Fall 1998

Eelgrass (Zostera marina L) as a sentinel accumulator of lead in Portsmouth Harbor, New Hampshire - Maine

Heidi Morrill Hoven
University of New Hampshire, Durham

EELGRASS (*Zostera marina* L.) AS A SENTINEL ACCUMULATOR OF LEAD IN PORTSMOUTH HARBOR, NEW HAMPSHIRE - MAINE

BY

HEIDI MORRILL HOVEN
B.S., University of Rhode Island, 1986
M.S., University of New Hampshire, 1992

Dissertation

Submitted to the University of New Hampshire
in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy

in

Natural Resources

September, 1998

UMI Number: 9907586

UMI
300 North Zeeb Road
Ann Arbor, MI 48103

This dissertation has been examined and approved.

Dissertation Director, Dr. Frederick T. Short, Research Associate Professor of Natural Resources and Marine Science

Dr. David M. Burdick, Research Assistant Professor of Natural Resources

Dr. George O. Estes, Professor of Plant Biology

Dr. Leland S. Jahnke, Associate Professor of Plant Biology

Dr. Frank D. Richardson, Senior Wetlands Inspector, New Hampshire Department of Environmental Services, Wetlands Bureau, Coastal Program

DEDICATION

To my grandfather, Austin Winfield Morrill, Jr., for nurturing my scientific interests during my childhood; and to Buckley, my dearest companion.

ACKNOWLEDGEMENTS

There have been many times along this path that I have reached out for help and support and found it. As much of a personal accomplishment this degree has been for me, I don't think it would have meant as much without the help and encouragement from others. I would first like to thank my Dissertation Director, Dr. Frederick Short, for his belief in my ability to become a marine scientist and for his patience during the times I didn't take the direct path. I would also like to thank my other committee members, Dr. David Burdick, Dr. George Estes, Dr. Leland Jahnke, and Dr. Frank Richardson for their helpful insight on my experimental design and analyses. I was fortunate to have been welcomed by Dr. Jahnke to use his laboratory for the physiological assays and I appreciate all I learned. I would also like to thank Dr. Henri Gaudette for welcoming me into his laboratory and for introducing me to the applications of Pb isotope ratios. I am especially grateful to Cathy Short for her editing of the dissertation and helpful critiques.

I have benefited greatly from the comraderie of my fellow graduate students: Ryan Davis, Pam Morgan, Kalle Matso, Beata Summer-Brason and Alison Bowden; and I'd like to thank each of you for being there for me at the various hurdles whether it was a helping hand in my field work, encouragement before the comprehensive and defense or watching me wade through the writing process and helping with editing. There were many students and volunteers who helped with seemingly endless sample processing, and I'd like to especially thank Sara Beagen, Lia Houk, Vidya Kesavan, Derick Gellis, Derick Sowers, Brad Agius, Sue Cobbler and Sara Hamilton for all their hard hours of work.

Finally, I owe the greatest thanks to my friends and family who did not know what they were committing to when I decided to venture one last time into academia. You have certainly shared the burdens and rewards along the way and I feel you have earned this degree with me. Thank you for your enduring love and support and know that you are an integral part of this Ph.D.

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EELGRASS (*Zostera marina* L.) AS A SENTINEL ACCUMULATOR OF LEAD IN PORTSMOUTH HARBOR, NEW HAMPSHIRE - MAINE

By

Heidi Morrill Hoven
University of New Hampshire, September, 1998

The potential of eelgrass, *Zostera marina* L., as a sentinel indicator of lead (Pb) pollution in the water column was investigated. Eelgrass was grown in mesocosms that contained ambient sediment collected from sites near the Portsmouth Naval Shipyard Jamaica Island Landfill (JIL) of Kittery, Maine, to test viability of eelgrass in the Shipyard's marine sediment. Eelgrass was also grown in mesocosms at three shoot densities in marine sediment of three Pb concentrations to determine to what extent Pb affects eelgrass growth and whether Pb uptake by eelgrass is affected by plant density. Additionally, eelgrass was used to detect water-borne sources of Pb. Eelgrass was deployed in the water column adjacent to water seepages along the JIL to see if Pb could be detected and whether the seepages near the JIL were indicated as sources of Pb to the estuary.

