Salt Marsh Restoration in Mugu Lagoon Sewage Ponds: 1999 Progress Report

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Executive Summary

On March 27, 1998, the Cal/EPA Regional Water Quality Control Board, Los Angeles Region, issued Cleanup and Abatement Order number 98-017 to the Naval Air Station (NAS) Point Mugu. This order demands cleanup of the sludge that occupies the NAS Point Mugu sewage oxidation ponds [pursuant to Section 13304 of the California Water Code]. The order further specifies that the project will begin with a scientific evaluation of the feasibility of using the sewage sludge as a soil amendment in restoring the area to the naturally functioning salt marsh habitat that originally occupied the 15-hectare site. This report describes work completed in 1999 on this scientific evaluation. Chapter 4 provides a concise summary of the results (minus the scientific details) as well as a discussion of the implications for restoration of the sewage ponds. The results of this work are presented in detail in Chapter 3.

A major focus of our scientific evaluation is a pilot restoration experiment that examines the influence of sludge concentration and soil matrix (clay or upland soil) on plant performance and movement of metals in the environment. This experiment was established in 1998 following construction in a small portion of one of the sewage ponds. The experiment consists of 3 m by 3 m experimental plots with two main treatments: (1) different concentrations of sludge (0%, 25%, 50% and 75%), and (2) different soil matrices (clay or upland soil) mixed with the sludge. Results from the experimental plots indicate that sludge concentration had little or no effect on plant performance. The cover of three common plant species at Mugu (*Salicornia*, *Frankenia*, or *Monanthochloe*) was not significantly influenced by sludge concentration in. Plant height also was generally not influenced by sludge concentration. These results corroborate the nonexistence of a sludge concentration effect on plant performance found in our earlier potted plant experiment (Vance *et al.*, in press). While plant growth did not increase with sludge concentration, it also displayed no decreasing trend. That is, growing in artificial sludge-containing mixtures evidently causes no harm to the three plant species tested, including the two most abundant salt marsh plant species at Mugu Lagoon.

We examined the effect of soil matrix on plant performance in several ways. The simplest test compares soils containing no sewage sludge, i.e. pure upland soil, pure clay, and pure wetland soil. In these pure soils, plants tended to perform better in clay than in either upland or wetland soil. Somewhat more complicated but also more powerful tests (due to larger sample sizes) compare plant performance in all soils containing upland soil or clay matrix, both in pure form and mixed with sewage sludge. Most of these comparisons revealed significantly better plant performance in clay or clay-containing mixtures.

The pilot restoration experiment also includes plots enriched with nutrients or organic matter in order to compare the effects on plant growth of sewage sludge with these more standard soil amendments. The results show that sludge enrichment in the pilot restoration experiment produced no less salt marsh plant growth than did the other two more widely used fertilization techniques, frequently repeated nutrient enrichment and one-time organic enrichment. That is, from the perspective of plant growth, Mugu Lagoon sewage sludge appears not to be a poorer soil amendment than either nutrients or naturally occurring organic matter.

However, the nutrient and organic enrichment experiments (and analyses) have limitations, so these conclusions should be regarded as suggestive rather than definitive.

In addition to data collected in the experimental plots, we have studied other aspects of the pilot restoration to gain insight into how to design a successful full restoration. We have examined natural recruitment of salt marsh plants into the pilot restoration because we probably will not be able to plant nursery-reared plants throughout all of the full restoration. We have also examined the use of the pilot restoration by fish and invertebrates, since success of the full restoration will depend in part on how well it supports fish and invertebrates.

Most aspects of a natural marsh seed bank appear to have developed in the pilot restoration site within only a few months after tidal flooding began. For example, at the wrack line where seeds occur most abundantly, the pilot restoration site contained natural abundance of combined plant species' seeds (mean $\pm$ 1 standard deviation was 3862 ± 2163 seeds m^{-2} at the pilot restoration site *vs*. 4194 ± 256 seeds m^{-2} in natural marsh). The pilot restoration had reduced abundance of *Salicornia virginica* seeds (1349 ± 1593 *vs*. 2270 ± 1535 seeds m^{-2}), elevated seed species evenness (0.57 ± 0.13 *vs*. 0.23 ± 0.16), and perhaps somewhat lower species richness (3.60 ± 1.34 *vs*. 5.75 ± 4.42 species).

Fish abundances in the Restoration Creek and in Creek A just downstream from the pilot restoration site were nearly identical, with California killifish being most abundant. Traps in the sedimentation pond that separates these two creeks contained both killifish and mudsuckers in appreciable numbers and also topsmelt. Some fish that occupy tidal creeks in salt marshes also venture out onto the salt marsh plain itself during high tides to feed. We employed fish traps to find out whether fish species that occupy Mugu Lagoon tidal creeks possess this behavior. Three traps placed on a natural marsh plain near the Creek A sampling site captured 52 killifish and no mudsuckers. In strong contrast, fifteen traps placed on the artificial marsh plain of the pilot restoration site up to 30 m from the tidal creek captured only 4 killifish and 2 mudsuckers. Thus, some fish are enter the pilot restoration's marsh plain at high tide, but many more killifish enter a natural marsh plain than the pilot restoration marsh plain.

We examined three aspects of invertebrate use of the pilot restoration: crab abundances, snail abundances, and abundance of siphon holes (used as an indicator of the abundance of organisms living in the mud). The two common crabs species found at Mugu, *Pachygrapsus crassipes* and *Hemigrapsus* sp., occurred in the pilot restoration, although their abundances were not high. There were no horn snails, *Cerithidea californica*, in the pilot restoration site in February or June 1999, but by November 1999 some individuals in intermediate size classes had begun to appear. The Restoration Creek's banks contained numerous siphon holes one year after construction. Siphon holes at the Restoration Creek and Creek A sites currently differ in only two ways: (1) Creek A contains holes greater than 40 mm diameter, whereas Restoration Creek has none greater than 29 mm diameter; and (2) Creek A has more 10-19 mm diameter holes than does Restoration Creek.

A number of important conclusions emerge from the data collected during 1999; these conclusions are summarized here, but discussed in more detail in Chapter 4. It is important to recognize the limitations of this study, and we encourage the reader to guard against over-

interpreting these conclusions. Failure to do so could, at worst, create serious environmental damage in the full sewage ponds restoration project. This investigation has two main limitations. The first, imposed by nature itself, is that ecological phenomena that arise during the first year in any newly established ecosystem do not necessarily predict accurately the ecological phenomena that will characterize that same ecosystem several years later. We feel that two years is the barest minimum observation period required to make realistic projections of the future behavior of a restored salt marsh. The second limitation, imposed mostly by budgetary constraints, is that this first year's study includes no information on environmental chemistry. Consequently, it provides an incomplete assessment of the environmental cost of using sewage sludge in salt marsh restoration. Completing this assessment is a high priority activity during the experiment's second year.

> **<u>Conclusion 1</u>: Mixing Mugu Lagoon sewage sludge with local upland soil or with material from the ponds' clay liner does not increase salt marsh plant performance.**

Consequently, using Mugu Lagoon sewage sludge in local salt marsh restoration will confer no discernible environmental benefit. Making the practical decision of whether to use the sludge in this way, rather than disposing it in landfills, will require weighing only the resulting financial savings against the resulting environmental cost.

> **<u>Conclusion 2</u>: Exposure to Mugu Lagoon sewage sludge does not cause obvious direct physiological harm to the most common plant and animal species that occupy local salt marsh and associated tidal creeks.**

Thus, the toxic substances present in this sludge cause no acute and pronounced ecological damage. Whether these substances might cause longer term harmful ecological effects through biomagnification remains to be evaluated.

> **<u>Conclusion 3</u>: As a substratum for salt marsh plant growth, the clay-bearing material contained in the Mugu Lagoon sewage ponds liner produces higher plant performance than do other local and readily available soils.**

This property arises from the ability of fine-grained soils to retard groundwater flow and hence to diminish loss of soil nutrients through leaching. Long-term retention of nutrient-bearing substances originally leached from decomposing sewage sludge might contribute to increased plant performance through elevated nutrient availability. If this clay material has captured nutrients leached from the sludge, however, then it may also have captured toxic substances leached from the sludge at the same time. If so, then the environmental benefit of using this clay in salt marsh restoration, namely increased plant performance, might be accompanied by an environmental cost in the form of increased toxic pollution of nearby ecosystems. Geochemical studies to evaluate this risk are presently underway.

Chapter 1:
Introduction

On March 27, 1998, the Cal/EPA Regional Water Quality Control Board, Los Angeles Region, issued Cleanup and Abatement Order number 98-017 to the Naval Air Station (NAS) Point Mugu. This order demands cleanup of the sludge that occupies the NAS Point Mugu sewage oxidation ponds [pursuant to Section 13304 of the California Water Code]. The order further specifies that the project will begin with a scientific evaluation of the feasibility of using the sewage sludge as a soil amendment in restoring the area to the naturally functioning salt marsh habitat that originally occupied the 15-hectare site. This order also emphasizes that failure to comply with any provisions of the order may subject NAS Point Mugu to further enforcement action pursuant to California Water Code Sections 13304, 13350, 13385, and 13386.

In response to this order, a group of UCLA ecologists, personnel from the NAS Point Mugu Environmental Division, personnel from Engineering Management Concepts, Inc., Navy personnel from the 31st Naval Construction Regiment (31st NCR) of Construction Battalion Center Port Hueneme, and others have, all under the direction of the NAS Point Mugu Environmental Division, launched this scientific evaluation. After completing construction of a pilot restoration experiment in a small portion of one of the ponds, we opened the site to tidal action on November 2, 1998, and began all manipulative experiments on December 11, 1998. Progress made during 1999 includes establishment of field and laboratory sampling procedures used in evaluating the progress of the pilot restoration experiment, sampling of the pilot restoration experiment in spring 1999 and fall 1999, establishment of several reference sites within the natural marsh at Mugu Lagoon, and sampling of sludge soils and potential matrix soils for use in the larger restoration of the 15-hectare sewage ponds site. This 1999 Progress Report describes both the nature of the sampling activities and also much of the resulting data from the pilot restoration experiment and associated studies.

4.1   Objectives of the Pilot Restoration

In about the early 1950's, Navy personnel created several large sewage oxidation ponds in a portion of the natural salt marsh habitat in the central basin of Mugu Lagoon, located within the Naval Air Station Point Mugu. These ponds received primary treated sewage and its associated heavy metal contamination for several decades, resulting in deposition of a heavy metal-containing sludge layer in each pond. There is some concern that this sludge could potentially be harmful to plant growth within the restoration itself and possibly also to diverse living organisms that inhabit neighboring ecosystems. Export of contaminants via tidal flushing could potentially exert significant effects on several ecosystem components ranging from immediate effects on local flora and fauna to somewhat delayed effects on organisms in higher trophic levels such as shorebirds and marine mammals. Further, because early life cycle stages of some commercially important marine species like halibut and shrimp often occupy estuarine habitat, entry of toxic substances into the estuarine food web could, through later bioaccumulation in these exploited species, eventually harm humans directly.

The cleaning up and restoration of the sewage pond area requires either removal of this sludge or its incorporation into the restored salt marsh. The high cost of sludge removal motivates exploration of the second alternative. Using nutrient-rich sludge as fertilizer may benefit the restoration effort by increasing plant growth and/or hastening ecological succession toward a mature marsh community.

The primary objective of the pilot restoration experiment is to evaluate the nature and magnitude of both the environmental benefits and the associated environmental costs of using this sewage sludge in the salt marsh restoration. A second objective is to discover the best practical restoration design for the remaining sewage pond area.

4.2   Restoration Experiments: A Brief History

As a first step, we conducted a small-scale experiment from March 1996 through June 1997 (Vance, *et al.*, in press). Perforated 1-gallon plastic pots were filled with artificially mixed soils containing sludge concentrations ranging from 0% to 70%. The pots were then placed into specially dug holes in a natural marsh area and then planted with one individual of either *Salicornia virginica* or *Frankenia grandifolia*, the two most common salt marsh plant species at Mugu Lagoon. After 15 months' growth, the above ground plant material was harvested and weighed, and both plant material and soil were analyzed for heavy metals.

We found that final plant mass correlated neither with sludge concentration nor with soil metal concentration. However, plant growth did depend on physical properties of the soil. Specifically, highest growth occurred in fine-grained soils with high organic content. We concluded that sludge containing heavy metals in low concentrations does not harm salt marsh plants. However, choice of the matrix soil with which the sludge is mixed can exert substantial effects on plant growth.

The pilot restoration experiment described in this report is the second phase of our examination. This experiment employs larger unlined plots to examine the effects of both sludge concentration and also identity of the soil matrix on salt marsh plant performance.

In 1997 and 1998, the 31st Naval Construction Regiment carefully prepared a portion of Pond 3 for this pilot restoration experiment. The 1998 Progress Report (Vance 1999) provides detailed descriptions of site excavation and preparation, sludge mixture preparation, planting, and experimental design. The experiment was designed to examine both whether plants benefit from the organic enrichment created by incorporating sludge into the soil and whether heavy metals contained in this sludge either migrate into neighboring environments or bioaccumulate in wetland flora or fauna in appreciable quantities.

The pilot restoration experiment's original design incorporated several related experiments. Unfortunately, however, during the critical process of planting and initial plant establishment in December 1998 and January 1999, extremely strong unseasonal Santa Ana wind conditions developed, and hot and dry desert air severely desiccated the young, vulnerable plants over an approximately 2-week period. We did implement an emergency watering pro-

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cedure during this period, but observations during subsequent months revealed that this watering failed to prevent heavy plant mortality. This mortality has forced early termination of some of the original experiments. Specifically, plant mortality of approximately 80% forced termination of the recruitment interaction experiment. Plant mortality ranging from 50% to 80% also forced termination of the competition experiment. Much of the plant mortality in these experiments resulted indirectly from soil compaction caused by repeatedly driving heavy soil-moving equipment over the site during construction. This compaction apparently reduced the soil's ability to absorb and retain moisture. Fortunately, the plots of the sludge enrichment experiment did not experience this fate. Earth-moving machinery never drove over these plots after their construction, and consequently their soil water-holding capacity was not compromised. Also, all plots in the nutrient enrichment experiment and most in the organic enrichment experiment lie in the well-watered plot rows nearest the restoration site's tidal creek. Though these plots did experience soil compaction, the plants placed there did not suffer much water stress during the Santa Ana winds. Thus, for these different reasons, the sludge enrichment, nutrient enrichment, and organic enrichment experiments survived the stressful period.

This report concerns mostly the sludge enrichment experiment and closely related studies. Chapter 2 describes in detail the sampling protocols that we employed to assess the performance of the pilot restoration. It also explains the basis and design of several associated studies that have been added since the 1998 Progress Report was prepared. Chapter 3 presents results and analysis of data collected during 1999 that best describe this experiment's progress. Chapter 4 draws from these results several conclusions of practical significance and states implications of these conclusions for the design of the future full sewage ponds restoration project.

Previous experience in southern California salt marsh restoration (Zedler 1996, 1997; Zedler and Callaway 1999, 2000) has shown that short term post-restoration monitoring does not (and cannot) provide completely reliable indications of long term marsh performance. For this reason, the pilot restoration experiment is scheduled to continue through two full post-restoration growing seasons. As this report describes trends observed during only the project's first year, all conclusions of practical importance stated in this report must be considered as preliminary and suggestive only. Definitive conclusions and final evaluation of practical implications must await full analysis of two years' restoration performance measurements. This information will appear in the 2000 project report.

Chapter 2:
Methods

The 1998 progress report (Vance 1999) describes the overall design, physical construction, and planting of the pilot restoration experiment. This chapter describes two additional classes of methods, those used to set up additional studies, and those employed to monitor the progress of all components of the pilot restoration experiment. The chapter's three sections describe methods associated with features of the physical environment, with the plants that occupy the physical environment, and with the animals that also occur there. One purpose for describing these methods in detail here is to ensure uniform measurements of all features of the pilot restoration experiment through its whole duration.

4.1 Physical Environment

4.1.1 Elevation, Tidal Inundation, and Sedimentation

The importance of constructing and maintaining proper elevational gradients to restore marsh habitat have been well documented (*e.g.*, Mitsch and Gosselink 2000, Zedler 1990). Our elevational monitoring began before construction of the pilot restoration and employs precise measurements.

Surveying

Conventional surveying employed a standard optical level and a reference marker on the berm between Ponds 1 and 3. This marker's elevation of 9.446 feet MSL was established by NAS Point Mugu Geodetic Office personnel using a mobile differential GPS receiver accurate to 0.5 cm in any direction. Each pilot restoration experiment plot's elevation was measured in the center and at all four corners for a total of five survey points per plot. As described in Vance (1999), each sludge plot was divided into four square quadrants containing different plant species or a species combination. The elevation of each quadrant was taken as the average of the elevation of the plot center and the elevation of the plot corner that lies within the quadrant. Each plant recruitment plot's elevation was measured at two points, the center of its upper edge and the center of its lower edge. Elevation of each smaller plot (*e.g.* snail plots) was measured at the plot center only.

Tidal Inundation

Knowledge of when and how long various portions of the Pilot Restoration site lie underwater is useful for planning the timing of field work and understanding the inundation regime experienced by the marsh surface and its plant and animal occupants. We monitored inundation by deploying TidbiT temperature loggers (Onset Computer Corporation). The loggers have a temperature resolution of $\pm\ 0.7°C$ and were set to record ambient temperature every 10 minutes. We deployed several loggers almost continuously between November 4,

1999, and April 4, 2000, with only a two-week data gap in January. Each logger was wrapped in a thin layer of latex to prevent fouling and then placed inside a PVC and plastic mesh housing (S. Anderson, *in prep*). This housing, once cable-tied to a PVC stake at a particular sampling location, allows water to flow freely over the temperature sensor while shading it from both direct sunlight and rain.

We placed loggers in the center and at the edge of Restoration Creek (the constructed tidal creek that runs east-west along the center of the pilot restoration site), on the restoration plain, and 1 m above the restoration plain. We then measured each logger's elevation. The creek bottom logger was never exposed to air and consequently produced a continuous record of creek water temperature. The logger 1 m above the marsh plain was never submerged and consequently created a continuous record of air temperatures. Loggers on the marsh surface were inundated with the rise and fall of the tides, and the inundation time of each logger was determined by comparison with the continuous records of air and creek temperatures. Abrupt change from air temperature to creek temperature occurred when the tide rose above the elevation of a logger, and change from creek to air temperature occurred when the tide level dropped below the elevation of the logger. This record was then compared to NOAA tide predictions (www.co-ops.nos.noaa.gov/cgi-bin/co-ops_qry.cgi) for the mouth of Mugu Lagoon (derived by applying correction factors to Los Angeles Harbor predictions), and the time lag between tidal flow at the mouth of Calleguas Creek and at the pilot restoration site was calculated.

An ancillary benefit of using temperature measurements to monitor tidal inundation is that the creek temperature record itself may eventually prove useful in interpreting seasonal abundance of fish or other organisms.

Sedimentation

To measure sedimentation rate, in November 1999 we established feldspar plots at several locations within and outside of the pilot restoration site (Fig. 2.1). The stark white powder contrasts sharply with the dark brown mud of the pilot restoration site and natural salt marsh. We established feldspar plots on smooth, plant-free, undisturbed marsh surface at low tide. Feldspar powder was sifted into a circular marsh area bounded by the perimeter of a short section of approximately 75 cm diameter PVC pipe placed onto the ground surface until the powder layer reached a thickness of about 2 cm. After stabilizing the feldspar with a fine spray of water, we removed the PVC boundary and marked the plot with pin flags. Plots in creek centers were established by removing as much water as possible from the PVC boundary and gently sifting feldspar onto the surface of the remaining water. This feldspar eventually settled down onto the creek bottom to create plots essentially identical to those established under drier conditions. Feldspar plots were placed at several locations on the restoration plain, in Restoration Creek, in the sedimentation basin, in Creek A, and in salt pans in the natural marsh adjacent to the restoration site.
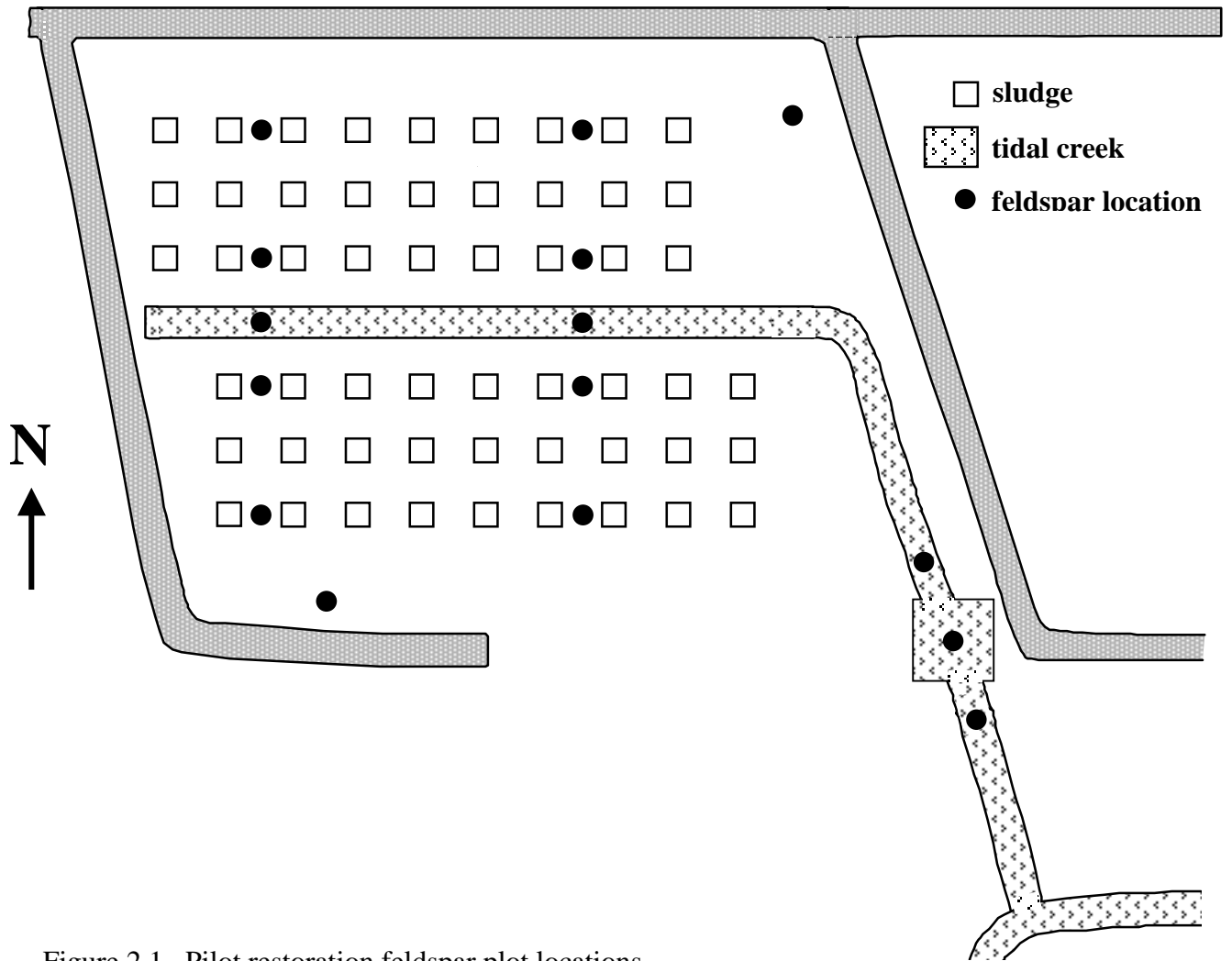
Figure 2.1. Pilot restoration feldspar plot locations.

