (0.032)	0.04 (0.003)
740	19.8 (0.19)	30.7 (0.19)	6.84 (0.076)	7.79 (0.040)	0.52 (0.184)

Water Quality Parameters During 21-Day Exposures to Bedded Point Reyes Reference Sediments, Mean (SE) (N = 20)

Storage Time days	Temp. °C	Sal. ppt	D. O. mg/L	pH	Total NH ₃ mg/L
30	20.0 (0.00)	30.0 (0.00)	6.49 (0.073)	8.09 (0.012)	0.40 (0.050)
65	20.0 (0.00)	30.0 (0.00)	6.66 (0.088)	8.11 (0.012)	6.5 (0.90)
113	19.8 (0.08)	30.7 (0.09)	6.47 (0.067)	8.24 (0.015)	1.1 (0.28)
194	20.0 (0.00)	30.2 (0.07)	6.48 (0.067)	8.17 (0.031)	2.1 (0.41)
286	20.5 (0.21)	32.0 (0.12)	6.69 (0.209)	8.21 (0.033)	0.59 (0.443)
334	21.0 (0.00)	30.7 (0.18)	7.25 (0.043)	8.40 (0.028)	3.3 (0.77)
427	19.7 (0.09)	31.0 (0.18)	7.24 (0.052)	8.32 (0.008)	5.8 (0.36)
469	20.0 (0.00)	30.4 (0.21)	8.02 (0.067)	8.19 (0.07)	4.0 (0.94)
621	20.9 (0.04)	30.2 (0.15)	7.34 (0.056)	7.75 (0.036)	0.05 (0.007)
740	19.8 (0.19)	30.6 (0.17)	6.74 (0.079)	7.66 (0.053)	0.26 (0.139)

**Water Quality Parameters During 21-Day Exposures to Bedded
Oakland Outer Harbor Sediments, Mean (SE) (N = 20)**

Storage Time days	Temp. °C	Sal. ppt	D. O. mg/L	pH	Total NH₃ mg/L
30	20.0 (0.00)	30.0 (0.00)	6.52 (0.063)	8.11 (0.017)	0.35 (0.050)
65	20.0 (0.00)	30.0 (0.00)	6.50 (0.060)	8.09 (0.011)	4.7 (1.04)
113	19.8 (0.08)	30.7 (0.09)	6.5 (0.064)	8.21 (0.017)	0.96 (0.203)
194	20.0 (0.00)	30.0 (0.00)	6.50 (0.052)	8.15 (0.021)	0.18 (0.019)
286	20.5 (0.17)	31.7 (0.12)	6.72 (0.124)	8.26 (0.040)	0.44 (0.291)
334	21.0 (0.00)	30.1 (0.09)	7.15 (0.034)	8.38 (0.029)	1.6 (0.56)
427	19.7 (0.09)	30.8 (0.15)	7.07 (0.052)	8.23 (0.017)	5.9 (1.14)
469	20.0 (0.00)	30.5 (0.18)	7.83 (0.077)	8.12 (0.021)	1.3 (0.46)
621	20.9 (0.04)	30.2 (0.15)	7.36 (0.064)	7.65 (0.020)	0.05 (0.007)
740	19.8 (0.19)	30.6 (0.15)	6.93 (0.070)	7.79 (0.046)	7.1 (1.59)

**Water Quality Parameters During 21-Day Exposures to Bedded
Oakland Inner Harbor Sediments, Mean (SE) (N = 20)**

Storage Time days	Temp. °C	Sal. ppt	D. O. mg/L	pH	Total NH₄ mg/L
30	20.0 (0.00)	30.0 (0.00)	6.55 (0.061)	8.10 (0.010)	0.38 (0.067)
65	20.0 (0.00)	30.0 (0.00)	6.60 (0.078)	8.10 (0.014)	1.3 (0.45)
113	19.8 (0.08)	30.7 (0.09)	6.63 (0.054)	8.24 (0.016)	0.18 (0.036)
194	20.0 (0.00)	30.0 (0.00)	6.64 (0.082)	8.19 (0.021)	0.44 (0.130)
286	20.5 (0.17)	31.7 (0.12)	6.72 (0.268)	8.28 (0.026)	0.11 (0.011)
334	21.0 (0.00)	30.1 (0.09)	7.08 (0.096)	8.34 (0.019)	0.23 (0.061)
427	19.7 (0.09)	30.8 (0.13)	7.15 (0.062)	8.25 (0.015)	0.92 (0.226)
469	20.0 (0.00)	30.6 (0.23)	7.87 (0.064)	8.13 (0.019)	0.64 (0.412)
621	20.9 (0.04)	30.2 (0.15)	7.29 (0.064)	7.68 (0.019)	0.10 (0.017)
740	19.8 (0.19)	30.6 (0.17)	6.77 (0.073)	7.76 (0.024)	0.22 (0.071)