We plan to collect the first sedimentation samples from feldspar plots in fall 2000. Sampling will employ a liquid nitrogen coring device to be borrowed from the Pacific Estuarine Research Laboratory in San Diego. This apparatus freezes a core within a feldspar plot that perfectly preserves the feldspar layer and overlying sediment. The frozen cores will be transported back to UCLA for measurement.

4.1.2 Soil

The purpose of measuring soil properties is to characterize similarities and differences of artificial soil mixtures and natural marsh soil and then to use these to interpret similarities and differences in plant performance in these soils. This section details both the soil collection methods in general and also specific protocols employed in the various parts of the pilot restoration experiment.

Soil Collection

Soil collection employed a rubber mallet, a PVC pipe (25 cm long, 25 mm inner diameter), zip-lock sandwich bags, a wooden dowel (~30 cm long, 7/8 inch diameter), and a portable cooler to store collected samples. Each sample destined for metals analysis was collected with its own PVC pipe which had been acid washed ahead of time. The acid washing procedure will be described below.

Soil samples were collected by placing a clean PVC pipe upright over the desired sampling area. The rubber mallet was then used to drive the PVC pipe vertically into the soil slightly deeper than needed for the actual sample. The protruding pipe end was temporarily sealed using either the palm of the hand or a thumb stuck into the end, and then the pipe was removed from the soil. The wooden dowel was used to push the sample out of the pipe onto a dry, clean surface. The required amount of soil was then measured and placed into a clean, labeled zip-lock bag. Samples were kept in the cooler in the field until they could be transported to a freezer for storage.

Sludge Plots

Soil was collected from all sludge plots in October 1999. A 10 cm core was collected from the center of each 0.75 m x 0.75 m quadrant, and these four cores were combined to form a single composite sample for each plot. Each composite sample was then stored in a freezer at -20°C until preparation for analysis. Each sludge plot soil sample will be analyzed for nutrient and organic content. Sludge plots in rows 5 and 6 (those closest to Restoration Creek) were also analyzed for sediment grain size composition and metals content.

Nutrient and Organic Enrichment Plots

Soil was collected in all ten nutrient addition plots, nine organic addition plots, and four control plots. A composite sample consisting of four 10 cm cores collected from the four plot quadrants was formed for each plot and then stored in a freezer at -20°C until preparation for analysis. Each was analyzed for nutrients, organic content, and sediment grain size composition.

Nutrient Addition

The plots employed in the nutrient enrichment experiment were situated between the sludge plots in the two plot rows closest to Restoration Creek. This choice was guided by the desire to minimize movement of fertilizer from the plots to which it was applied and into adjacent plots subject to different treatments. We felt that any nutrients leached from fertilized plots immediately adjacent to the creek would most likely migrate down into the creek itself, thereby contaminating no other experimental plots. As will be discussed in Chapter 3, this plot placement complicates statistical comparisons of the nutrient addition treatment to sludge enrichment treatments.

Nutrient addition was accomplished by dissolving measured amounts of commercial fertilizers in Restoration Creek water and then every two weeks carefully sprinkling it onto the nutrient enrichment plots by hand. This operation took place at low tide, allowing at least 1 hour for the nutrients to infiltrate the plot's soil before the returning tide flooded the area again.

Past fertilization experiments in various North American wetlands (Sullivan and Daiber 1974, Broome, et al. 1975, Gallagher 1975, Valiela, et al. 1985, Osgood and Zieman 1993) have revealed that nitrogen is the nutrient usually in shortest supply for salt marsh plants. However, because nitrogen enrichment always has the potential to induce secondary limitation by another nutrient, most likely phosphorus, our fertilizer treatment involved adding phosphorus as well as nitrogen.

The amounts of N and P added were chosen by experience and also to coordinate with previous and ongoing nutrient enrichment experiments at Mugu Lagoon and other southern California salt marshes. Elsewhere at Mugu Lagoon, addition of 15 g m^{-2} of N and 1.5 g m^{-2} of P every 2 weeks (for a N:P ratio of 10:1) stimulates growth by well-established mature individuals of *Salicornia virginica* (Boyer *et al.* in prep.), and even after this addition, N remains the nutrient in shortest supply. Since the pilot restoration experiments involved small young plants, we considered a somewhat lower addition rate appropriate, a rate half way between that appropriate for large, old plants and zero. Thus, we chose to add 7.5 g m^{-2} of N and 0.75 g m^{-2} of P every two weeks throughout the year. This addition has greatly stimulated *Salicornia virginica* growth even in the poor sandy soil of Mugu Lagoon's L Avenue restoration. For nitrogen, we used 46-0-0 Hydro Viking Ship brand urea which is 46% N by weight. For phosphorus, we used 0-45-0 Argee brand Super Triple Phosphate which is 18% P by weight. Appropriate quantities of these (120.3 g urea and 30.8 g Super Triple Phosphate for each nutrient enrichment plot of area 7.38 m^2) were dissolved in 100 ml of distilled water in the lab and added to 2 liters of Restoration Creek water prior to sprinkling onto each plot. (These plots have area 7.38 m^2 rather than 9 m^2 because a plant shortage at planting time forced reducing the *Frankenia* and *Monanthochloe* subplots to square arrays of only 9 plants that occupied an area of 1.2 m × 1.2 m or 1.44 m^2 each instead of the 16-plant arrays that occupied the other two 1.5 m × 1.5 m subplots.)

Plot fertilization commenced on February 23, 1999, approximately 2 months after planting, and took place every two weeks throughout 1999 (and beyond).

The organic enrichment plots and the organic enrichment procedure are fully described in Vance (1999). Organic enrichment was performed just once at the time of planting. Organic matter in the form of healthy *Enteromorpha* sp., a green alga that occurs commonly at Mugu Lagoon, was mixed throughout a column of soil immediately beneath each plant in the organic enrichment plots. The amount of *Enteromorpha* used produced the same soil organic matter concentration immediately beneath each plant as occurred in the 75% sludge in upland soil plots. These organic enrichment plots also lay between sludge plots mostly in the plot rows nearest Restoration Creek.

Both nutrient enrichment plots and organic enrichment plots occupy pure upland soil that had been previously compacted by the heavy earth-moving machinery used to construct the pilot restoration site. This physical difference from the sludge plots complicates comparisons of both nutrient enriched plots and organic enriched plots to sludge enriched plots, as will be discussed in Chapter 3.

Metals Migration

Soil was collected near the sludge plots within rows 5 and 6 that contain 50% sludge or 75% sludge. These are plots 3, 7, 9, and 15 in row 5 and plots 7, 9, 11, and 17 in row 6. Sample cores were collected at distances of 1, 2, and 3 m towards the tidal creek from each plot's lower edge (Fig. 2.2). At each distance, a composite sample of four 3-cm long cores was collected within a 15 cm^2 area and stored in a freezer at -20°C until preparation for analysis. Each soil sample will be analyzed for metals content.

A Potential Matrix Soil

A potential matrix soil for use in forming sludge mixtures in the full salt marsh restoration project is the soil immediately east of the sewage ponds. To evaluate this potential, we collected nine samples to be analyzed for grain size composition and metals content. During sampling, we found that soil in this area is extremely compacted and contains much gravel and associated small rocks.

Soil sampling east of Pond 2 took place on November 12, 1999. Three transects were established in an east-west orientation along the outside edge of the eastern berm of Pond 2 and marked with pin flags. The northernmost transect was numbered "1", the middle transect "2", and the southernmost transect "3". These transects were spaced approximately 25 meters apart so that the entire area east of Pond 2 between the two service roads running along the northern and southern boundaries of the pond could be sampled evenly. Along each transect, three stations were established such that each station lay approximately 5 meters from its nearest neighboring station. At each station, the soil was sampled to a depth of 10 cm. Each individual sample was placed in a zip-lock bag. The samples were then transported to UCLA and stored frozen at -20°C for future analysis.
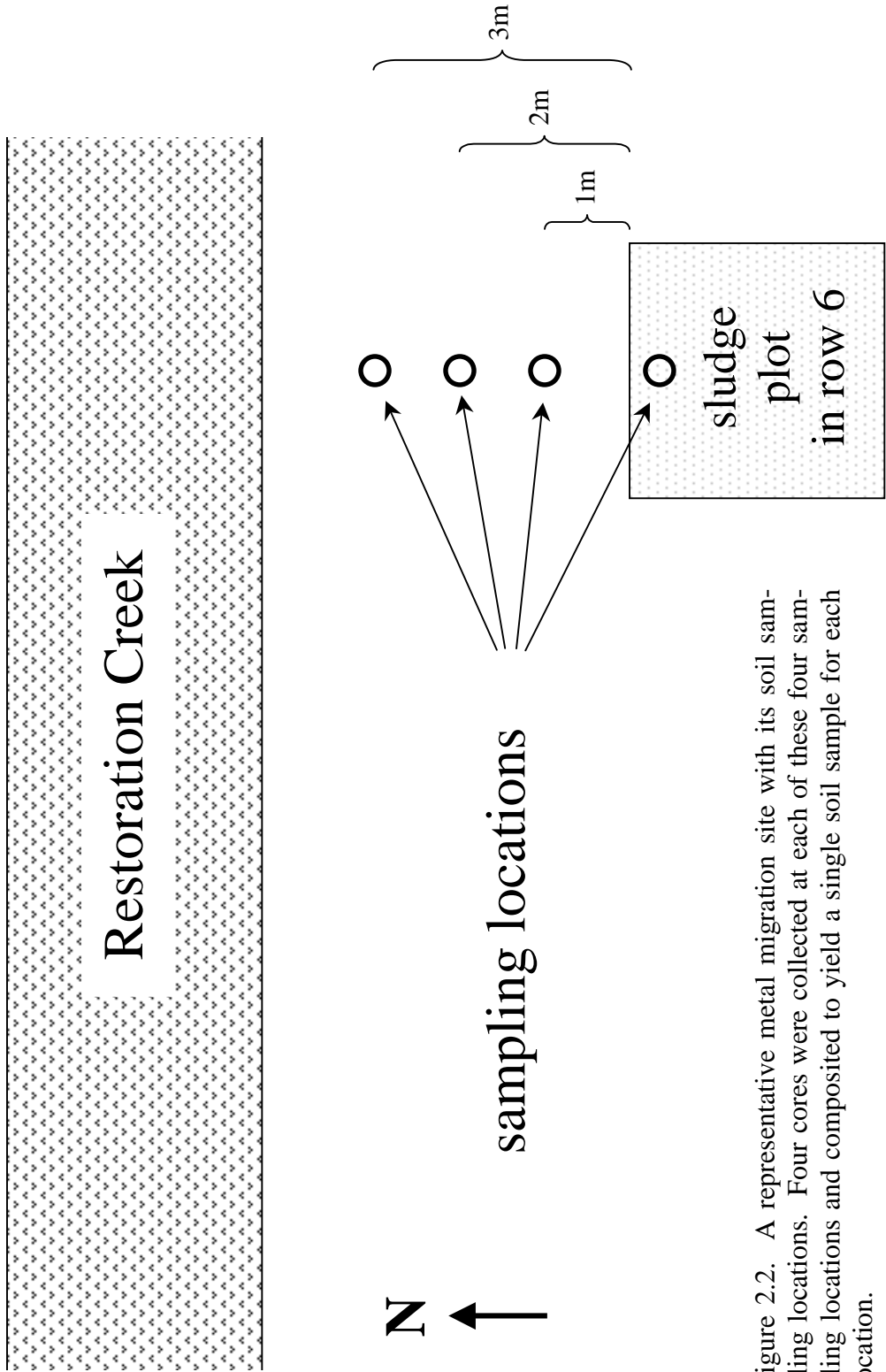
Figure 2.2. A representative metal migration site with its soil sampling locations. Four cores were collected at each of these four sampling locations and composited to yield a single soil sample for each location.

Sludge and Clay

We resampled the sewage ponds to determine the present distribution of metals there to guide later sludge mixture preparation for the full restoration project. We established sampling grids in Pond 2 and in the remaining portions of Ponds 1 and 3. The remaining area of Pond 1 contained a 2x2 grid of 4 stations. Pond 2 contained a 2x3 grid of 6 stations. The remaining portion of Pond 3 contained a 2x2 grid plus one additional station at the far east corner for a total of 5 stations. Two samples were collected at each station, one at the surface of the sludge layer and one in the clay layer just beneath the sludge layer. Sludge samples were collected on November 12, 1999, and clay samples were collected on May 26, 2000. Each sample, consisting of a single 10 cm core, was transported to UCLA in a zip-lock bag and stored at -20°C prior to analysis.

4.1.3 Laboratory Procedures

Acid Washing

Prior to collecting soil, water, or living organisms or their tissues for metals analysis, we attempted to remove any pre-existing metal-containing residues from all containers and any other equipment that might later contact the samples. The cleaning procedure, called acid washing, involves immersing all such equipment (new as well as used) in a nitric acid solution. Our sample containers were 30 cm long 1-inch diameter PVC tubes for soil and ICHEM Nalgene bottles for water, organisms, and tissue samples. Acid washing these containers followed this procedure:

1. All acid washing must be done in covered plastic buckets in a fume hood.

2. Mix the acid solution by volume: 10% trace metal grade nitric acid, 90% deionized water (Milli-Q) water. Mix enough solution to immerse the containers completely.

3. Immerse the containers in the acid solution overnight.

4. Flush the containers with deionized (Milli-Q) water for 3-4 hours.

5. Remove the containers from the water while wearing powder-free rubber gloves.

6. Place the containers in a drying oven at 70 ℃ until they become dry, which usually requires 4 to 6 hours.

7. Store the containers in new dust-free zip lock bags until use.

Metals Sampling Preparation Procedure

Following field collection, samples destined for heavy metal analysis were transported to UCLA for preparation. The chemical analysis itself lies outside UCLA ecologists' expertise. During 1999, this analysis was performed under the supervision of David Kimbrough, Water

Quality and Laboratory Supervisor, Castaic Lake Water Agency. Pre-analysis preparation follows this procedure:

1. The minimum dry mass of each soil or sediment sample is 50 grams.

2. The minimum volume of each water sample is 500 ml.

3. No sample may contact any metal object (shovel, etc.) at any time.

4. Immediately after collection, each sample must be placed in the shade, preferably in an ice chest.

5. Each water sample must be acidified as soon as possible after collection. Add very small volumes of concentrated trace metal grade nitric acid incrementally, mixing the sample thoroughly after each addition, until sample pH falls below 2, as determined by either pH paper or a pH meter. Typically, about 0.2 ml of acid is required per 100 ml of sample with initial pH near 7.

6. Each water and sediment sample must be frozen between field collection and laboratory analysis.

Sediment Particle Size Analysis

Each soil or sediment sample destined for particle size analysis must contain at least 50 gm dry mass. Sample preparation and analysis by a modification of Bouyoucos' (1962) hydrometer method employs this procedure:

1. Remove all twigs, shells, rocks, and roots over 2 mm in greatest dimension.

2. Dry the sample.

    a. Cut, label, and weigh an aluminum foil square formed into a sample holding pan.

    b. Place the sample into the foil holding pan and weigh them together to obtain gross wet weight.

    c. Place the holding pan into a drying oven maintained at 55°C if the sample is to be analyzed later for nutrients, or at 70°C if not, until the sample is dry. Make sure that the whole sample, and not just the surface, is dry.

    d. Weigh the foil holding pan and dried sediment. Subtract this gross dry weight from the gross wet weight to determine the sample's water content.

3. Remove 50 g of dried soil from the holding pan and place it in a beaker.

4. Prepare sodium metaphosphate solution by adding 50 gm of dry sodium metaphosphate to 1 liter of distilled water and stirring it vigorously until it dissolves.

15

5. Add 100 ml of sodium metaphosphate solution to the beaker containing the sample, and then add 200 ml of distilled water .

6. Place the beaker on a shaker at 125 rpm for 24 hours. Make certain that all clumps of sample are broken apart by hand to avoid overestimating sand content.

7. Pour the beaker's contents into a glass cylinder with a 1-liter mark, and use distilled water in a wash bottle to wash all remaining sample from the beaker into the cylinder.

8. Add more distilled water to the cylinder to bring the total volume of sample plus water to 1 liter.

9. Make a blank by adding 5 gm of sodium metaphosphate to 1 liter of distilled water and mixing vigorously.

10. Measure water temperature in the cylinder; it must lie between 16.5°C and 24.4°C for accurate results.

11. Place parafilm over the cylinder top, and invert the cylinder several times to suspend the soil thoroughly.

12. Immediately after mixing, record the time and lower a hydrometer into the cylinder.

13. After exactly 40 seconds, read the hydrometer.

14. If multiple samples are being analyzed in a batch, rinse the hydrometer between samples.

15. After exactly two hours, make another hydrometer reading and another temperature reading.

16. To calculate the fraction of the sample's mass that consists of sand (particles > 62 μm diameter), silt (particles between 2 μm and 62 μm diameter), and clay (particles < 2 μm diameter), employ the following equations. Note that precise use of this procedure employs 1 liter of mixture and 50 gm dry weight of soil (or sediment).

$$\text{corrected hydrometer reading} = \text{hydrometer reading of sample}$$
$$- \text{ hydrometer reading of blank}$$

$$\text{temperature adjusted hydrometer}$$
$$\text{reading (TAHR)} = [(\text{temperature} - 20°) \times 0.35]$$
$$+ \text{corrected hydrometer reading}$$
$$\% \text{ silt and clay} = (\text{TAHR at 40 sec} \times \text{liters of mixture})/\text{grams of dry soil}$$

$$\% \text{ sand} = 100\% - \% \text{ silt and clay}$$

$$\% \text{ clay} = (\text{TAHR at 2 hours} \times \text{liters of mixture})/\text{grams of dry soil}$$

$$\% \text{ silt} = \% \text{ silt and clay} - \% \text{ clay}$$

Soil Organic Content Procedure

We measured soil organic content as percent mass loss following oxidation of organic matter. As this procedure shares some steps with the particle size analysis procedure, we usually performed both at the same time using a sample large enough to accommodate both. Organic content measurement requires no specific sample size, but we typically employ samples of about 25 g dry mass. Organic content measurement employed this procedure:

1. Perform steps 1-2 of the particle size analysis procedure.

2. If desired, remove 50 g of dry sediment to be used for grain size analysis, put it in a beaker, and proceed with Step 4 of that procedure. For organic content analysis, perform the steps that follow on the sample that remains.

3. Weigh an empty crucible.

4. Employ a mortar and pestle to grind apart all the conglomerates in the dried sample, and then place the ground sample in the crucible.

5. If the sample has already been dried at 55°C or 70°C in Step 2c, then dry it further at 100°C for 3 hours. If it has not been dried previously, dry it at 100°C for 24 hours. In either case, allow the dried sample to cool in a desiccator.

6. Weigh the cooled sample in its crucible.

7. Calculate sample dry mass as the mass measured in Step 6 minus the mass measured in Step 3.

8. Oxidize the sample's organic matter by heating the sample in its crucible to 400°C for 10 hours in a muffle furnace, and then allow it to cool in the desiccator.

9. Weigh the oxidized sample, or ash, in its crucible.

10. Calculate the organic dry mass as the sample dry mass minus the ash dry mass.

11. Calculate the organic fraction, expressed in percent, as the organic dry mass divided by the sample dry mass.

Tidal Creeks

To evaluate metal movement within Tidal Creek A that floods and drains the pilot restoration site, we collected samples of both creek sediment and creek water. We established one sampling station in Restoration Creek, four stations in Creek A at increasing distances from the restoration site, and for comparison, one station each in Creek C, Creek D1, and Calleguas Creek.

Creek sediment was collected on 10 November 1999 by hand pushing a 250 ml acid washed Nalgene sampling bottle through the upper 3cm of sediment lying in the creek's center until sediment filled the bottle. These sediment samples were stored at -20°C until analysis.

Creek water was sampled on the same dates at these same stations during both incoming and outgoing tides. Samples of 500 ml were collected in acid washed Nalgene bottles and then acidified in the field to pH 2.0 or lower by adding about 0.5 ml of trace metal grade nitric acid. Within a few days, the samples were transported to the Castaic Lake California Water Agency analytical laboratory for metals analysis.

4.2   Plants

Full convergence to the natural condition of any restored salt marsh to natural condition, if it occurs at all, will take place very gradually over several decades (e.g., Simenstad and Thom, 1996). The main structural component of the salt marsh ecosystem is the vegetation itself, and consequently the trajectory of vegetation changes through time provides the best indicator available of salt marsh ecosystem development. We have devised simple measures of plant performance that we will employ throughout long-term monitoring of the sewage ponds pilot restoration experiment, of the full sewage ponds restoration project to follow, and also of several restored and reference marshes scattered throughout the Naval Air Station Point Mugu. This section outlines the methods for performing these various measurements.

4.2.1 Percent Cover

The percent cover protocol estimates the fraction of the ground area covered by each plant species. A portable 0.5 m x 0.5 m acrylic laser board supported by four independently adjustable legs was positioned parallel to the substratum over the center of each subplot to be sampled. For each subplot, 49 evenly distributed points in a 7 x 7 grid were selected from the array of 625 holes on the laser sampling board. A laser pointer was then inserted into each of the 49 holes such that the laser beam pointed down in a direction perpendicular to the substratum. The identity of the plant or other surface contacted first by the laser beam was recorded. This uniform sampling method virtually eliminates human bias and provides very accurate samples of complex plant canopies.

For each plant species, foliage at the laser beam contact point was classified into one of three categories. "Green" foliage is healthy, photosynthetic, green tissue. "Brown" foliage is alive

but not photosynthetic; this category includes the basal portions of most living stems. "Dead" foliage is nonliving tissue, including *Salicornia* seed pods; dead stems are often identified by breaking them apart and observing the total absence of flexible green tissue in the stem interior. If the laser beam did not contact plant tissue, the identity of the material it did contact was recorded. We recognized three non-plant materials. "Algae" is green and photosynthetic *Ulva* or *Enteromorpha*. "Debris" is organic but not living, or it is man-made. Wrack or other debris on top of plants was recorded as such rather than being moved. "Bare ground" is the soil surface.

In addition to the plants we transplanted into experimental plots, additional plants germinated from naturally dispersed seeds began to appear in the pilot restoration site after the first few months of tidal circulation. Through the fall 1999 sample (but not thereafter), we were able to distinguish these invaders from the experimental plants. If the laser beam first contacted an invader, its identity was noted, but then the invader was moved aside to allow identification of the experimental plant or other material that the laser beam then contacted first. Only the latter datum was employed in evaluating experimental results.

While measuring plant cover, we qualitatively evaluated flowering condition of the experimental plants by placing them into one of four flowering categories: "none" when no flowers were present, "few" when 1-10 flowers were present, "some" when either 10-20 flowers were present or more than 20 flowers were present but highly localized beneath the laser board, and "many" when more than 20 flowers were present and not highly localized.

4.2.2 Plant Heights

The plant height protocol quantifies the vertical extent of the target plant species. We employed two different measures of plant height. To obtain both, the laser board was placed over the center of the subplot to be sampled. The first measure, maximum height, is the vertical height of the tallest individual beneath the laser board. The second measure, mean height, was the average distance from the ground of the plant part contacted first by the laser beam at each of three haphazardly chosen grid points. These points were always chosen far from each other. They always fell on different plants when individuals were clearly distinguishable, and they probably usually did so even when neighbors intertwined so much that individuals could no longer be distinguished.

In measuring plant heights, we considered only laser beam contacts with green or brown plant tissue; we ignored contacts with dead plant tissue. All plant heights were measured to the nearest centimeter. A height of "0 cm" was recorded if the laser beam fell on a horizontal runner less than 0.5 cm tall. If the tallest individual of an experimental species was located outside the plot region covered by the laser board, then the height of that plant was recorded, and the plant was identified as an "edge" plant. Also, the tallest plant under the laser board was recorded and the plant was identified as "in". Edge plants were used in height measurements only if fewer than three individuals occurred beneath the laser board.

4.2.3 Plant Quality Assessment

Individual survivorship and subjective judgement of the condition of experimental plants were recorded for all plots in the pilot restoration experiment. The number of experimental plants of each species alive at the time of the census was recorded for each subplot within each plot. Individual plants were then classified into five qualitative categories: 0 (dead), 1 (mostly brown, nearly dead), 2 (some green tissue present), 3 (some green tissue and recent growth present), 4 (green tissue and vigorous growth present).

4.2.4 Seed Bank Development and Plant Recruitment

Seed bank development and plant recruitment were examined at several sampling stations established between January and April 1999. These sampling stations were spaced 20.5 m apart from one another along the length of Restoration Creek. Low elevation (2.14 feet MSL $\pm$ 0.14 feet SD) stations were established on the marsh plain 1 m from the creek bank edge and below sludge plot rows 5 and 6. High elevation (2.84 $\pm$ 0.25 feet MSL) stations were located as high as possible on the restoration marsh plain, above competition plot rows 0 and 11 and approximately 3 m from the restoration plain edge. Wrackline elevation (3.16 $\pm$ 0.16 feet MSL) sampling stations were established just onto the pond berm from the restoration plain edge. Stations at each elevation were placed on both the north and south banks of the marsh plain (Fig 2.3).

For comparison, we located similar sampling stations in natural marsh near Creek A just to the south of the pilot restoration site. These stations were placed at low (0.64 $\pm$ 0.21 feet MSL), high (1.91 $\pm$ 0.20 feet MSL), and wrackline (2.37 $\pm$ 0.19 feet MSL) elevations, although the greater variation in natural creekside topography made locating all three elevations at equal distances from the creek impossible. Long-creek sampling station spacing was also more variable, ranging between 40 m and 120 m between stations. The site itself contains healthy vegetation and appears indistinguishable from creekside habitat throughout the rest of the central basin.

Each sampling station consists of five immediately adjacent 0.25 m^2 quadrats arranged in a line parallel to the tidal creek. Within each array, two plots called coring plots were devoted to seed bank examination, and three plots called cover plots were examined in place for the presence of new plant recruits. Within each cover plot, we estimated percent cover by eye (*i.e*., without using the laser board) of each plant species, algae, wrack, and bare ground. Also, we counted the number of recruits of each plant species present there when these were not obscured by overlapping plants or wrack.

From each coring plot, we collected four randomly placed soil cores (diameter = 10 cm, depth ~ 5 cm). The position of each was recorded to avoid coring any area more than once during the recruitment study. All holes created by removing cores were filled with soil collected nearby. Each soil core was individually potted with steam-sterilized soil (Supersoil, Rod McLellan Co.) in nursery pots and then transported to a UCLA greenhouse for incubation. Core orientation and integrity were maintained throughout the entire collection and incubation process.
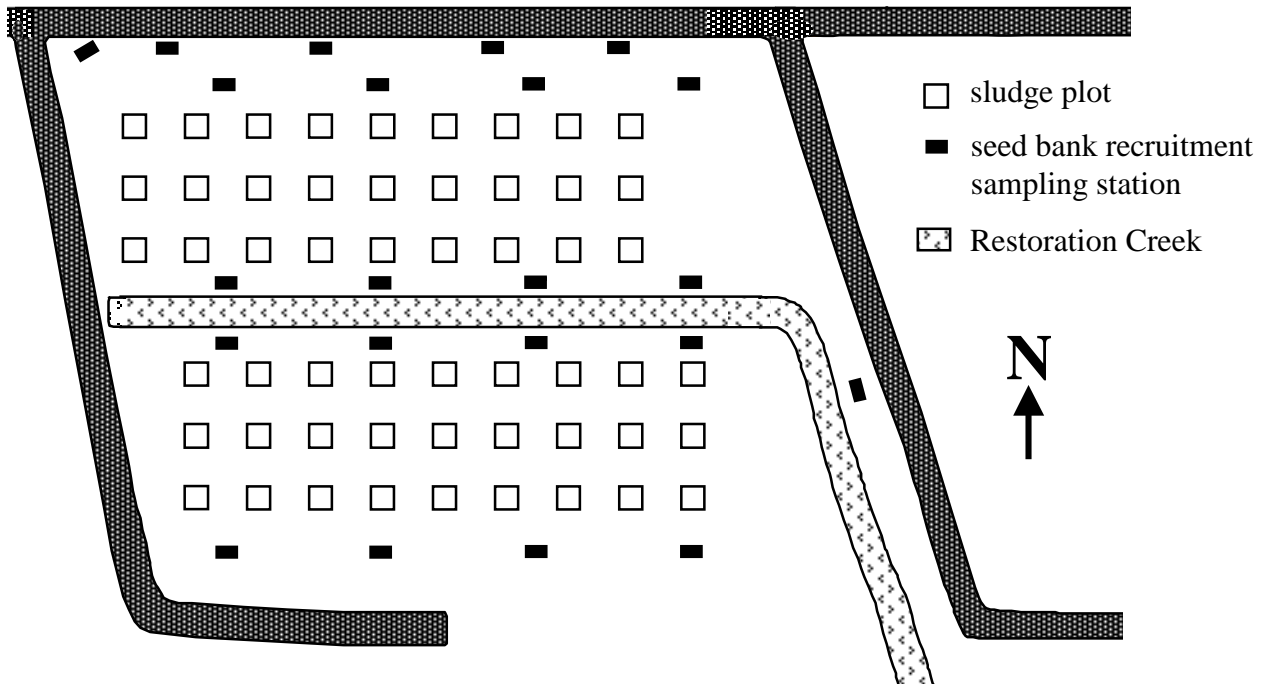
Figure 2.3. Pilot restoration seed bank and recruitment sampling stations. Each station consists of five adjacent square $0.25m^2$ plots. The central and both end plots are seed bank plots. The remaining two are recruitment plots.

In the greenhouse, cores were misted with fresh water for 10 minutes (enough to saturate the soil) twice daily. Germinated seedlings were counted and identified every 2 or 3 weeks for at least 3 months. While some species like *Salicornia virginica* are easily identifiable within a few days of germination, others like grasses can require up to several months of growth for proper identification. Hickman (1993), Mason (1957), and Munz (1974) were used to identify plant species, and our nomenclature follows Hickman (1993).

4.2.5 Plant Metals Uptake

Collection of plant tissue samples for heavy metals analysis did not take place during the fall 1999 sampling period. This task will be performed in 2000.

4.3 Animals

Evaluation of restoration success requires examining animals as well as plants. In the fall 1999 sample, we examined four groups of animals: fish, crabs, snails, and the animals that create siphon holes on the banks of tidal creeks.

4.3.1 Fish and Crabs

We employed two methods to estimate fish abundance, traps and seines, both of which also capture crabs. Traps were used to obtain estimates of the abundance and species composition of fish and crabs that occupy several areas of the pilot restoration site and adjacent natural wetland during high tide. Commercially available minnow traps (Gee Minnow Traps, 9 inches x 17.5 inches with a mesh size of ¼ inch) were deployed on both the north and south banks of the pilot restoration site. Three traps were placed at high elevations (approximately 2.6 ft MSL) and three more at low elevations (approximately 2.1 ft MSL). Three more traps were placed in Restoration Creek at locations aligned with the traps on both banks (Fig. 2.4). Additionally, single traps were deployed in the creek just seaward of the sedimentation basin, at the junction of Creek A and Restoration Creek, and at a point midway between these. Traps were baited with standardized amounts of dry dog food (Kibbles and Bits) and deployed at each location just before tidal inundation and recovered just after re-emergence.

Fish collected from these traps were identified to species, and their standard lengths were measured to the nearest millimeter. Crabs incidentally caught in the traps were identified to species and sexed, and their carapace widths were measured to the nearest millimeter at their widest points.
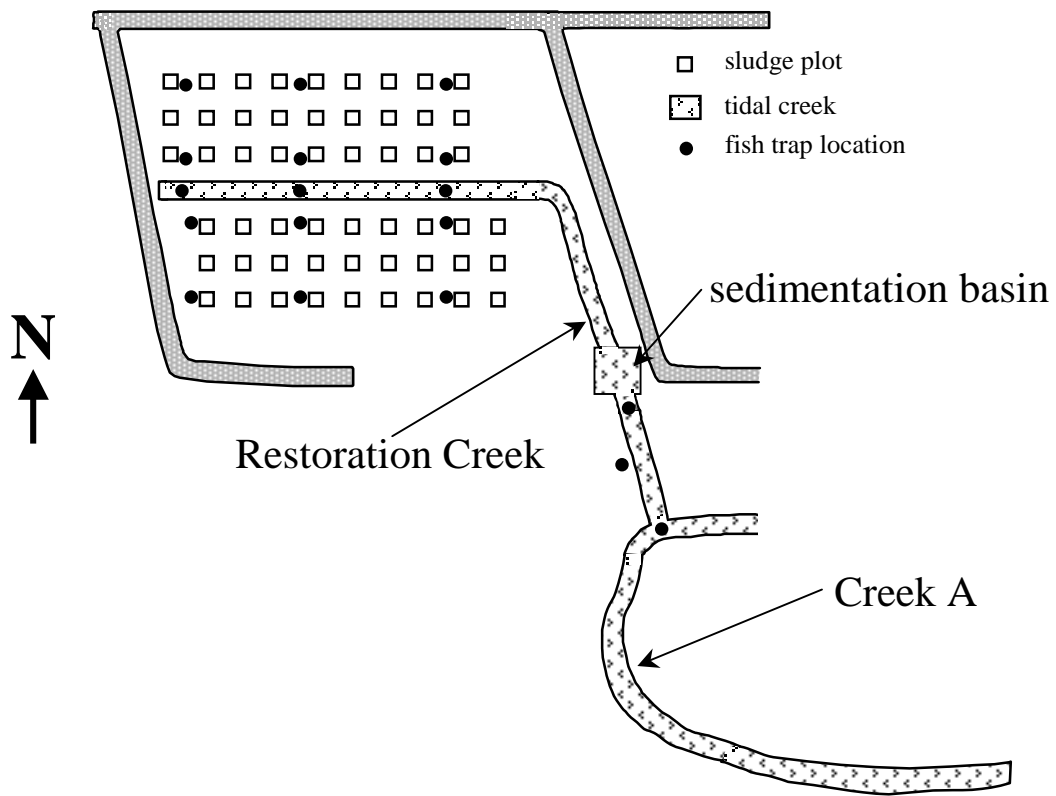
Figure 2.4. Fish trap locations.

Seine nets were used to obtain estimates of the abundance and species composition of fish and crabs that occupy Restoration Creek and natural tidal creeks. Seining took place at two sites in Restoration Creek, two sites in Creek A that drains the pilot restoration, and at four sites in Creek C that experiences no influence of sewage sludge (Fig. 2.5).

Before seining, the 10-meter length of creek to be sampled was isolated by simultaneously placing two bottom-weighted blocking nets across the creek at the sampling site's upstream and downstream ends. These blocking nets were put in place on a falling tide just as the water level dropped below the upper edges of the creek bank so that all fish were confined to the tidal creek itself. With the blocking nets in place, two people pulled a 10-meter seine through the stream from one creek bank to the other, being careful to draw the ends of the seine along the upstream and downstream blocking nets. After each sweep of the seine, all fish and crabs caught were collected for identification and measurement. Fish were identified to species, and their standard lengths were measured to the nearest millimeter. Crabs were identified to species and sexed, and their widest carapace widths were measured to the nearest millimeter. Seining continued until the seine collected no more fish or crabs. Finally, the downstream blocking net was pulled along the creek until it reached the upstream blocking net and then both nets were removed from the creek containing any fish or crabs not captured by the seine.

Some fish and crabs collected during seining were preserved in acid-washed glassware for future heavy metals analysis. For the common species *Fundulus parvipinnus, Gillichthys mirabilis, and Clevelandia ios*, only the first 100 specimens collected were preserved.

4.3.2 Snails

Cerithidea californica snails were sampled along the banks of three tidal creeks, Restoration Creek, Creek A, and the tidal creek that drains Mugu Lagoon's L Avenue Restoration (Fig. 2.6). Four sampling stations were established at various intervals between 10 m to 30 m apart along each tidal creek. In the spring 1999 sample, transects began at the edge of the vegetation canopy adjacent to the creek bank and ran contiguously to the creek center. Beginning in fall 1999, each sampling station consisted of three transects oriented perpendicular to the creek's axis, one 3 m upstream from the station's permanent marker stake, a second right at the marker stake, and a third 3 m downstream from the stake. The sampling units were 50 cm x 10 cm quadrats placed contiguously along transects extending from 1.5 m above the creek edge down to the creek's center (Fig 2.7).

All living snails (only *Cerithidea californica* in spring 1999; *Cerithidea californica*, *Melampus olivaceus*, and *Acteocina inculta* beginning in fall 1999) within each quadrat were counted, and, for the central transect only, their lengths were measured to the nearest 5 mm. We also recorded subjective estimates of percent cover of algae and plants and the slope of the substratum surface within each quadrat. The first 100 *Cerithidea* individuals in the 20-25 mm size class encountered were collected for parasite analysis.
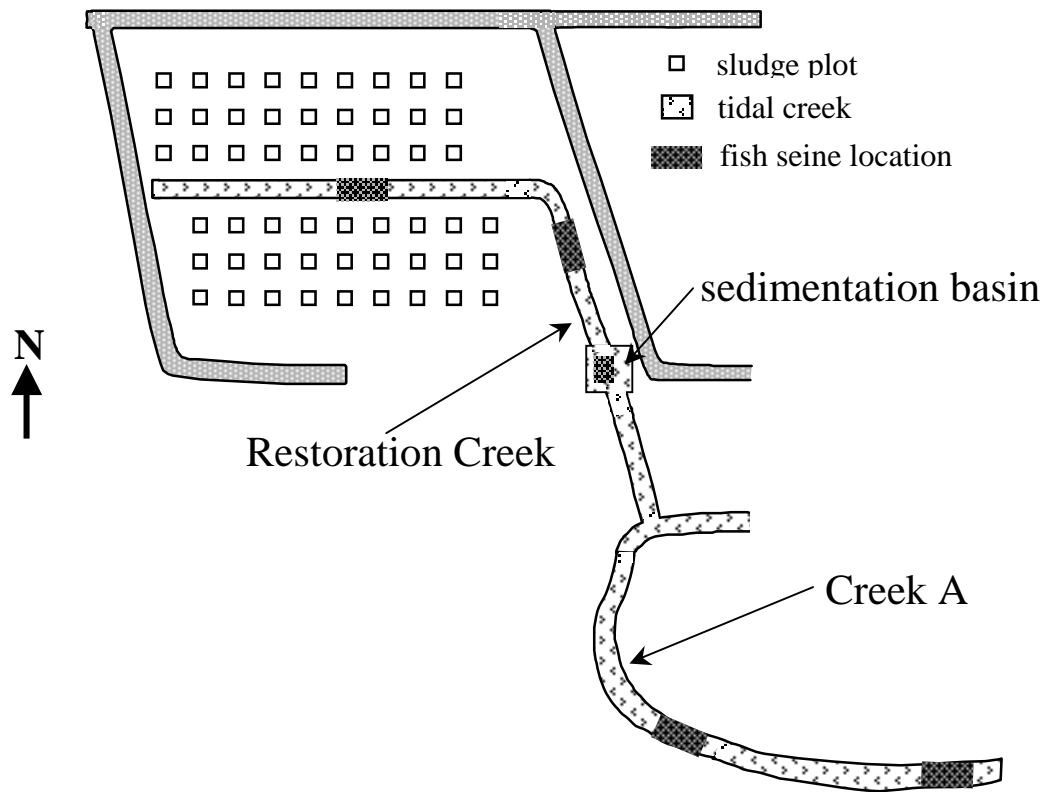
Figure 2.5. Fish seine locations. Creek C seine locations lie approximately 1 km to the west of this diagram and are not shown.