**Water Quality Parameters During 21-Day Exposures to Bedded
Oakland Contaminated Sediments, Mean (SE) (N = 20)**

Storage Time days	Temp. °C	Sal. ppt	D. O. mg/L	pH	Total NH ₃ mg/L
30	20.0 (0.00)	30.0 (0.00)	6.66 (0.062)	8.09 (0.012)	0.44 (0.056)
65	20.0 (0.00)	30.0 (0.00)	6.64 (0.067)	8.10 (0.010)	3.9 (0.93)
113	19.8 (0.08)	30.7 (0.09)	6.59 (0.070)	8.24 (0.014)	2.0 (0.39)
194	20.0 (0.00)	30.0 (0.00)	6.74 (0.083)	8.05 (0.039)	2.6 (0.68)
286	20.3 (0.13)	31.8 (0.09)	6.86 (0.060)	8.27 (0.033)	0.17 (0.018)
334	21.0 (0.00)	30.1 (0.09)	7.19 (0.038)	8.51 (0.021)	0.89 (0.307)
427	19.7 (0.09)	30.8 (0.13)	7.06 (0.048)	8.35 (0.016)	11.5 min. = 2.3 (1.35) max. = 19.8
469	20.0 (0.00)	30.6 (0.23)	7.81 (0.088)	8.23 (0.012)	4.7 (1.32)
621	20.9 (0.04)	30.2 (0.15)	7.28 (0.082)	7.81 (0.022)	0.05 (0.004)
740	19.8 (0.19)	30.6 (0.17)	6.84 (0.074)	7.95 (0.045)	1.5 (0.58)

Appendix C Summary Data for Reference Toxicant Tests

**Mean Cadmium Concentration (mg/L) and Percent Survival of *N. arenaceodentata* During Reference Toxicant Tests, Mean (SE)
(N = 5)**

Time¹	CD** Concentration	Survival
29	5.9 (0.05)	80 (11)
65	2.7 (0.03)	100 (0)
69	5.8 (0.04)	70 (11)
114	5.5 (0.04)	90 (9)
149	3.4 (0.04)	100 (0)
192	3.1 (0.04)	50 (14)
285	6.2 (0.03)	80 (11)
334	6.2 (0.03)	80 (11)
474	5.6 (0.21)	100 (0)
653	5.7 (0.09)	83 (14)
740	5.3 (0.02)	68 (14)

¹ Time in days relative to date of sediment collection.

Water Quality Parameters (Controls Only) During Reference Toxicant Tests, Mean (SE) (N = 5)

Time ¹	Time °C	Sal. ppt	D. O. mg/L	pH
29	20.0 (0.00)	30.0 (0.00)	7.14 (0.021)	8.31 (0.035)
65	20.0 (0.00)	30.0 (0.00)	6.26 (0.075)	8.20 (0.017)
69	19.8 (0.09)	30.7 (0.10)	6.66 (0.054)	8.24 (0.017)
114	20.0 (0.00)	30.0 (0.00)	6.46 (0.162)	8.14 (0.039)
149	20.0 (0.00)	30.0 (0.00)	6.63 (0.077)	8.19 (0.035)
192	20.0 (0.00)	30.0 (0.00)	6.54 (0.016)	8.11 (0.035)
285	20.0 (0.00)	32.0 (0.00)	7.17 (0.038)	8.32 (0.015)
334	20.0 (0.00)	29.0 (0.00)	6.70 (0.010)	8.41 (0.002)
474	20.0 (0.00)	30.6 (0.27)	7.77 (0.092)	8.19 (0.018)
653	20.0 (0.00)	30.0 (0.00)	6.00 (0.000)	7.53 (0.012)
740	20.0 (0.00)	30.0 (0.00)	6.90 (0.058)	7.72 (0.088)

¹ Time in days relative to date of sediment collection.

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Mean percent survival of *N. arenaceodentata* was high (80 to 100 percent) in all sediments. Significant mortality was only observed in a single instance (i.e., 0-percent survival in OC sediment on Day 427) and may have been related to high levels of ammonia (mean total ammonia = 11 mg/L) measured in the overlying water. Mean total ammonia levels in the overlying water of Sequim Control, PRR, OO, and OC sediments showed a cyclical pattern with increasing storage time. Higher ammonia levels were measured in these sediments during tests initiated on Days 65, 427, and 740. Significant differences in growth rate over time were observed in tests with both the OI and OC sediment. In addition, the OC sediment showed a slight, but not statistically significant, trend towards increasing growth with storage time.