sedimentation basin

Creek A

Restoration Creek

N

sludge plot
tidal creek
Cerithidea and si-
phon hole sites

□
▨
●
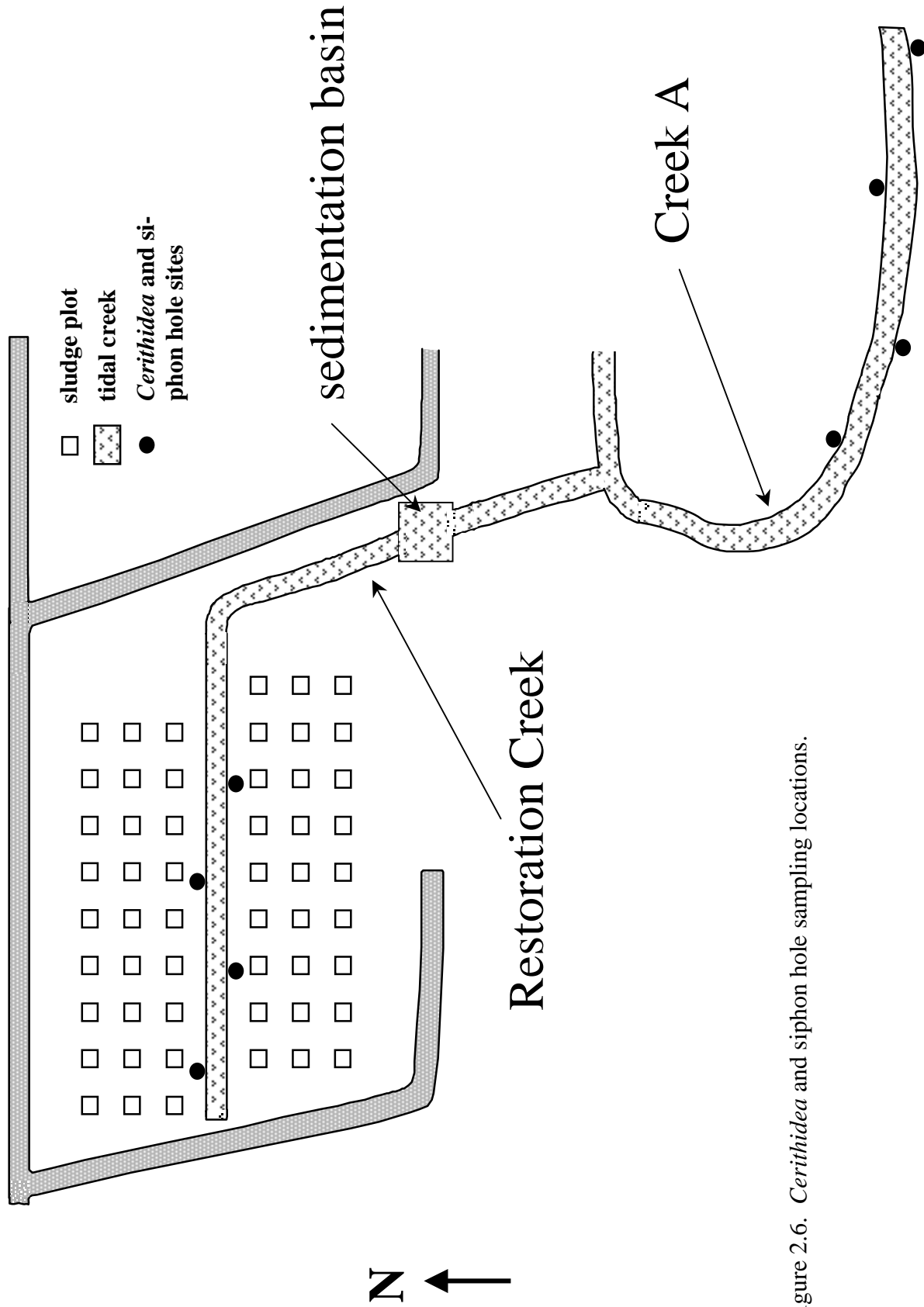
Figure 2.6. *Cerithidea* and siphon hole sampling locations.
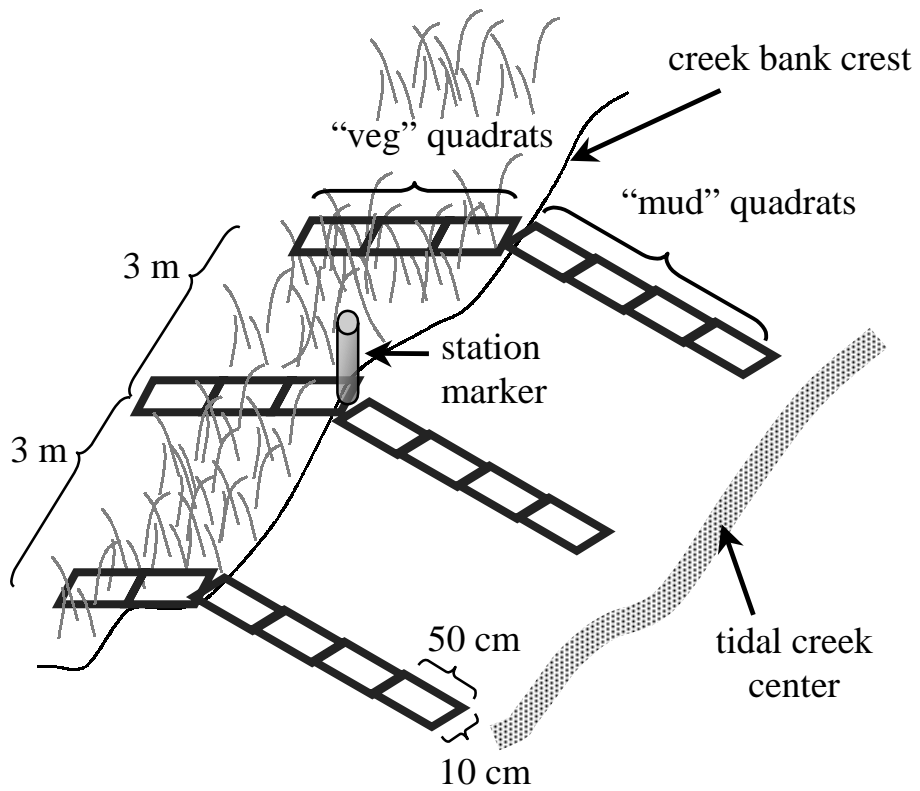
Figure 2.7. A *Cerithidea* sampling station.

Parasite analysis was performed within days of collection by Todd Huspeni of the University of California at Santa Barbara. Snails were re-measured to nearest millimeter, cracked open, and examined for trematode parasites following well established protocols (Huspeni, *et al*. in prep). Abundance of each trematode species was recorded for each snail.

4.3.3 Siphon Holes

Numerous circular holes ranging from 2 or 3 mm up to 30mm in diameter occur in the mud of tidal creek banks in Mugu Lagoon. We suspect that these are siphon holes excavated by clams and polychaetes, but we have not yet attempted to identify the animals involved. However, since very little extra effort was required, we counted and measured siphon holes while sampling snails in these same habitats. Sampling was conducted along the same three band transects at each of the four sampling stations at Restoration Creek and Creek A and at a single station at each of two sites (C34 and C37). This time the sampling units were contiguous 50 cm x 50 cm quadrats within which we counted the number of siphon holes in several size classes (<4, 5-9, 10-19, 20-29, 30-39, and >40 mm diameter).

4.3.4 Metals Bioaccumulation

The fall 1999 sample included collection of fish, crabs, and snails for heavy metals analysis. However, at this writing, these samples remain in frozen storage. Their chemical analysis will take place along with analysis of more recently collected material in 2000.

Chapter 3:
Results

During 1999, we conducted two full samples of the pilot restoration experiment, one in spring and one in fall. Unfortunately, full analysis of both samples proved beyond the capacity of our present workforce to complete in the available time. Consequently, we elected to examine, in addition to some physical data collected continuously over several months, the data from the fall 1999 sample only. As these data were collected one full year after planting, they provide the best indication available during 1999 of the outcome of the pilot restoration experiment. This chapter presents these data and their associated statistical analysis.

The major sections of this chapter describe results concerning the physical environment, the plants that live there, and the animals that live with them. We present no chemical data at this time, as only a small fraction of the analysis has actually been completed to date. The 2000 Progress Report will treat this subject extensively.

4.1 Physical Environment

4.1.1 Elevation and Sedimentation

The Pilot Restoration site subsided somewhat shortly after the onset of tidal exchange. Elevation measurements were made on October 29, 1998 prior to initial flooding on November 2 and then again on November 24, 1998. Between these dates, the northern plain of the restoration sank an average of 6.6 cm, the restoration creek banks by 6.4 cm, and the restoration creek center by 7.9 cm. This elevational decrease was almost entirely reversed, however, by the end of the first year. By November 11, 1999, the site had returned almost to its elevation before flooding. At that time, the northern plain lay only 2.3 cm below its pre-flooding elevation, creek banks 0.8 cm below, and the creek center just 1.9 cm below its pre-flooding elevation. These results show that it is possible, even with complications introduced by flooding, to achieve excellent control of elevation in salt marsh restoration.

The only elevational feature of potential concern is the creek depth. To ensure adequate seawater flow to the entire restoration site, we exercised great care during site construction to make sure that the creek was deep enough. Although the creek does flood the marsh plain in exactly the desired way, it has turned out to be slightly too deep. The creek actually retains some standing water at low tide, thereby creating somewhat unnatural ecological conditions there.

The first sedimentation data from the feldspar plots will be collected in fall, 2000. Visual inspection reveals, though, that at the time of this writing the feldspar plots are covered by at least a thin layer of sediment.

4.1.2 Inundation, Local Tide Correction, and Restoration Creek Temperature

Interpretation of tidal inundation times from recorded temperature data has proved challenging. Among other complications, many tidal inundations occurred when air and creek temperatures did not differ measurably, thereby obscuring transitions from air to water or water to air.

Nevertheless, the temperature data did reveal a consistent time lag in incoming tides. Elevation-calibrated incoming tides in the restoration site were delayed by an average of 44 minutes, with a standard deviation of only 6.9 minutes, after incoming tides at the mouth of Mugu Lagoon (sample size = 8 incoming tides). Outgoing tides proved much more variable. The average delay of 41.5 minutes for outgoing tides essentially matched the lag time of incoming tides. The variability was so high, with a standard deviation of 55.7 minutes and a coefficient of variation of 134% (sample size = 6 outgoing tides), however, that the time lag of any particular outgoing tide cannot be predicted with much confidence. This qualitative difference in predictability of incoming and outgoing tides appears common in tidal wetlands. Many factors such as quadratic bottom friction, limited tidal amplitude, and variable wind direction can all diminish the predictability of outgoing tides (R. Luettich, University of North Carolina, Chapel Hill *pers. com.*).

To determine the best times for fieldwork, we calculated tidal correction factors for the onset of tidal flooding and ebbing at the pilot restoration site (Table 3.1).

Restoration Creek temperature varied from November 1999 through March 2000, of course. While the daily temperature range differed somewhat from month to month, maximum and minimum temperatures (Table 3.2) occurred at consistent times of day (Fig. 3.1).

4.2 Plants

4.2.1 Sludge Enrichment Experiment

Statistical analysis to appear shortly employed several conventions motivated by the nature of the data. Each plant species was analyzed separately. Whenever an interaction or a covariate proved strongly nonsignificant (P > 0.1), that element was removed from the statistical model, and then the calculations were repeated. In these cases, reported results concern the reduced statistical model. Three metrics evaluate plant performance in single-species plots: percent cover, mean canopy height, and maximum canopy height. Graphs of mean canopy height employ a single value for each subplot, the calculated average of measured heights at three haphazard locations, in calculating means and variances for each soil treatment. To achieve greatest statistical power, however, statistical analysis of canopy height employs the three individual measurements from each subplot. All graphs of percent cover display untransformed data, while associated tests employ the arcsine transformation to achieve normality. Because plot elevations did not remain constant within any row of the plot array, statistical tests employed plot elevation as a covariate rather than row membership as a categorical factor.

Table 3.1. Mugu Lagoon pilot restoration site tidal corrections. These factors correct the widely available Los Angeles Harbor tide chart data. Calculations are based on the southern bank of Restoration Creek (1.78 feet MSL). Mean ± 1 standard deviation appear in the units reported in most tide charts (MLLW).

	Time lag between LA Harbor and the Mugu pilot restoration site	LA Harbor tidal height when tide begins to flood or ebb at the Mugu pilot restoration site
Incoming Tide	47 ± 6.9 minutes	5.37 ± 0.25 feet
Outgoing Tide	52.5 ± 55.7 minutes	2.44 ± 1.8 feet

Table 3.2. Restoration Creek temperature extremes. Fig. 3.1 gives sample sizes.

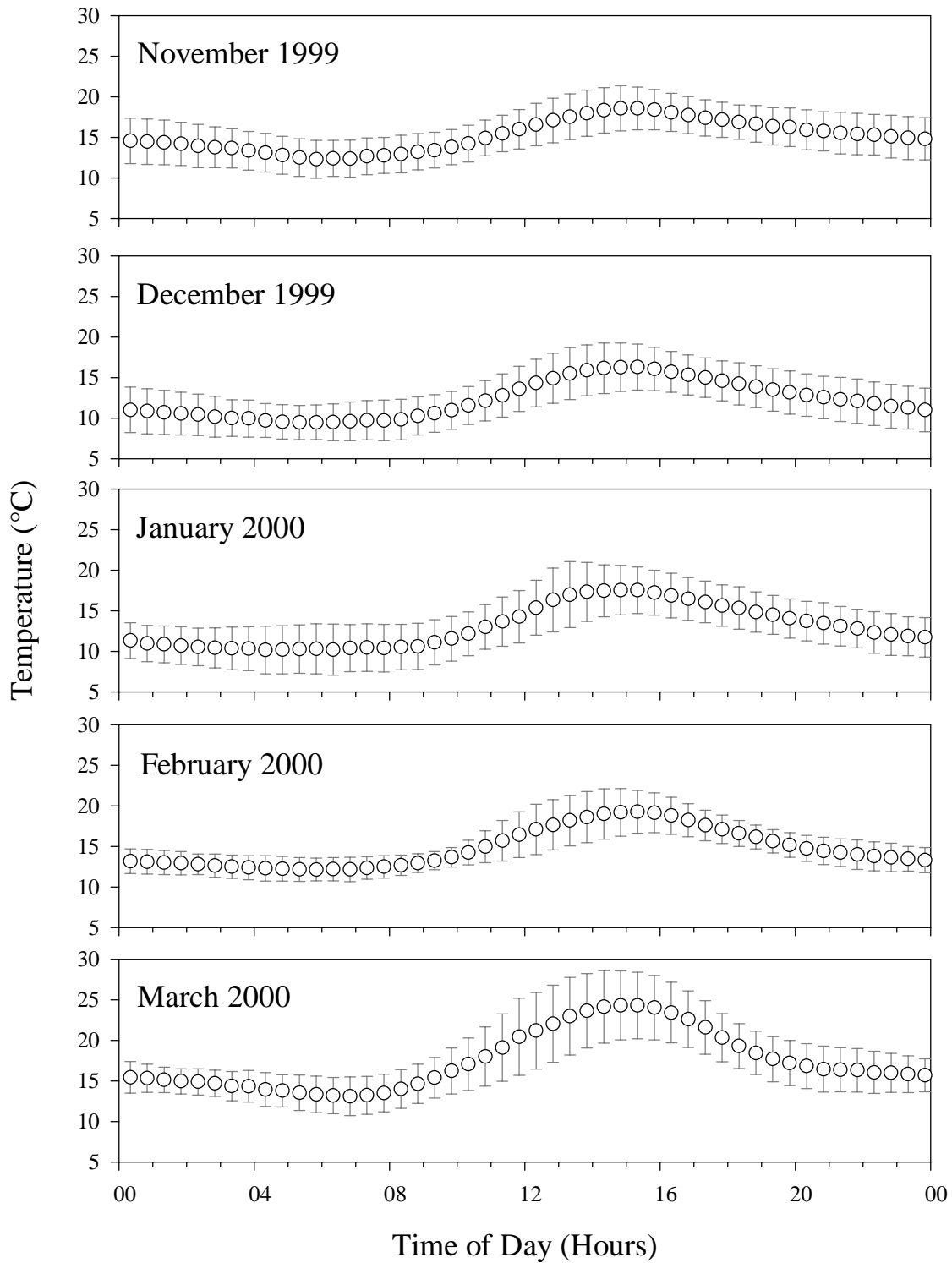|	Monthly Maximum (ºC)	Monthly Minimum (ºC)
November 1999	23.40	5.93
December 1999	21.22	4.37
January 2000	27.23	4.06
February 2000	26.00	8.42
March 2000	29.97	6.72

Figure 3.1. Restoration Creek water temperatures. Mean temperature ± 1 standard deviation was calculated for each 10-minute interval of the day. Sample size is the number of days of the month except November with a sample size of 26 and January with 21.

Sludge Concentration Effect

Sludge concentration exerted little or no effect on plant performance. Plant percent cover (Fig. 3.2) was not significantly influenced by sludge concentration in *Salicornia*, *Frankenia*, or *Monanthochloe*. In fact, percent cover rarely differed by more than 20% over the entire range of sludge concentrations in either soil matrix. Mean plant height (Fig. 3.3) also was not significantly influenced by sludge concentration in either *Salicornia* or *Monanthochloe*. Sludge concentration did exert a significant effect on *Frankenia* mean height when growing in clay-containing mixtures (ANCOVA: $F_{3,56} = 3.025$, $p = 0.037$), but only because the 0% and 75% sludge concentrations differed; even with this species, mean plant height did not display a monotonic trend with increasing sludge concentration. Maximum plant height (not displayed graphically) was not significantly influenced by sludge concentration in any of these three species. These results corroborate the nonexistence of a sludge concentration effect on plant performance found in our earlier potted plant experiment (Vance *et al.*, in press).

We note here, just as in the potted plant experiment, that while plant growth did not increase with sludge concentration, it also displayed no decreasing trend. That is, growing in artificial sludge-containing mixtures evidently causes no harm to the three plant species tested, including the two most abundant salt marsh plant species at Mugu Lagoon.

Soil Matrix and Plot Elevation Effects

The simplest test of soil matrix effects on plant performance compares soils containing no sewage sludge, i.e. pure upland soil, pure clay, and pure wetland soil. In these pure soils, plants tended to perform better in clay than in either upland or wetland soil (Fig. 3.4). Soil type significantly affected *Frankenia* maximum height (ANCOVA: $F_{2,12} = 4.301$, $p = 0.039$), and it nearly significantly affected *Frankenia* percent cover (ANCOVA: $F_{2,14} = 3.646$, $p = 0.053$) and *Salicornia* canopy height (ANCOVA: $F_{2,50} = 2.720$, $p = 0.076$). Few other comparisons achieved or closely approached statistical significance, however, primarily because of our low replication (only 6 replicates per soil type).

Somewhat more complicated but also more powerful tests (due to more degrees of freedom) compare plant performance in all soils containing upland soil or clay matrix, both in pure form and mixed with sewage sludge. Most of these comparisons revealed significantly better plant performance in clay or clay-containing mixtures. In *Salicornia*, both percent cover (ANCOVA: $F_{1,39} = 7.571$, $p = 0.009$) and mean height (ANCOVA: $F_{1,135} = 5.007$, $p = 0.027$) were significantly greater in clay, and maximum height (ANCOVA: $F_{1,39} = 3.355$, $p = 0.075$) was nearly so. In *Frankenia*, all three plant performance measures were significantly greater in clay (percent cover ANCOVA: $F_{1,40} = 6.370$, $p = 0.016$; mean height ANCOVA: $F_{1,107} = 16.802$, $p < 0.001$; maximum height ANCOVA: $F_{1,30} = 7.314$, $p = 0.011$). Finally, *Monanthochloe* achieved significantly higher percent cover in clay (ANCOVA: $1,40 = 7.480$, $p = 0.009$).

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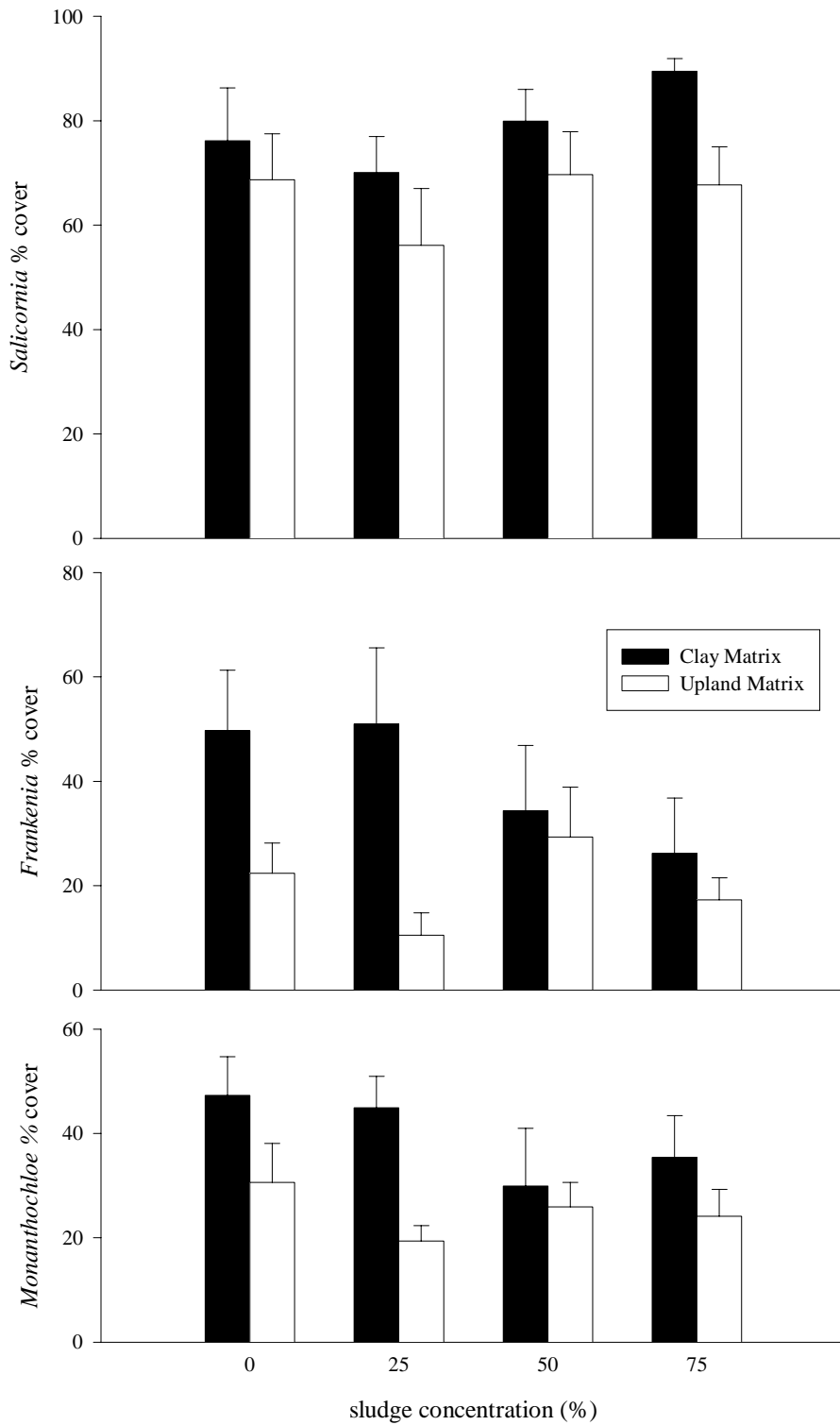
Figure 3.2. Mean cover in fall 1999 of plants growing in soils with different sludge concentrations. Error bars represent 1 standard error, with n = 6 for each bar.
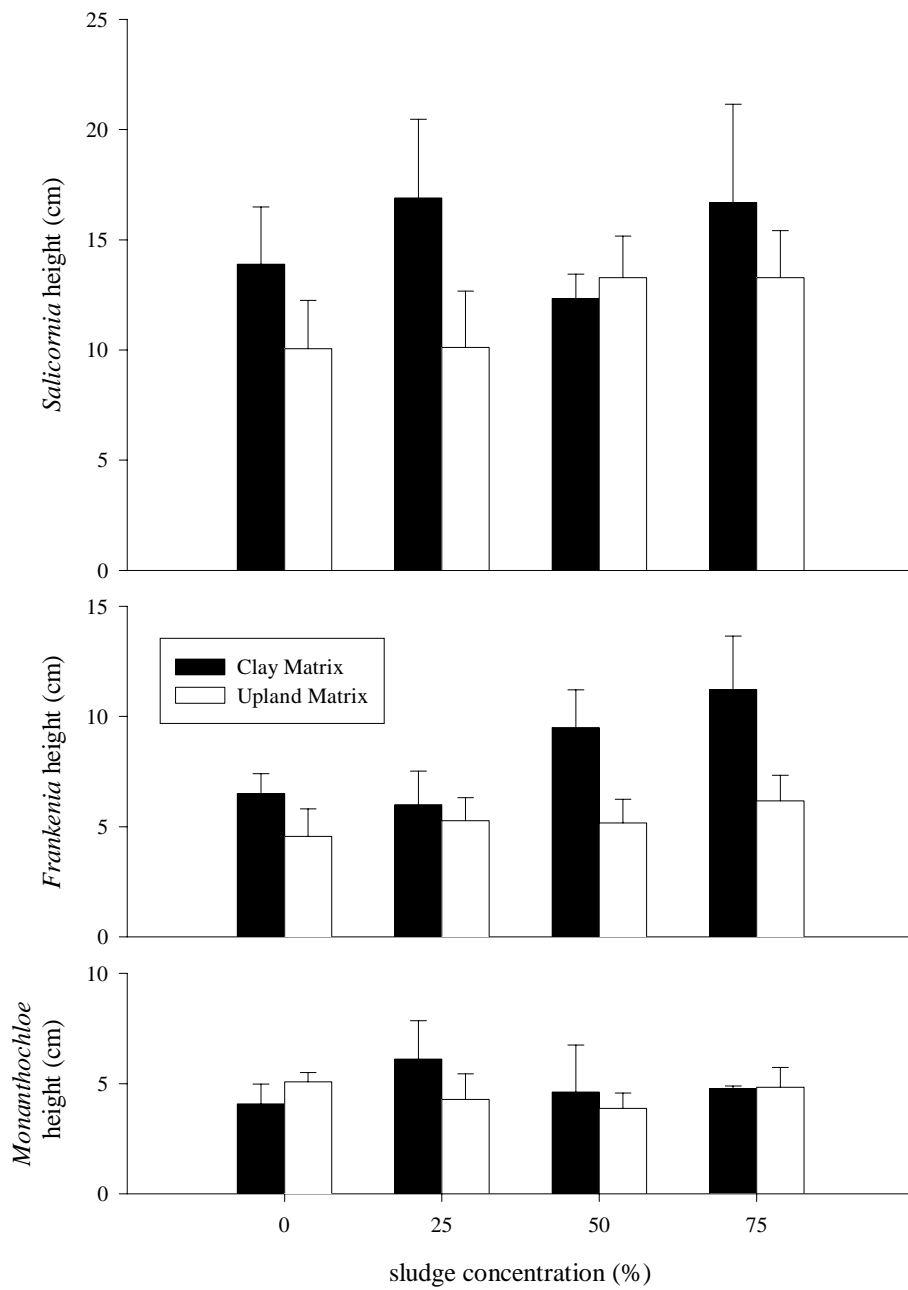
Figure 3.3. Mean height in fall 1999 of plants growing in soils with different sludge concentrations. Error bars represent 1 standard error, with n = 6 for each bar.

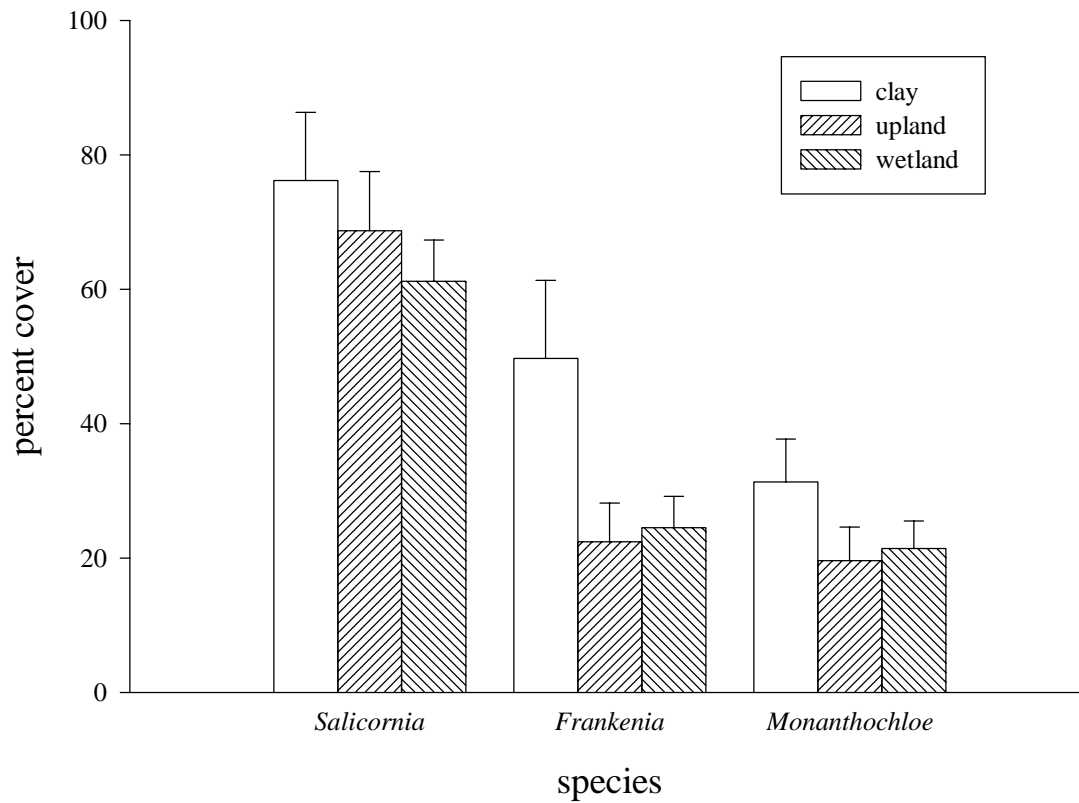Figure 3.4. Effect of pure soils on mean percent cover in fall 1999 of plants growing in single-species subplots. Error bars represent 1 standard error. Sample size is 6 for each bar. Within each species, with this sample size no effect proved statistically significant in these pure soil comparisons.

Fig. 3.5 displays effects of both soil matrix material and elevation on plant cover at the same time. Analysis of covariance reveals that plant cover significantly increases with elevation in *Salicornia* ($F_{1,45} = 8.879$, $p = 0.005$) but not in the other species. The same analysis establishes that plant cover in clay matrix significantly exceeds plant cover in upland soil matrix for all three species ($F_{1,45} = 7.806$ and $p = 0.008$ for *Salicornia*, $F_{1,45} = 6.570$ and $p = 0.014$ for *Frankenia*, and $F_{1,45} = 7.315$ and $p = 0.010$ for *Monanthochloe*). This statistical analysis employed arcsine transformed cover data, but the straight lines shown for simplicity in Fig. 3.5 did not. Plant cover data in the six natural wetland soil plots, though not examined statistically, appear in Fig. 3.5 for visual comparison.

Our qualitative field observations suggest that size differences between plants growing in clay-containing mixtures and upland soil-containing mixtures are becoming more pronounced as the total growing time for the plants increases. This tendency of better performance in clay seems likely to become increasingly distinct as the plants continue to grow.

4.2.2 Nutrient and Organic Enrichment Experiments

The pilot restoration experiment included plots enriched with nutrients and plots enriched with organic matter in order to compare the effects on plant growth of sewage sludge with effects of these more standard soil amendments. As described in Section 2.1.2, spatial constraints in the pilot restoration site prevented a completely balanced statistical design. All nutrient enrichment and most organic enrichment plots lay at the lowest elevation locations in the plot array adjacent to Restoration Creek. Sludge-containing plots, by contrast, differed in both sludge concentration and elevation. Without virtually eliminating statistical replication, it is not possible in statistical tests to hold both sludge concentration and also elevation constant at the same time. Accordingly, the statistical tests to follow hold just one of these factors constant at a time and thereby examine two somewhat different sets of comparisons.

The first set of comparisons concerns sludge plots with sludge concentration held constant at 75%, the highest concentration employed in the experiment. Statistical replication is achieved by combining into a single sample plots with the same soil mixture from all elevations. This operation allows comparing nutrient enrichment and organic enrichment effects with sludge enrichment effects, albeit employing a heterogeneous sample (*i.e.*, from different elevations) of the latter. We shall display fully only the results for *Salicornia virginica*, the most common salt marsh plant species at Mugu Lagoon. For *Salicornia*, this comparison includes six treatments: (1) 75% sludge in clay matrix, (2) 75% sludge in upland soil matrix, (3) natural wetland soil, (4) nutrient enriched upland soil, (5) organically enriched upland soil, and (6) control plots containing pure, unenriched upland soil.
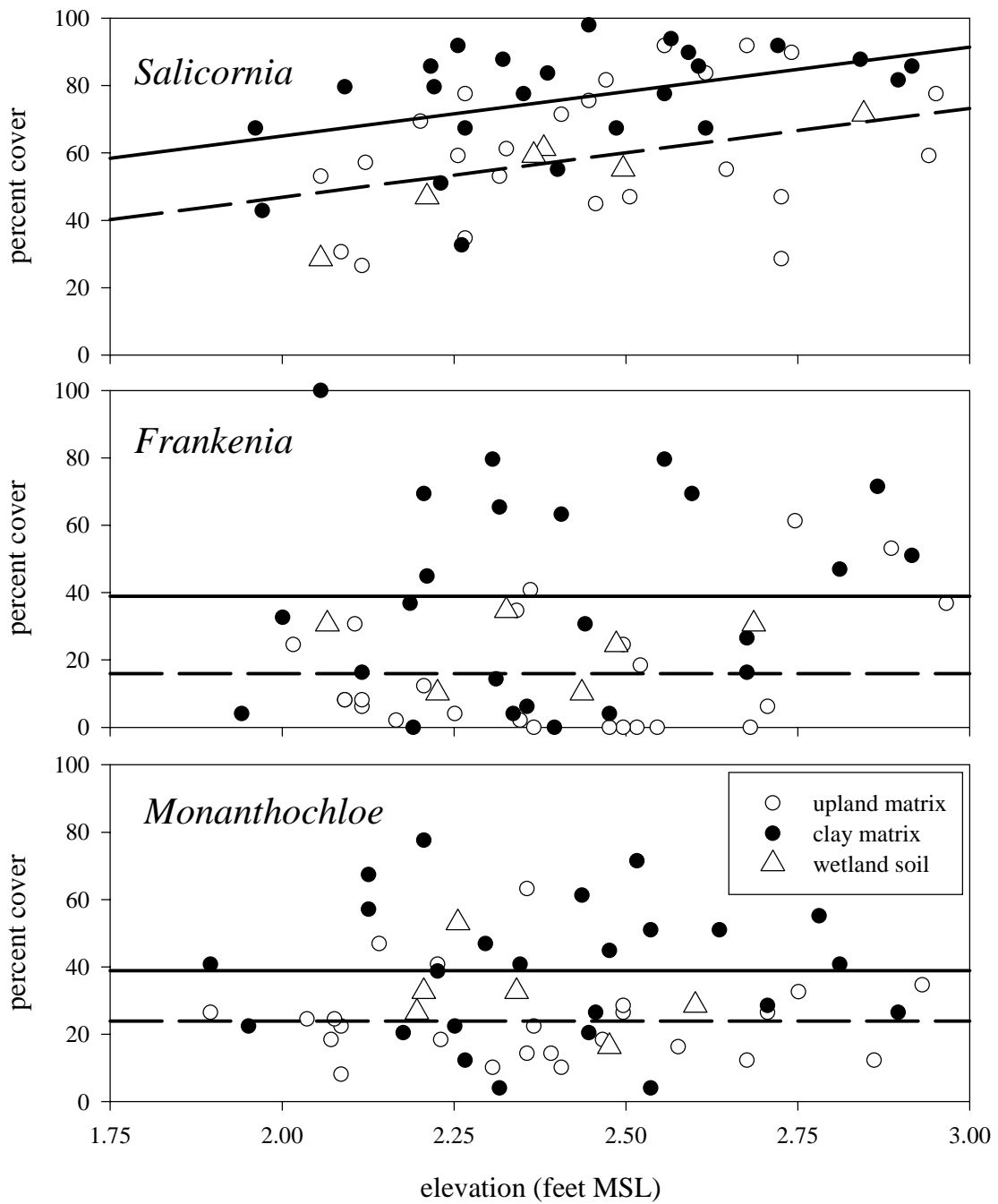
Fig 3.5. Effects of matrix material and elevation on plant cover in the sludge enrichment experiment. See text for explanation.

Table 3.3. Effects of soil type on *Salicornia* growth: constant sludge concentration (75%) but variable plot elevation. Mean height analysis employed the arcsine transformation. Significant p-values appear in bold typeface.

Factor	S of S	df	MS	F	*p*
Salicornia cover					
soil type	4664	5	933	7.071	**0.000**
error	4749	36	132		
Salicornia mean height					
soil type	424	5	85	2.590	**0.043**
error	1146	35	33		

Table 3.4. Effects of soil type on *Salicornia* growth: "constant" elevation (plot rows 5 and 6) but variable sludge concentration. Mean height analysis employed the arcsine transformation. Control plots, which lie outside rows 5 and 6, were excluded from this analysis. Significant p-values appear in bold typeface.

Factor	S of S	df	MS	F	*p*
Salicornia cover					
soil type	1614	4	404	2.508	0.061
error	5310	33	161		
Salicornia mean height					
soil type	190	4	47	1.115	0.367
error	1361	32	42		

Figure 3.6. *Salicornia virginica* percent cover in nutrient and organic enrichment treatments (rightmost two bars) compared with other treatments. The sludge enrichment and wetland soil treatments (leftmost three bars) are comprised of single sludge concentrations (75% or 0% sludge), but over a range of elevations (one plot from each of six plot rows). Error bars represent 1 standard error. Sample sizes from left to right are 6, 6, 6, 4, 10 and 10.

Figure 3.7. *Salicornia virginica* mean height in nutrient and organic enrichment treatments (rightmost two bars) compared with other treatments. Sludge concentrations, plot elevations, error bars, and sample sizes as in Fig. 3.6. Pairwise comparisons reveal no significant difference between any pair of treatments.

Greatest between-treatment differences in the entire experiment appear with *Salicornia* growth measured as percent cover (Fig. 3.6). One-way analysis of variance (Table3.3) reveals a significant treatment effect. Pairwise *a posteriori* comparisons using Tukey's Honest Significant Difference test reveal that growth in the 75% sludge in clay matrix treatment significantly exceeds growth in the organic enrichment, control, and wetland soil treatments; and growth in the nutrient enrichment treatment significantly exceeds growth in the control treatment. No other significant differences exist in this comparison. Measuring *Salicornia* growth as mean height produces a similar qualitative pattern but of smaller magnitude (Fig. 3.7). One-way analysis of variance (Table 3.3) again reveals a treatment effect, but this time *a posteriori* pairwise comparisons reveal no significant differences between any pair of treatments.

The second set of comparisons involves sludge enrichment plots with elevation held constant and roughly equal to elevation in the nutrient enrichment and most organic enrichment plots. In this comparison, statistical replication was achieved by combining into one sample all plots containing clay matrix and into another sample all plots containing upland soil matrix. This operation allows comparing nutrient enrichment and organic enrichment effects with effects of soil matrix, albeit employing a heterogeneous sample (*i.e.*, with different sludge concentrations) of the latter. Again we shall display the full results only for *Salicornia virginica*. For *Salicornia*, this comparison includes five treatments: (1) soils containing clay, (2) soils containing upland soil, (3) wetland soil, (4) nutrient enriched upland soil, and (5) organically enriched upland soil.

Neither measure reveals pronounced between-treatment plant growth differences. One-way analysis of variance (Table 3.4) of *Salicornia* percent cover (Fig. 3.8) reveals no significant treatment effect, and it follows that no *a posteriori* pairwise comparisons are significantly different. Analysis (Table 3.4) of *Salicornia* mean height (Fig. 3.9) also reveals no significant treatment effect and hence again no significantly different pairwise comparisons.

Similar tests of both kinds performed on *Frankenia grandifolia* and *Monanthochloe littoralis* revealed even fewer significant between-treatment differences than occurred in *Salicornia*, and all were small in magnitude.

Taken together, these results support an important conclusion: sludge enrichment in the pilot restoration experiment produced no less salt marsh plant growth than did the other two more widely used fertilization techniques, frequently repeated nutrient enrichment and one-time organic enrichment. That is, from the perspective of plant growth, Mugu Lagoon sewage sludge appears not to be a poorer soil amendment than either nutrients or naturally occurring organic matter.

These nutrient and organic enrichment experiments have limitations. One is low replication and the resulting low power of all statistical tests. Another is heterogeneity in the sludge mixture treatments pointed out above that increases within-treatment variance. Third, the very localized organic enrichment procedure employed here may have produced no more than a fraction of the benefit that could potentially arise from larger scale organic fertilization.
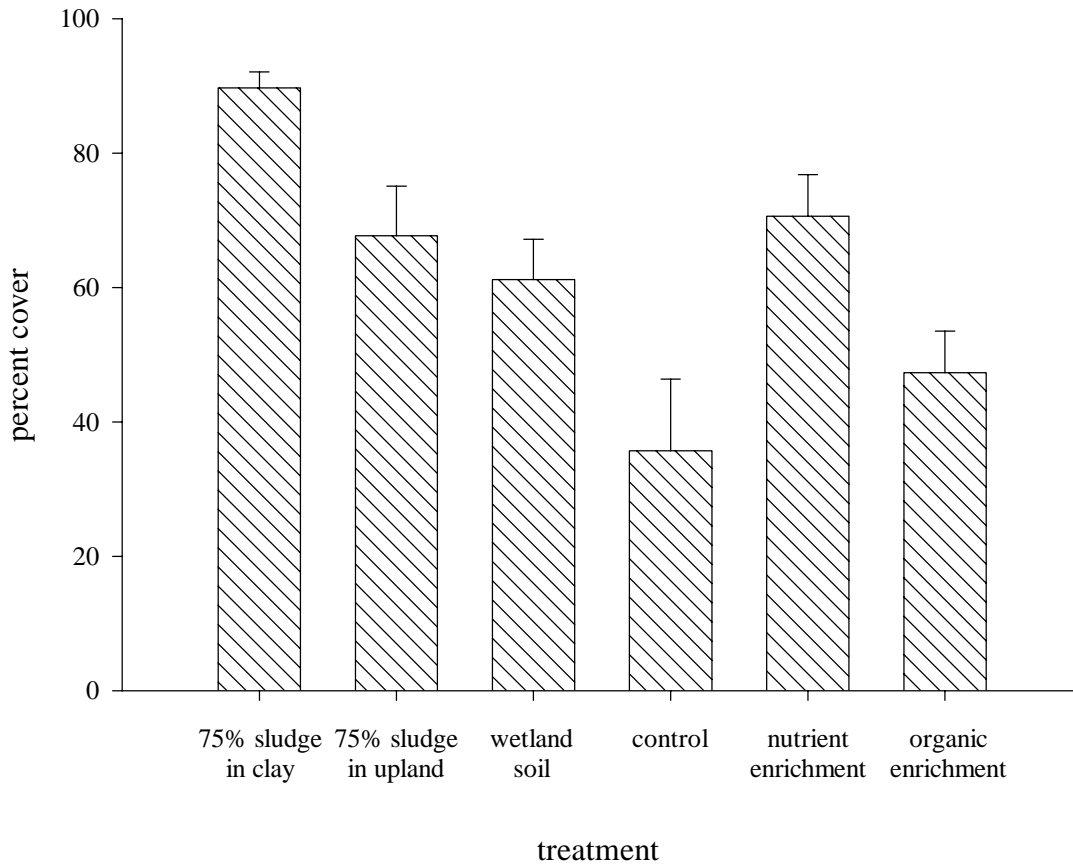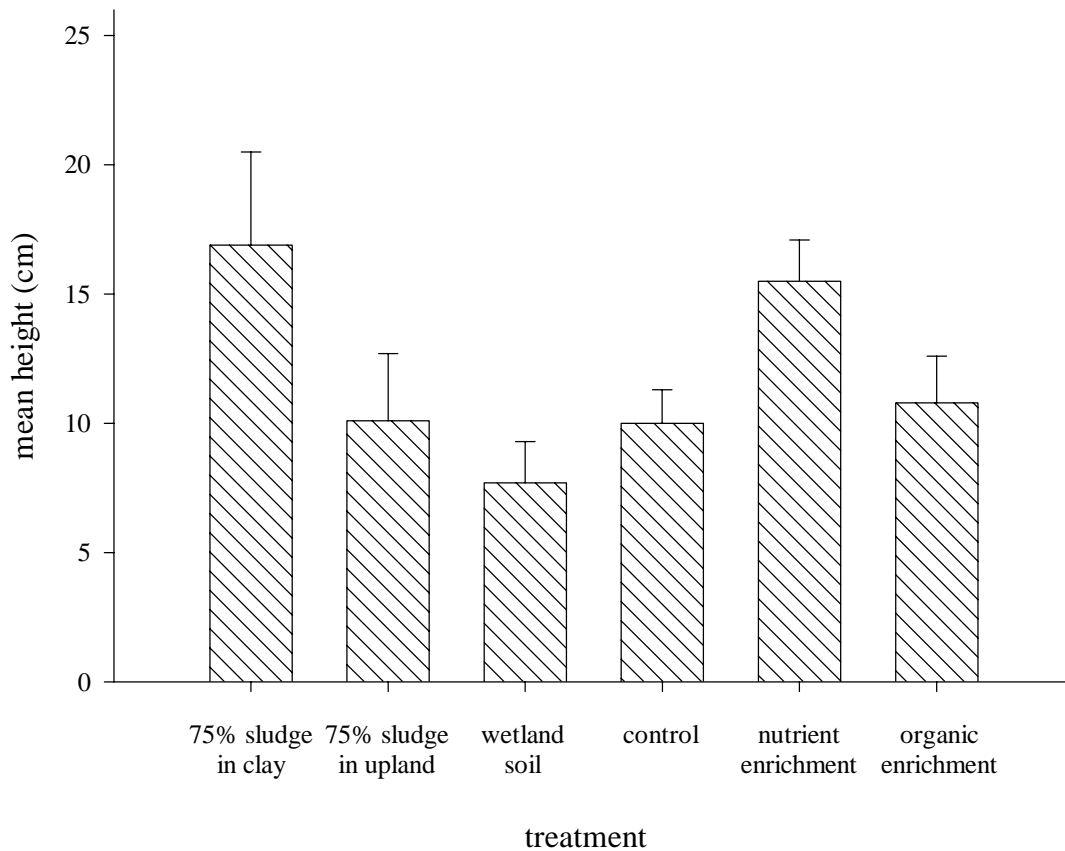
Figure 3.8. *Salicornia virginica* percent cover in nutrient and organic enrichment treatments (rightmost two bars) compared with other treatments. The sludge enrichment treatments (leftmost two bars) concern just one elevation (all plots in rows 5 and 6 which lie adjacent to Restoration Creek) but a range of sludge concentrations (0% to 75%). Error bars represent 1 standard error. Sample sizes from left to right are 8, 8, 2, 10 and 10. Pairwise comparisons reveal no significant difference between any pair of treatments.
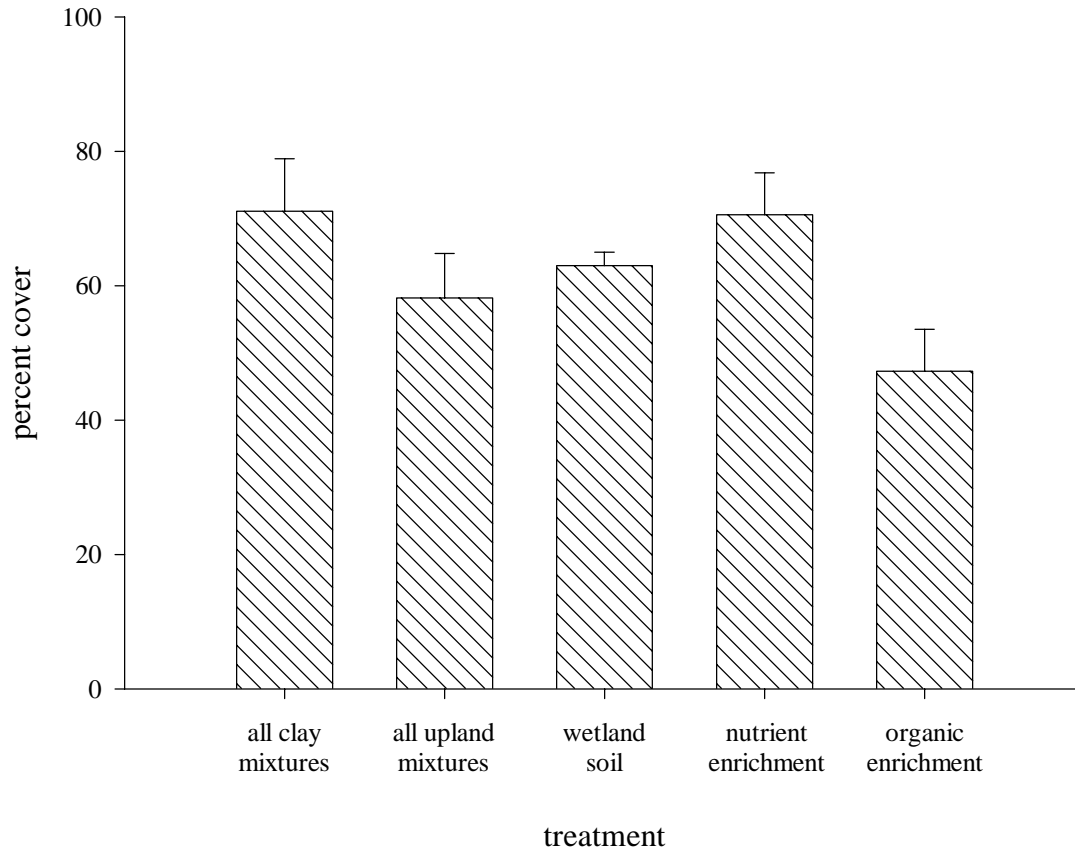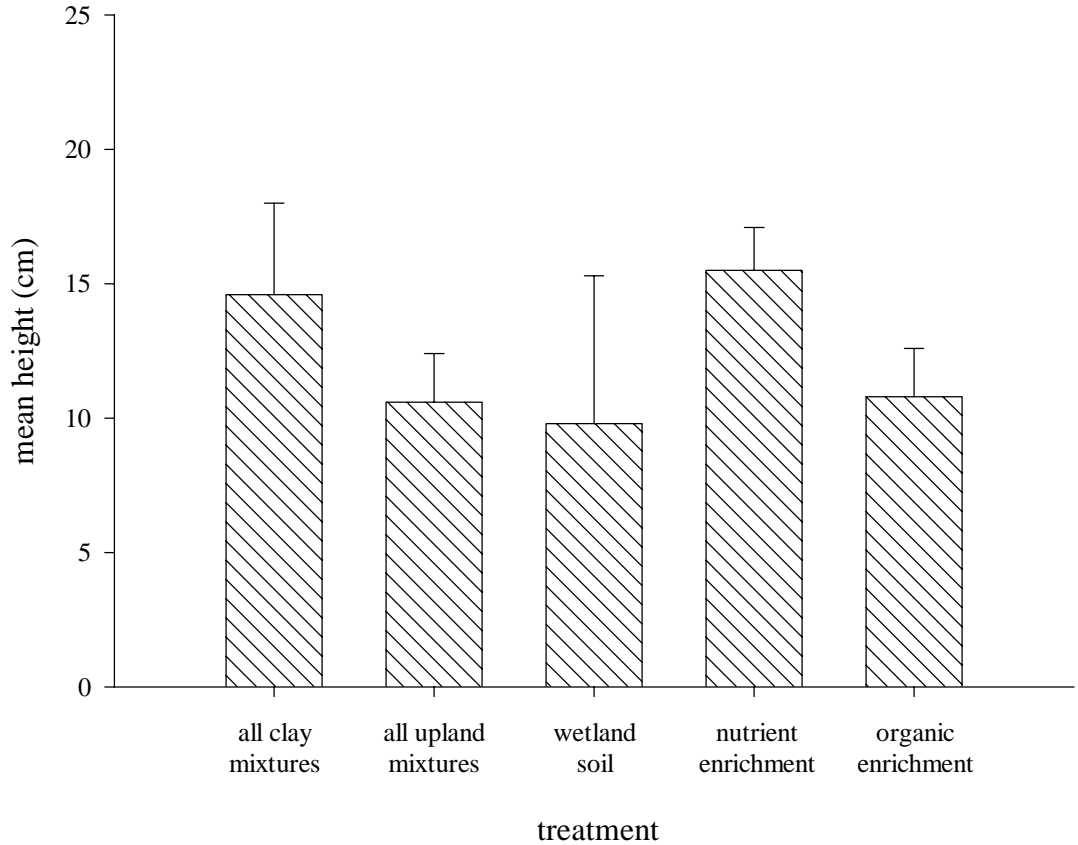
Figure 3.9. *Salicornia virginica* mean height in nutrient and organic enrichment treatments (rightmost two bars) compared with other treatments. Plot elevation, sludge concentrations, error bars, and sample sizes as in Fig. 3.8. Pairwise comparisons reveal no significant difference between any pair of treatments.

A fourth and more fundamental problem is that nutrient and organic enrichment plots differed from sludge enrichment plots in a physical way: they experienced soil compaction by earth-moving machinery during restoration site construction which the sludge enrichment plots did not. Compaction reduced soil water-holding capacity that protects small, delicate plants from desiccation. Because all nutrient enrichment and most organic enrichment plots occupied relatively well-watered low elevation sites near Restoration Creek, desiccation did not produce the same disastrous mortality that caused elimination of the competition and recruitment interaction experiments that occupied higher elevation sites. Partial desiccation might have inhibited plant growth somewhat, however, and thereby may have diminished the positive effects of nutrient and organic enrichment. If so, then the conclusion that sludge enrichment caused no poorer growth than nutrient and organic enrichment might not have arisen solely through soil amendment differences.

These caveats weaken the experimental tests described here, unfortunately, and they force the conclusions of this section to be regarded as suggestive rather than definitive.

4.2.3 Recruitment

In the 1999 samples, the recruitment plot array, rather than the individual recruitment plot, was selected as the unit of replication. (This convention will change with the 2000 samples to be described in the 2000 project report.) For each plot array, seed abundance was calculated as the average of the seed counts in the two coring plots, and plot array seed diversity was calculated after first pooling the seeds in these coring plots.

Although we also collected seedling abundance data from each plot array's three cover plots, we will postpone this subject's presentation until the 2000 progress report by which time seedling abundance can be compared to seed abundance in a meaningful way.

The analysis to follow examines three features of the seed bank: seed abundance of all plant species combined, seed abundance of *Salicornia virginica*, and seed species diversity. Four indices measure different aspects of species diversity: species richness (α), Smith and Wilson's (1996) index of evenness (E_{var}), and two indices of heterogeneity, the Shannon-Weiner index (H') that emphasizes rare species, and the reciprocal of Simpson's index (N_2) that emphasized common species.

Spring 1999 soil cores from the restoration and natural marsh sites contained 14 seed species and 1,134 individual seeds, the great majority of which are native salt marsh species (Table 3.5). The species list in the combined spring 1999 and fall 1999 samples includes many rare exotic species, all of which frequent disturbed soil (Table 3.6).

The spatial pattern in seed abundance seemed more correlated with sampling station elevation than with sampling station along-creek distance from the creek terminus, with seed abundance increasing dramatically with increasing elevation to a maximum at the wrack line (Table 3.7, Figs. 3.10, 3.11). Significant correlations explained more of the variance in natural marsh than in the pilot restoration site. No aspect of species diversity was strongly correlated with

either elevation or along-creek distance (Table 3.7, Fig.3.12) except evenness in the natural marsh which decreased with increasing elevation.

Total seed abundance appeared related to every indicator of cover measured. Total vegetative cover, wrack cover, and bare ground were all significantly correlated with total seed abundance. This relationship did not hold for the most numerous seed bank species, *Salicornia virginica*, however, nor for any of the diversity measurements (Table 3.8).

Newly formed soil seed banks within the pilot restoration site differed from longer established seed banks in nearby natural marsh only in *Salicornia* seed abundance (Table 3.9). That is, most aspects of a natural salt marsh seed bank appear to have developed in the restoration site within only a few months after tidal flooding began. We cannot appropriately evaluate the location effect on species richness because of a significant location-elevation interaction. A hint at this interaction can be seen in the simple regressions already presented, however. Elevation seems to explain 42% of the variance in the natural marsh but only 15% in the pilot restoration site (Table 3.7).

Because of this interaction and especially because seeds are often quite scarce at lower elevations, it seems appropriate to compare the pilot restoration with natural marsh by examining seeds at the wrack line where they occur most abundantly. At this elevation, the pilot restoration site contained natural abundance of combined plant species' seeds (mean ± 1 standard deviation was 3862 ± 2163 seeds m^{-2} at the pilot restoration site *vs.* 4194 ± 256 seeds m^{-2} in natural marsh) but reduced abundance of *Salicornia virginica* seeds (1349 ± 1593 *vs.* 2270 ± 1535 seeds m^{-2}) and elevated seed species evenness (0.57 ± 0.13 *vs.* 0.23 ± 0.16) (Table 3.9). There is a hint that the pilot restoration site contains somewhat depressed species richness (3.60 ± 1.34 *vs.* 5.75 ± 4.42 species), though this difference is not significant (Table 3.9).

4.3   Animals

4.3.1   Fish

After just one year of existence, the pilot restoration site contained what appears to be a remarkably natural fish fauna. Fish sampling employed two methods: trapping on three consecutive days, and seining on a single day. Fish traps captured similar samples in Restoration Creek and in Creek A just downstream from the pilot restoration site. Both sets of samples were strongly dominated by the California killifish *Fundulus parvipinnis*, and the Restoration Creek sample also contained a single individual of a second species, the longjaw mudsucker *Gallichthys mirabilis*, that did not appear in the Creek A trap sample. Traps deployed in the sedimentation pond that separates these two creeks contained both killifish and mudsuckers in appreciable numbers and also the topsmelt *Atherinops affinis*. Table 3.10 presents the total catch by traps at all three locations. It reveals that the physically similar shallow sites in Restoration Creek and Creek A support very similar fish faunas but that species composition and species abundances differ substantially in the deeper sedimentation pond. Figure 3.13 displays the average number of killifish caught during 3 consecutive days per trap, a measure of relative abundance (or catch per unit effort) at the three sites. It reveals almost identical killifish relative abundances in Restoration Creek and Creek A but far fewer fish during high tide on the restoration plain than on the natural marsh plain.

Table 3.5. Spring 1999 seeds.

habitat	all seeds	*S.virginica* seeds	species richness	% *Salicornia virginica*	% salt marsh natives
pilot restoration site	705	217	14	31%	95%
natural marsh	429	282	13	66%	99%

Table 3.6. Spring and winter 1999 seeds that could be identified to species.

species	common name	habitat	origin	pilot rest site	Natural marsh
Atriplex traingularis	spearscale	wetland	native	x	x
Bromus madritensis	foxtail	disturb	exotic	x	
Conyza canidensis	horseweed	disturb	native	x	
Cotula coronopifolia	brass button	disturb	exotic	x	
Cyperus eragrostis	tall flatsedge	wetland	native	x	x
Epilobium ciliatum	willow herb	?	native	x	x
Frankenia salina	alkali heath	wetland	native		x
Jaumea carnosa	Jaumea	wetland	native		x
Juncus balticus	Baltic rush	wetland	native	x	
Melilotus indica	sourclover	disturb	exotic	x	
Mesembryanthemum crystallinum	ice plant	disturb	exotic	x	x
Polygonum lapathifolium	willow weed	disturb	native		x
Polypogon monspeliensis	annual beard grass	disturb	exotic	x	
Salicornia virginica	pickleweed	wetland	native	x	x
Scirpus maritimus	alkali bulrush	wetland	native	x	x
Triglochin concinna	arrow-grass	wetland	native		x

Table 3.7. Effect of elevation and along-creek distance on seed banks in spring 1999. Simple linear regressions were performed on non-transformed data for all variables and on log transformed[†] density data. Statistically significant *p*-values and associated correlation coefficients appear in bold typeface.

	Pilot Restoration		Pilot Control	
	p-value	r^2	*p*-value	r^2
Elevation				
Overall Seed Density[†]	**0.020**	**0.243**	**0.000**	**0.882**
Salicornia Seed Density[†]	**0.022**	**0.235**	**0.001**	**0.789**
Overall Seed Density	**0.003**	**0.370**	**0.003**	**0.735**
Salicornia Seed Density	**0.030**	**0.215**	**0.018**	**0.574**
Richness (α)	0.079	0.146	0.058	0.421
Evenness (E_{var})	0.330	0.047	**0.003**	**0.728**
Heterogeneity (H')	0.680	0.009	0.766	0.695
Heterogeneity (N_2)	0.900	0.001	0.406	0.101
Distance				
Overall Seed Density[†]	0.779	0.004	0.793	0.011
Salicornia Seed Density[†]	0.478	0.026	0.628	0.035
Overall Seed Density	**0.048**	**0.181**	0.731	0.018
Salicornia Seed Density	0.055	0.172	0.338	0.131
Richness (α)	0.651	0.010	0.750	0.015
Evenness (E_{var})	0.326	0.048	0.803	0.010
Heterogeneity (H')	0.317	0.644	0.276	0.639
Heterogeneity (N_2)	0.201	0.080	0.390	0.107

Figure 3.10. Effects of elevation and along-creek distance on total seed abundance in spring 1999. Statistically significant regressions are shown.

Figure 3.11. Effects of elevation and along-creek distance on *Salicornia virginica* seed abundance in spring 1999. Statistically significant regressions are shown.
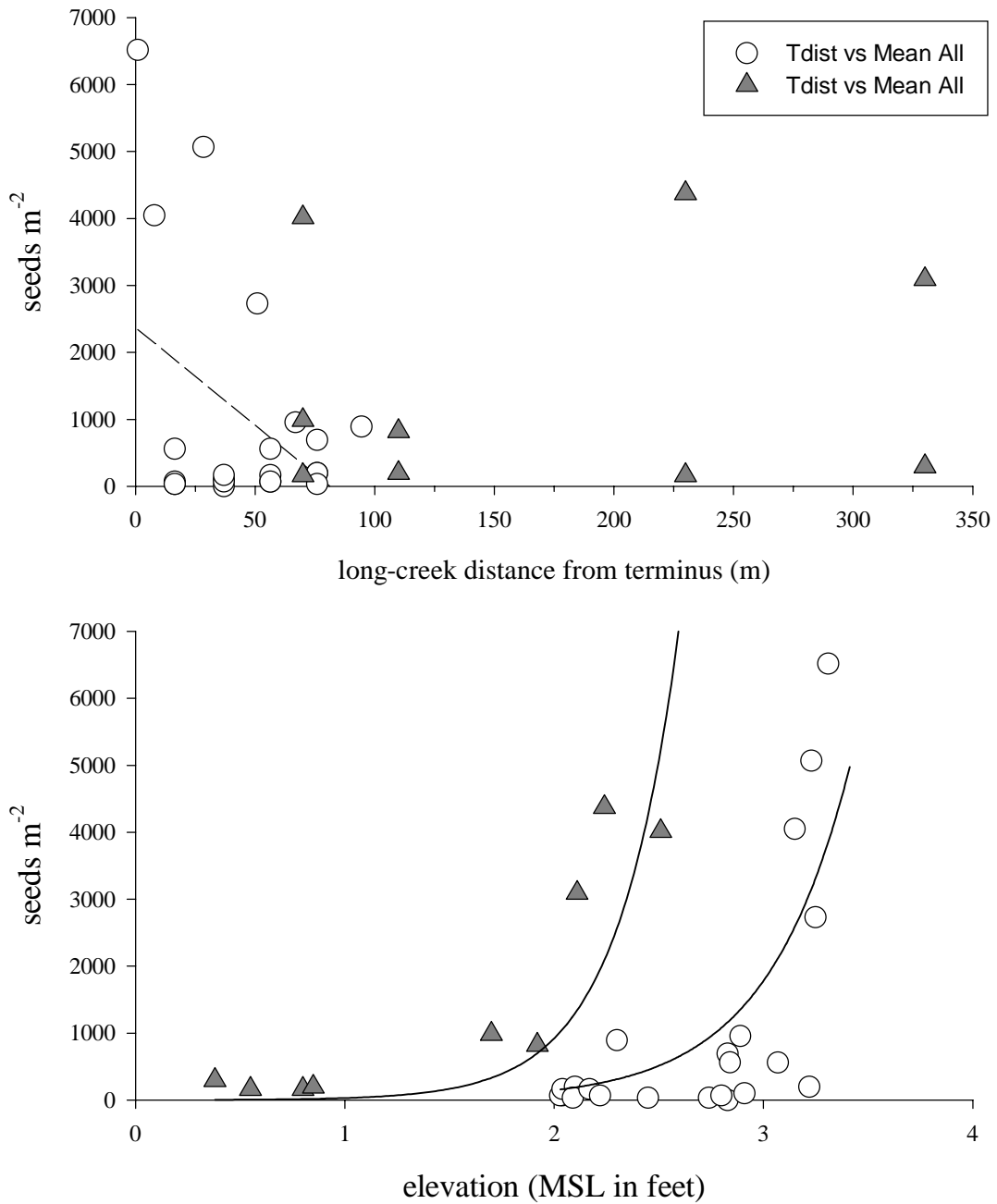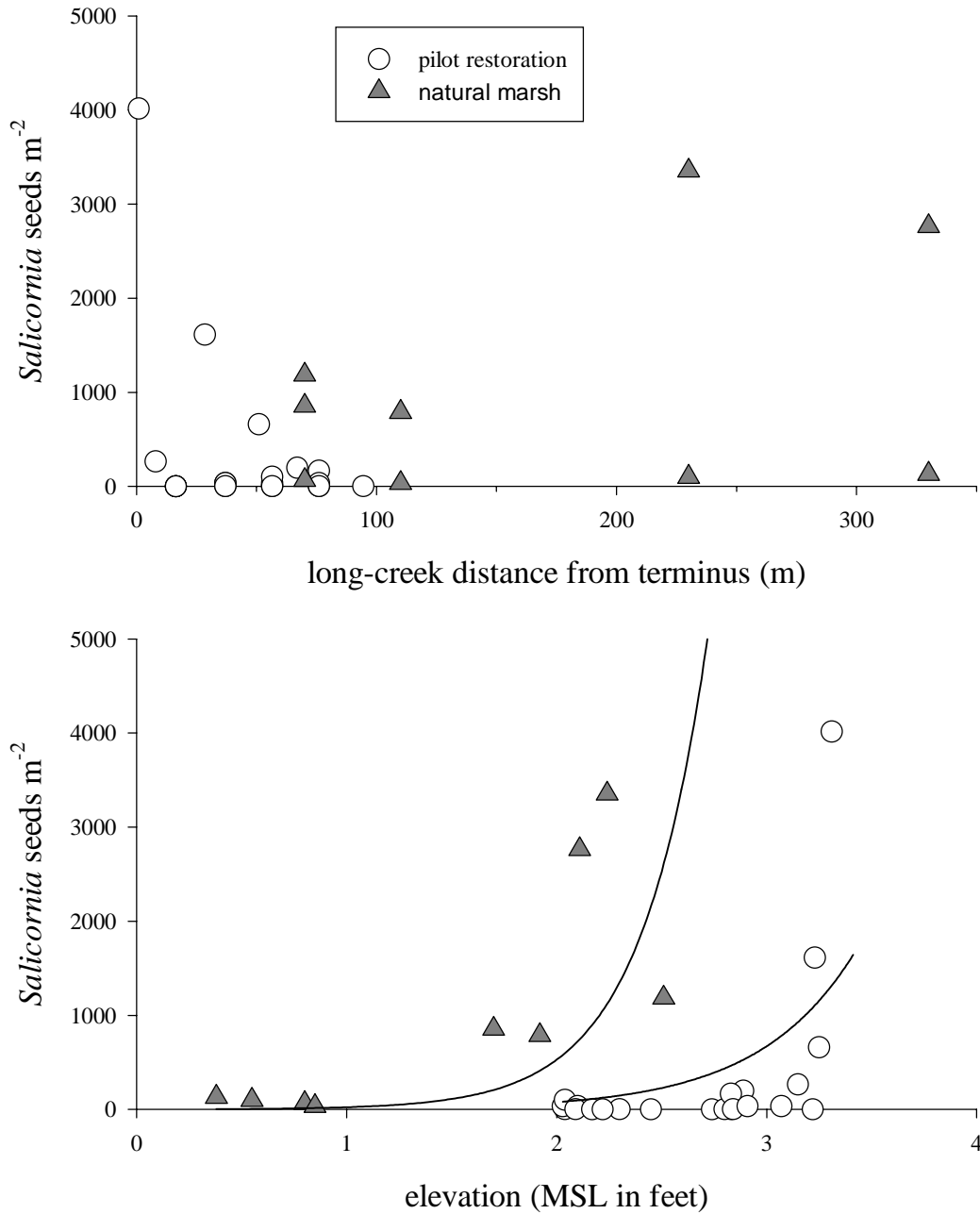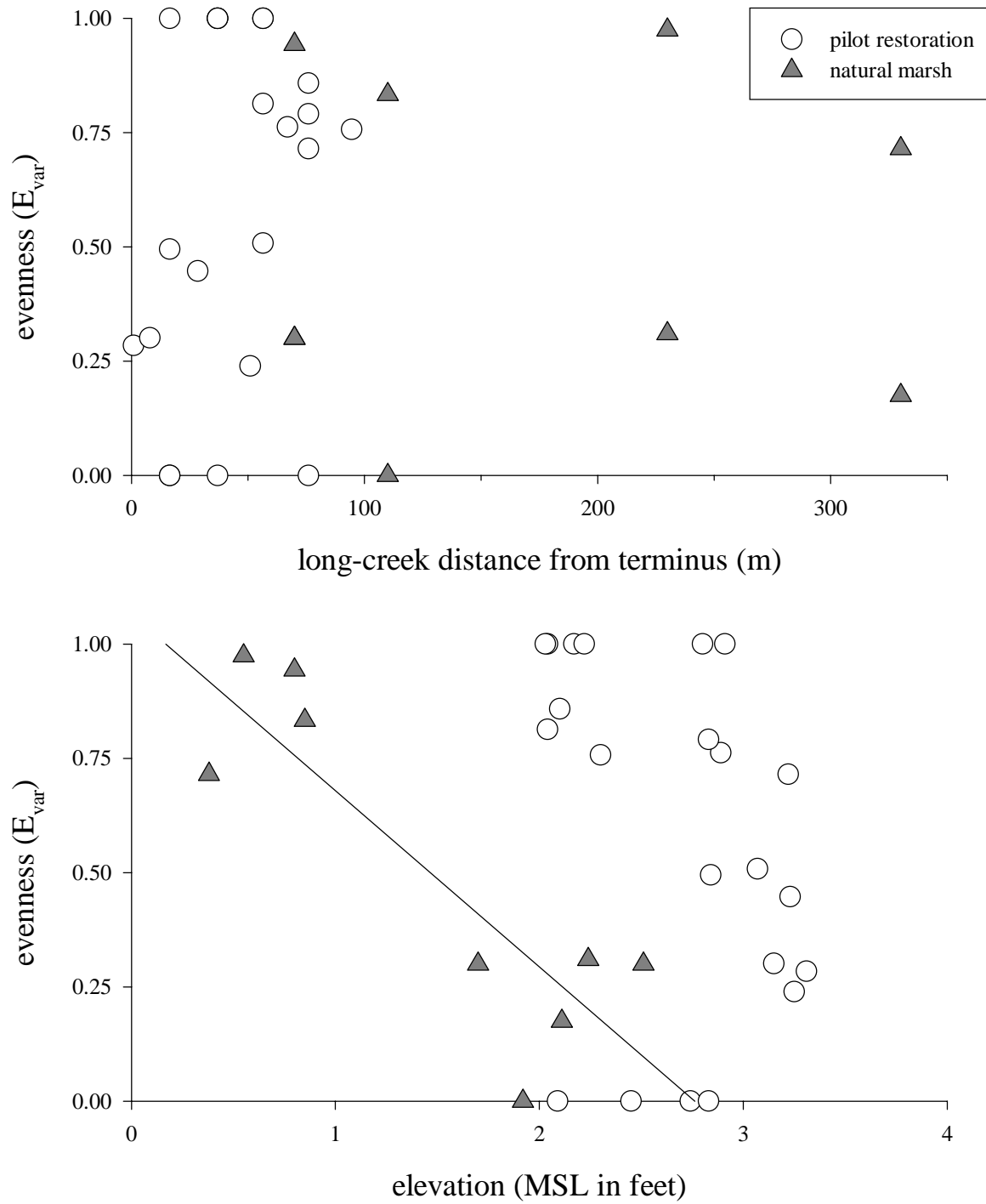
Figure 3.12. Effects of elevation and along-creek distance on seed evenness in spring 1999. Statistically significant regressions are shown.

Table 3.8. Effect of cover on seed banks in spring 1999. Linear regressions were performed on arcsine transformed cover and untransformed seed abundance data from the pilot restoration site. Statistically significant *p*-values and associated correlation coefficients appear in bold typeface.

	vegetative cover		wrack cover		bare ground	
	p-value	r^2	*p*-value	r^2	*p*-value	r^2
total seed abundance	**0.007**	**0.309**	**0.005**	**0.332**	**0.001**	**0.425**
Salicornia seed abundance	0.301	0.053	0.163	0.095	0.132	0.110
richness (α)	0.289	0.056	0.528	0.020	0.406	0.035
evenness (E_{var})	0.399	0.036	0.567	0.567	0.470	0.026
heterogeneity (H')	0.067	0.158	0.186	0.186	0.099	0.130
heterogeneity (N_2)	0.823	0.003	0.718	0.718	0.706	0.007

Table 3.9. Pilot restoration-natural marsh comparisons in spring 1999. Two-way ANOVAs were performed on categorical data. The second row for each factor represents a second ANOVA run after dropping the non-significant interaction factor. Statistically significant *p*-values and associated correlation coefficients are shown in bold type.

	location *p*-value	elevation *p*-value	location × elevation *p*-value
total seed density	0.151	0.000	0.377
	0.137	**0.000**	–
Salicornia seed density	0.018	0.001	0.159
	0.027	**0.001**	–
richness (α)	0.002	0.000	**0.002**
	–	–	–
evenness (E_{var})	0.210	0.070	0.289
	0.304	0.172	–
heterogeneity (H')	0.986	0.085	0.172
	0.955	0.260	–
heterogeneity (N_2)	0.782	0.365	0.300
	0.817	0.683	–

Table 3.10. Fish and crabs caught in traps and seines over the course of three consecutive deployment days and in seines deployed during a single day.

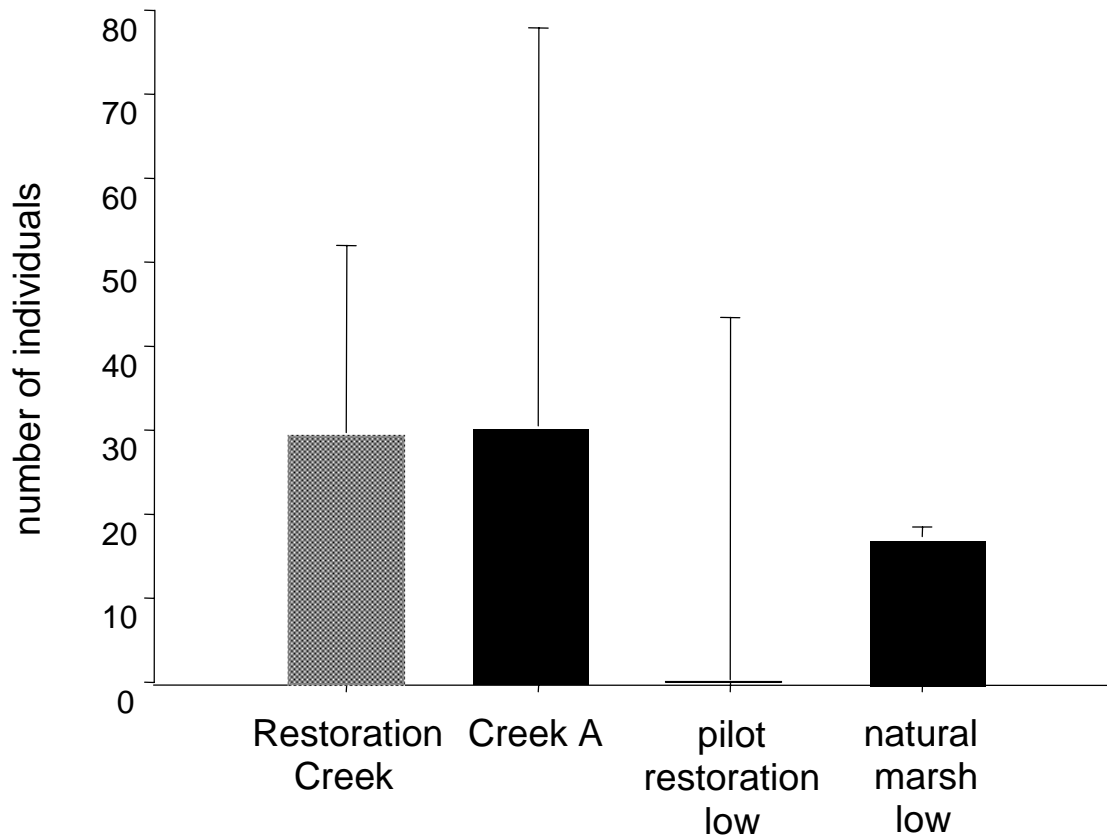| Fish Traps	Effort (# traps)	*Fundulus parvipinnis*	*Gillichthys mirabilis*	*Atherinops affinis*	*Pachygrapsus crassipis*	*Hemigrapsus spp.*
Restoration Creek	10	297	1	0	0	16
Creek A	2	61	0	0	1	0
restoration site – low tide	15	4	2	0	1	0
natural marsh – low tide	3	52	0	0	1	0
sedimentation pond	3	43	111	25	3	0
restoration site – high tide	18	5	0	0	1	1
Total		**462**	**114**	**25**	**7**	**17**
Fish Seines	(# pulls)					
Restoration Creek, west arm	4	363	11	22	0	4
Restoration Creek, south arm	6	72	7	12	1	1
Creek A, site 1	4	27	3	0	0	0
Creek A, site 2	4	17	2	1	0	0
sedimentation pond	1	206	0	3	0	10
reference site C37	1	0	0	0	1	0
reference site C40	1	0	0	0	0	0
Total		**685**	**23**	**38**	**2**	**15**

Figure 3.13. Mean (± 1 SD) abundance of *Fundulus parvipinnis* trapped in the Restoration Creek, in natural Creek A nearby, in low elevation sites in the pilot restoration plain, and in low elevation sites in the nearby natural marsh plain.

Fish seine samples in Restoration Creek and Creek A (Table 3.10), collected on days when traps were not present, reveal somewhat different patterns of total fish abundance in the 10 m long creek segments seined. Both the western and southern arms of Restoration Creek contained all three fish species in appreciable numbers, but seined killifish were about five times more abundant in the western arm. The two nearby seined sites in Creek A contained both killifish and mudsuckers, but only one contained a single *Atherinops* individual. As Figure 3.14 reveals, seined killifish were much less common in the natural Creek A than in Restoration Creek. Although the size of the sedimentation pond prevented quantitative seining, a single seine pull there (Table 3.10) revealed considerably higher killifish abundance than the fish trap data imply.

The Restoration Creek and Creek A samples suggest that there might be a difference in killifish population size structure between these two sites. Figure 3.15(a and b) display the size structure of the fish collected in traps. Small fish below about 35 mm standard length appear to be much more common in Restoration Creek (Kolmogorov-Smirnoff test, p = 0.016). On the other hand, the fish seine data in Figure 3.15(c and d) do not differ so dramatically between these creeks though again small fish are significantly more numerous (p = 0.005) in Restoration Creek. Because our repeated seine pulls capture nearly all individuals in a creek segment while traps capture only some of them, the smaller between-site difference in the seine data may better describe reality. Other explanations cannot be excluded, however. It is possible, for example, that the trap data accurately reflect a true between-site difference that happened to be not so pronounced on the particular day that seining took place.

Because Creek A and Restoration Creek may both contain elevated heavy metal concentrations derived from sludge leaching, for comparison we also seined Creek C that lies in a different drainage within Mugu Lagoon's central basin and lacks exposure to sewage sludge. Unfortunately, the two 10 m creek segments sampled yielded only one killifish each. This extremely low abundance compared with Restoration Creek and Creek A probably reflects physical site differences unrelated to chemical contaminants. In any case, these samples by themselves provide no basis to evaluate heavy metal effects on fish that occupy tidal creek water potentially influenced by sludge-derived heavy metals.

Some small-bodied fish that occupy tidal creeks in salt marshes also venture out onto the salt marsh plain itself during high tides, presumably to feed. We employed fish traps to find out whether fish species that occupy Mugu Lagoon tidal creeks possess this behavior and if so whether they visit the marsh plain within and outside the pilot restoration site equally. Three traps placed on a natural marsh plain near the Creek A sampling site captured 52 killifish and no mudsuckers (Table 3.10). In strong contrast, fifteen traps placed on the artificial marsh plain of the pilot restoration site up to 30 m from the tidal creek captured only 4 killifish and 2 mudsuckers. These data support two obvious conclusions, that at least two Mugu Lagoon tidal creek fish species enter the marsh plain at high tide, and that many more killifish enter a natural marsh plain than the pilot restoration marsh plain. This difference in marsh usage may arise from increased food availability and/or increased protection from avian predators provided by the dense vegetation of the natural marsh.
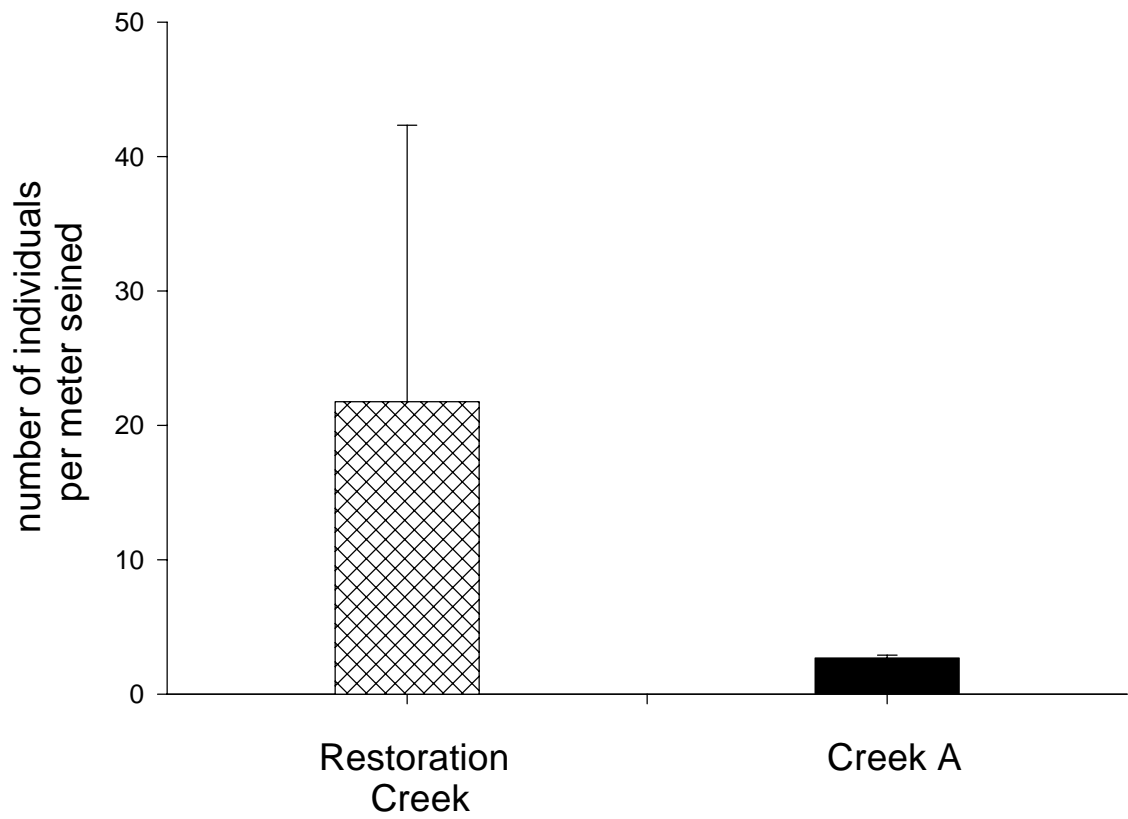
Figure 3.14. Mean abundance of seined *Fundulus parvipinnis* in Restoration Creek and in the nearby natural Creek A.
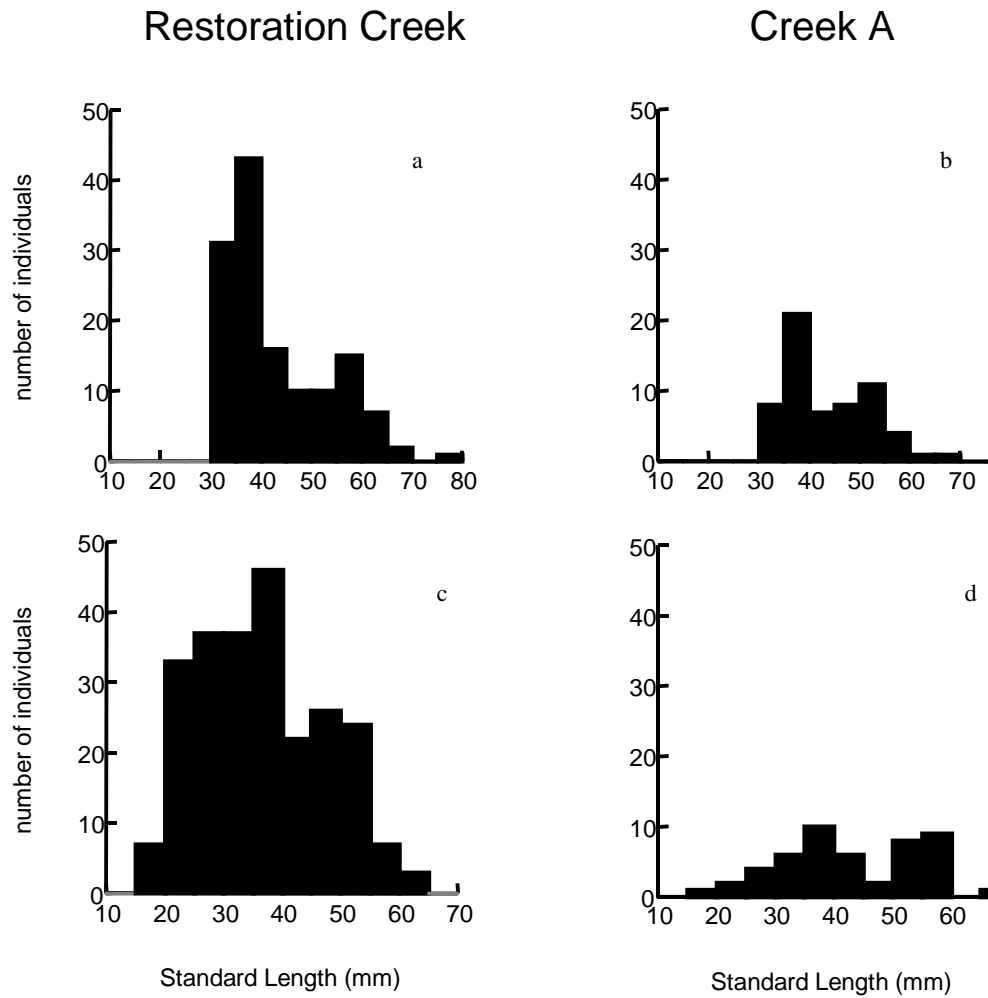
Restoration Creek

Creek A

Figure 3.15. Size distributions of *Fundulus parvipinnis* in Restoration Creek and in nearby natural Creek A obtained in (a) and (b) trap samples and in (c) and (d) seine samples.

Taken together, the fish data presented in this section reveal that fish populations with a close to natural size structure can occupy artificial tidal creeks soon after construction but venture in appreciable numbers out onto artificially constructed salt marsh plain probably only after vegetation there has reached natural density. Although these data do not provide a rigorous test (due to the absence of suitable reference sites) of whether toxic substances leached from sludge cause these fish any harm, the data do establish that the pilot restoration site can support an apparently healthy fish population with no stark and immediately obvious differences from populations that occupy areas in somewhat more natural condition.

4.3.2 Crabs

Table 3.10 of the previous section displays abundances of the two crab species found to occupy Restoration Creek and Creek A during fish trapping and seining. The very few *Pachygrapsus crassipes* individuals collected came mostly from Creek A, while the some-what larger number of *Hemigrapsus* sp. individuals appeared mostly in Restoration Creek. Because both species' sample abundances were not very large, it is not clear whether sample differences reflect underlying species distributional differences. What is clear is that at least some crabs are capable of living in the tidal creek closest to sludge-containing soil and pre-sumably most affected by any toxic substances that may have leached from the sludge during the preceding 12 months.

4.3.3 Snails and Parasites

Creek A in the natural marsh south of the pilot restoration site has contained a dense population of *Cerithidea californica* for many years. Although absolute abundances varied through time, population size structure seems to have remained roughly the same during three sampling periods in February 1999, June 1999, and November 1999 (Fig. 3.16). Samples in the pilot restoration site itself contained no *Cerithidea* in February or June 1999, but by No-vember 1999 some individuals in intermediate size classes had begun to appear.

Quantitative sampling of the trematode parasites of *Cerithidea* in the pilot restoration did not take place in 1999, as too few snails in the 20-25 mm size class that bear most parasites were present then. Parasite sampling did occur at Mugu Lagoon's L Avenue restoration site, however (data not shown in this report). This exercise revealed that the sampling procedure can adequately measure parasite abundance in the pilot restoration
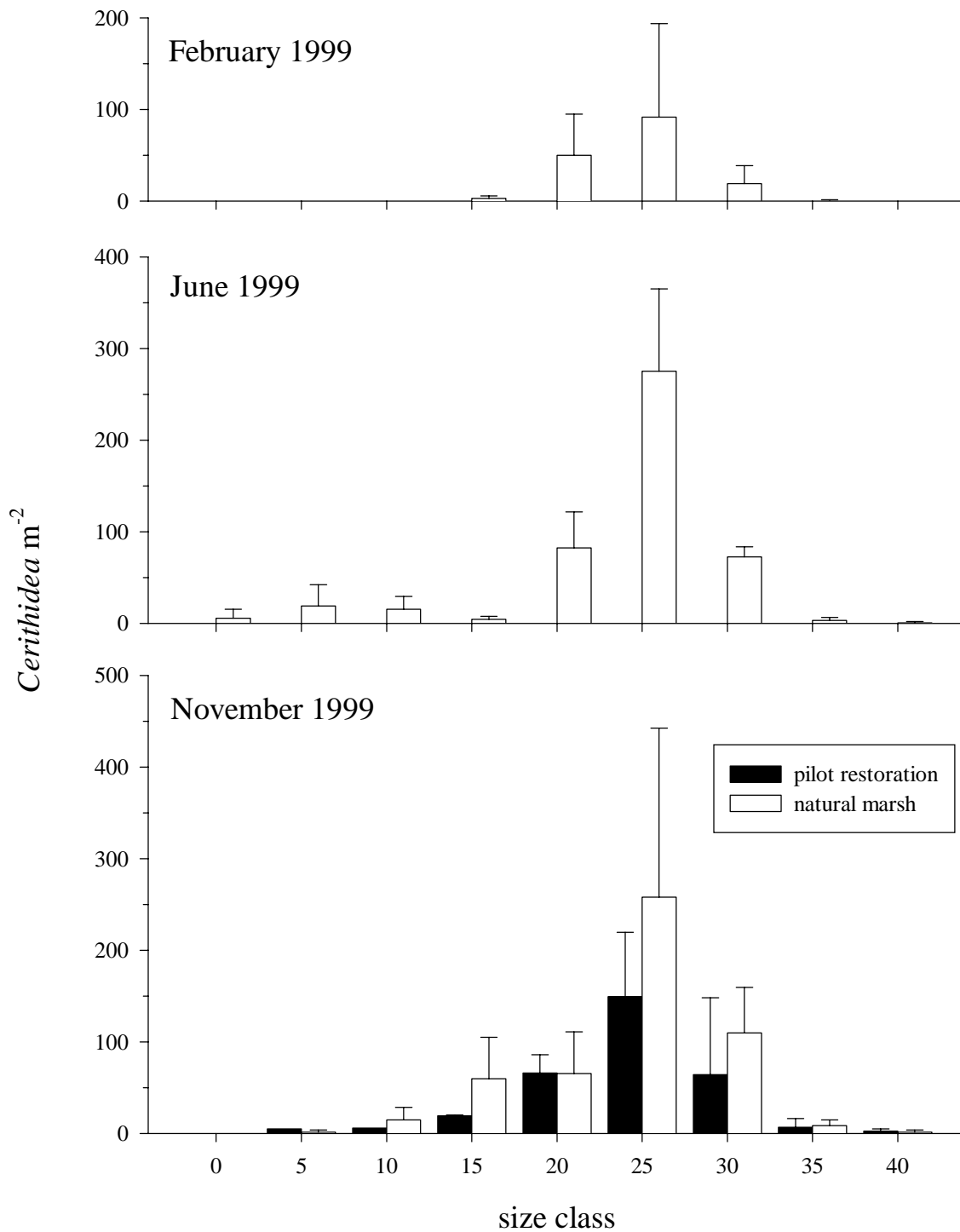
Figure 3.16. *Cerithidea californica* size class abundances in creek bank transects. Each histogram represents an average of four samples. Error bars represent 1 standard deviation. site when snails appear in sufficient numbers there to make parasite sampling worthwhile.

4.3.4 Siphon Holes

Restoration Creek's banks contained numerous siphon holes one year after construction (Fig. 3.17). Restoration Creek and Creek A sites currently differ in only two ways (Table 3.11): (1) Creek A contains holes greater than 40 mm diameter, whereas Restoration Creek has none greater than 29 mm diameter; and (2) Creek A has more 10-19 mm diameter holes than does Restoration Creek (t test, $p = 0.003$).

These comparisons between Restoration Creek and Creek C reference sites are somewhat equivocal due to differences in the physical environments. Creek C bank architecture is somewhat different from the other creeks, and Creek C is also wider and deeper. Restoration Creek will probably never achieve the morphology or siphon hole density of Creek C.

We expect siphon hole size range and overall density in Restoration Creek to continue to increase over time and eventually even to become statistically identical to the siphon holes in Creek A.
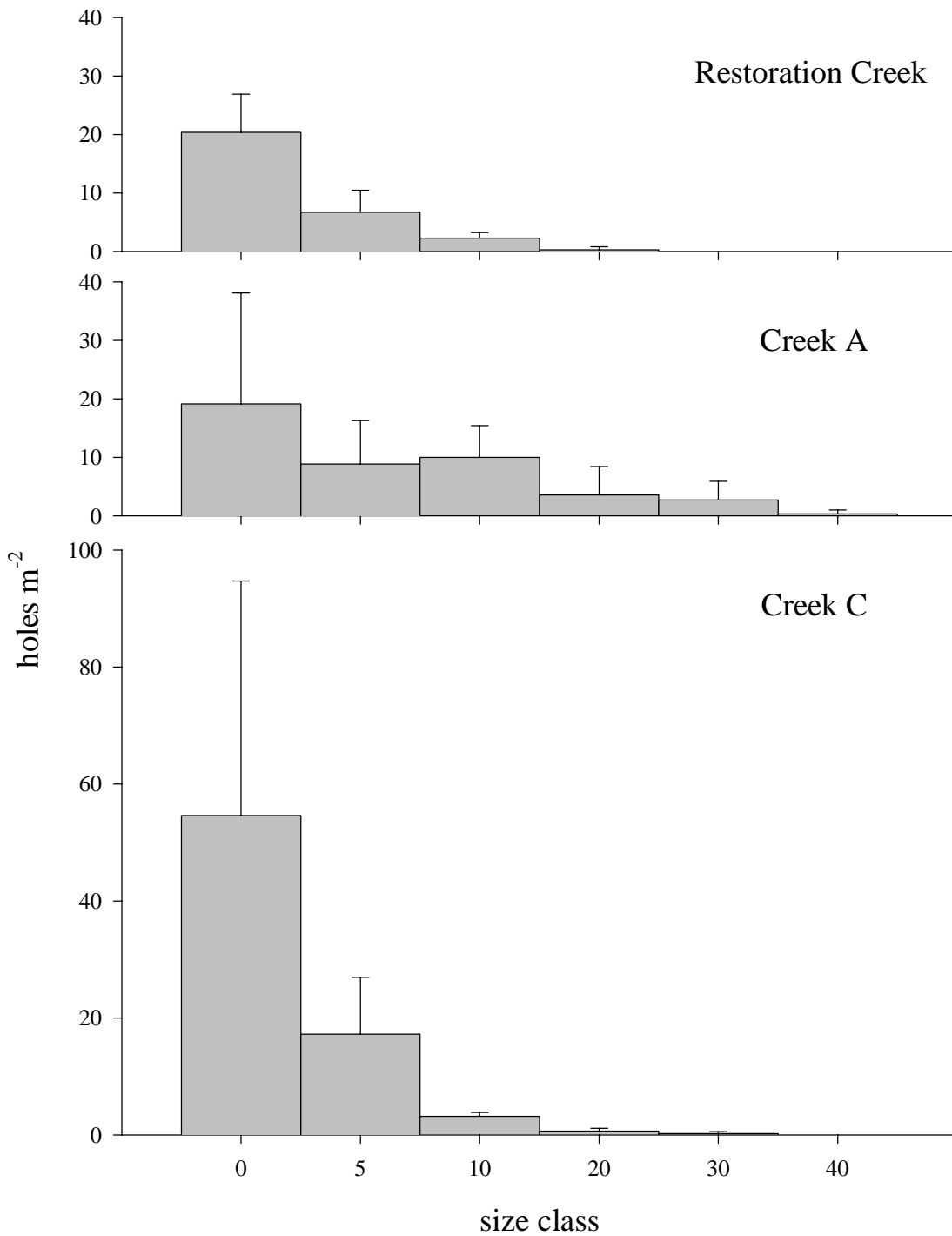
Figure 3.17. Siphon holes in Restoration Creek, Creek A, and Creek C. Size class 0 represents 0-4 mm holes, size class 5 is 5-9 mm holes, size class 10 is 10-19 mm holes, size class 20 is 20-29 mm holes, size class 30 is 30-39 mm holes, and size class 40 is holes at least 40 mm in diameter. Bars are means, and error bars represent 1 standard deviation. These samples come from four sites in Restoration Creek, four in Creek A, and two in Creek C.

Table 3.11. Two-way ANOVA and two-sample *t*-tests compare siphon hole densities between Restoration Creek and Creek A sites. Significant differences are indicated in boldface type.

source	SS	*df*	MS	*F*	*p*
Site	227.256	1	227.256	2.018	0.158
Hole Size	6349.427	5	1269.885	11.278	**<0.000**
Site x Hole Size	278.149	5	55.630	0.494	0.780
error	14863.603	132	112.603		

Hole Size Class	*t*	*df*	*p*
< 4 mm	0.122	15.3	0.905
5-9 mm	-0.649	18.9	0.524
10-19 mm	-3.608	12.3	**0.003**
20-29 mm	-1.805	11.2	0.098
30-39 mm	-	-	-
> 40 mm	-	-	-

Chapter 4:
Restoration Implications

This report describes biological events that have taken place during the first year of the Mugu Lagoon sewage ponds pilot restoration experiment. The results presented in Chapter 3 incorporate enough detail and sufficient statistical replication to support three scientifically solid conclusions that relate directly to the future full sewage ponds restoration project. This chapter states these conclusions, discusses their scientific foundation, articulates their practical importance in sewage ponds restoration, and draws from them three useful restoration guidelines.

Though factually correct, these conclusions emerge from an investigation of limited scope. Because of the study's limitations, we encourage the reader to guard against over-interpreting these conclusions. Failure to do so could, at worst, create serious environmental damage in the full sewage ponds restoration project. This investigation has two main limitations. The first, imposed by nature itself, is that ecological phenomena that arise during the first year in any newly established ecosystem do not necessarily predict accurately the ecological phenomena that will characterize that same ecosystem several years later. We feel that two years is the barest minimum observation period required to make realistic projections of the future behavior of a restored salt marsh. The second limitation, imposed mostly by budgetary constraints, is that this first year's study includes no information on environmental chemistry. Consequently, it provides an incomplete assessment of the environmental cost of using sewage sludge in salt marsh restoration. Completing this assessment is a high priority activity during the experiment's second year.

4.1   Sewage Sludge as a Soil Amendment

The potential environmental benefit of using sewage sludge as a soil amendment in salt marsh restoration derives from organic enrichment. Salt marsh plants require phosphorus- and especially nitrogen-containing nutrients for healthy growth. In a fully established salt marsh, soil nutrients come in large part from the decay of older vegetation containing organically bound nutrients that have accumulated in the marsh, mostly from upland sources, very gradually over a period of many decades. In artificially constructed salt marshes, however, plants necessarily depend upon whatever nutrients may exist in the soil used in marsh construction. Typically, soils available for this purpose contain much lower nutrient concentrations than does natural salt marsh soil, and accordingly plant growth in constructed marshes often does not compare favorably with growth in natural marshes. Other than sewage sludge, soils available near the Mugu Lagoon sewage ponds are particularly poor in nutrients, and consequently incorporating sewage sludge into the soil of this particular restoration has the potential to improve salt marsh plant performance substantially. The organic matter in the sludge could serve as a kind of slow-release nutrient supply during the constructed marsh's first few years.

Unfortunately, however, measurements in the pilot restoration experiment revealed that growth of three different salt marsh plant species did not vary with sludge concentration over the range of 0% sludge to 75% sludge. Furthermore, sludge failed to stimulate plant growth when mixed with either upland soil or material mined from the sewage ponds' clay liner. We suspect that this failure arose from the fact that Mugu Lagoon sewage sludge actually contains surprisingly little organic matter (Vance *et al.* in press). This sludge dates back mostly to the 1970's and 1980's when the ponds received all sewage produced by the naval base, and most of its organic matter has long since decomposed into simpler nutrient-containing compounds vulnerable to leaching via ground water movement. With most nutrients removed by leaching, what remains in the sludge layer is not a particularly effective fertilizer.

Whatever the cause, the potential benefit of using Mugu Lagoon sewage sludge in salt marsh restoration appears negligible. Consequently, from an environmental perspective, the desirability of using this sludge in the sewage ponds restoration will depend mostly on the resulting environmental cost. Environmental chemistry studies to evaluate this cost are presently underway.

This first year's biological measurements do reveal that the environmental cost will not be immediate and severe. Sludge appears not to harm salt marsh plant growth. Though plants growing in up to 75% sludge concentrations did not perform any better than plants growing in sludge-free soils, they did not perform any worse either. Indeed, the sludge-exposed plants performed no worse even than non-exposed plants fertilized with either naturally occurring organic matter or frequently applied commercial fertilizers known to stimulate *Salicornia virginica* growth in Mugu Lagoon's L Avenue restoration experiment. Together these experiments establish that toxic substances in Mugu Lagoon's sewage sludge cause no pronounced, direct physiological harm to plants growing in sludge-containing soil mixtures.

Animals too show no signs of pronounced, direct physiological harm caused by several months' exposure to this sewage sludge. Snail invasion of the pilot restoration site is not yet completed, but the *Cerithidea californica* present in late 1999 appeared as healthy as snails elsewhere in Mugu Lagoon. A similar statement applies to crabs. Fish seem not only not to avoid the pilot restoration site but to thrive in Restoration Creek, particularly the killifish *Fundulus parvipinnis* whose abundance there exceeds population abundance in superficially comparable tidal creeks elsewhere in Mugu Lagoon. Also, the silversides *Atherinops affinis* appeared much more abundant in the pilot restoration site's sedimentation pond than anywhere else in the tidal creeks we have examined.

These observations establish only that sewage sludge causes no discernible harm to the estuarine plants and animals that contact it. This finding is not particularly surprising for sludge that contains such small amounts of toxic substances. The main danger of these toxic substances actually lies in their escape into the larger estuarine and adjacent oceanic environments where biological uptake followed by biomagnification can produce physiologically damaging concentrations in higher trophic level species. Vulnerable species include some endangered species (*e.g.*, the light-footed clapper rail), other species esthetically valued by humans (*e.g.*, the harbor seal), and indeed humans themselves through consumption of commercially harvested organisms (*e.g.*, California halibut). Assessing the magnitude of this

threat must involve thorough study of sludge effects on various aspects of environmental chemistry.

4.2 Clay as a Soil Amendment

Previous experience in southern California (Langis *et al.* 1991, Gibson *et al.* 1994) has revealed that sandy soil provides a very poor substratum for a constructed salt marsh. Exactly this same conclusion arose from our earlier Mugu Lagoon experiment with potted plants, as plant growth correlated more strongly with abundance of fine particles than with sludge content (Vance *et al*. in press). Sandy soil allows rapid groundwater movement, and any nutrients initially present may leach out quickly. Regular nutrient enrichment can enhance plant growth in sandy soil, but poor plant performance quickly follows cessation of regular nutrient enrichment because dissolved nutrients have such a short residence time (Boyer and Zedler 1998). Even enrichment with organic matter fails to stimulate plant growth much in sandy soil because although organic decomposition does release nutrients gradually, once these enter the dissolved state they wash away quickly (Gibson *et al.* 1994). Because excessively sandy soil virtually always supports poor plant performance, it is desirable in salt marsh restoration to use finer grained soil whenever possible.

Both the upland soil and the "clay" used as soil matrix in the sludge-bearing soil mixtures were finer-grained than the soil employed in the pot experiment, but the "clay" contains more clay-size and silt-size particles than the upland soil does. This difference (for which we have data not displayed in this report) almost certainly accounts for the observation that plants grew better in clay-containing soil mixtures than in upland-containing soil mixtures. This observation arose repeatedly in different plant species and using several different measures of plant performance.

There is also a second potential reason, not yet documented, for this difference. The clay was obtained from the original sewage pond liner, and it is possible that much of the dissolved nutrients and soluble nutrient-containing organic compounds that arose from sludge decomposition over the years remained trapped in the clay liner after being leached from the ponds' sludge layer. If so, then the clay-stimulated improvement in plant performance actually amounts to a sludge-enrichment effect, but enrichment of the clay by the sludge occurred over a period of many years and on a molecule-by-molecule basis. We are presently examining whether this clay material has higher organic and/or nutrient content than the upland soil does.

The growth experiments also revealed that clay supports better plant performance even than natural wetland soil itself! This unexpected difference probably arises from the suspected higher than usual sand content of "natural" wetland soil at Mugu Lagoon, particularly in the central basin, compared to soil in a (hypothetical) completely natural salt marsh in southern California. Elevated sand content, if indeed it occurs, probably arose from greatly increased sand deposition in the central basin associated with increased soil erosion in the Oxnard Plain during the past 150 years of agriculture. In any case, plant performance in sewage ponds' clay is very likely closer to natural plant performance in Mugu Lagoon than occurs in the present-day agriculturally modified wetland there.

The practical implication of this finding is that incorporating the sewage ponds' clay liner into the soil of the restored marsh will probably produce the healthiest salt marsh plants that the area is capable of supporting. However, see the cautionary comments following conclusion 3 in the next section and guideline 3 of Section 4.4.

4.3   Conclusions

The thoughts of the previous two sections, and the data in Chapter 3 upon which they depend, support this study's three conclusions of practical importance.

> **<u>Conclusion 1</u>: Mixing Mugu Lagoon sewage sludge with local upland soil or with material from the ponds' clay liner does not increase salt marsh plant performance.**

Consequently, using Mugu Lagoon sewage sludge in local salt marsh restoration will confer no discernible environmental benefit. Making the practical decision of whether to use the sludge in this way, rather than disposing it in landfills, will require weighing only the resulting financial savings against the resulting environmental cost.

> **<u>Conclusion 2</u>: Exposure to Mugu Lagoon sewage sludge does not cause obvious direct physiological harm to the most common plant and animal species that occupy local salt marsh and associated tidal creeks.**

Thus, the toxic substances present in this sludge cause no acute and pronounced ecological damage. Whether these substances might cause longer term harmful ecological effects through biomagnification remains to be evaluated.

> **<u>Conclusion 3</u>: As a substratum for salt marsh plant growth, the clay-bearing material contained in the Mugu Lagoon sewage ponds liner produces higher plant performance than do other local and readily available soils.**

This property arises from the ability of fine-grained soils to retard groundwater flow and hence to diminish loss of soil nutrients through leaching. Long-term retention of nutrient-bearing substances originally leached from decomposing sewage sludge might contribute to increased plant performance through elevated nutrient availability. If this clay material has captured nutrients leached from the sludge, however, then it may also have captured toxic substances leached from the sludge at the same time. If so, then the environmental benefit of using this clay in salt marsh restoration, namely increased plant performance, might be accompanied by an environmental cost in the form of increased toxic pollution of nearby ecosystems. Geochemical studies to evaluate this risk are presently underway.

4.4   Preliminary Restoration Guidelines

These conclusions inspire three restoration guidelines, the first two subject to confirmation, modification, or replacement by further scientific findings to appear in the 2000 project report.

> **<u>Guideline 1</u>: The most locally effective and economical soil preparation technique for the full sewage ponds restoration project is to mix the sewage pond sludge with the clay-bearing material that lies directly beneath it.**
>
> **<u>Guideline 2</u>: From the perspective of sludge remediation, the sewage ponds restoration project can be restricted to just the land area presently occupied by the ponds because the clay layer occurs only there. For completeness, the area presently occupied by the sewage pond berms should also be restored, but this area will contain neither sewage sludge nor material from the clay liner. It is not necessary to extend the sewage ponds restoration beyond the outer limits of the berms.**
>
> **<u>Guideline 3</u>: Before implementing Guidelines 1 and 2, their environmental cost should be thoroughly evaluated and compared to salt marsh restoration that would employ only local upland soil. This evaluation must include measuring toxic substance content in all relevant ecosystem components.**

These components include samples, each from several areas, of (1) sludge, (2) clay liner, (3) natural salt marsh soil near the sewage ponds, (4) soil from within and near to pilot restoration plots through time (to evaluate heavy metal leaching and retention), (5) tidal creek water, (6) tidal creek mud, (7) tissue of plants growing in pilot experimental plots, (8) tissue of plants growing in natural salt marsh near the sewage ponds, (9) tissue from tidal creek snails, (11) tissue from tidal creek fish, and (12) tissue from some abundant animal species that lives within tidal creek mud.

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