Mesocosm research showed that eelgrass grew well in Shipyard marine sediment, however physiological evidence indicated eelgrass experienced some level of toxicity when exposed to high levels of Pb. Shoots growing in sediment with high Pb concentrations had significantly lower levels of chlorophyll and low Fv/Fm values. Additionally, as shoot density increased, Pb uptake decreased in both rhizomes and leaves of shoots growing in Pb enriched sediment, indicating a density effect on Pb uptake.

Leaves of deployed eelgrass accumulated Pb relative to reference shoots (with background Pb levels) at five of the six stations sampled. Highest Pb concentration occurred in shoots that were deployed closest to water seepages, suggesting that the seeps were a source of Pb and that eelgrass tissue Pb reflected Pb exposure. Analysis of stable isotopic composition of Pb in deployed eelgrass leaves and Pb in seep water and sediment indicated that two seepages near JIL were sources of anthropogenic Pb to Jamaica Cove.

If eelgrass habitat is restored in Pb contaminated sediment, high eelgrass densities could be planted to bioaccumulate Pb, while maintaining a low tissue Pb burden per plant. Additionally, using Pb isotope ratios in eelgrass expands the use of eelgrass as a sentinel accumulator in the detection of water-borne sources of Pb.

CHAPTER I

GENERAL INTRODUCTION

Background

The Portsmouth Naval Shipyard, located on Seavey Island in the Portsmouth Harbor of New Hampshire and Maine, supports marine and estuarine habitats such as salt marsh, intertidal mudflat and rocky shore, as well as submerged eelgrass meadows in depositional areas around the island. Centrally situated in the harbor, the Portsmouth Naval Shipyard (Shipyard) provides repair, overhaul, and refueling of nuclear submarines. The majority of the Shipyard's activities are industrial in nature, primarily occurring in and around three active drydocks. During the last several decades, industrial waste was disposed of by various methods at the Shipyard, many of which were inconsistent with today's waste management regulations. Solid waste management units (SWMUs), identified by an on-shore study, identified waste disposal sites on the Shipyard that might require remediation (McLaren / Hart, 1992). The SWMUs include formerly used disposal areas and industrial waste outfalls, underground storage tanks, active storage areas, and a 25 acre landfill that was used between 1945 and 1975 for waste disposal that included hazardous materials (Johnston *et al.*, 1994).

The Shipyard was issued a Hazardous and Solid Waste Amendments Corrective Action Permit under the Resource Conservation and Recovery Act in 1991. Continued use of the SWMUs under the guidelines of the Corrective Action Permit required an Ecological Risk Assessment (ERA) of the lower

Piscataqua River and Great Bay Estuary. The purpose of the ERA was to assess whether hazardous materials used at the Shipyard imposed any ecological risks to the estuarine environment. The ERA listed lead (Pb) as one of the contaminants of concern in the lower Piscataqua River sediment, exceeding several times the background concentrations of the estuary. During the last 50 years, the Shipyard used Pb for batteries, ballast and radioactive shielding in nuclear submarines. At the time the permit was issued, the Jamaica Island Landfill (JIL) was one of the primary sites at the Shipyard that was suspected to be a contaminant source due to its inadequate construction. Of particular relevance to the present study, areas of the Shipyard that showed biota (blue mussels, *Mytilus edulis* L., and eelgrass, *Zostera marina* L.) and sediment with elevated levels of Pb were proximal to the JIL.

The JIL was established during the early 1940s by filling in an intertidal mudflat long before the ecological value of wetlands was appreciated and before the ramifications of groundwater transport of contaminants were understood. The landfill was not lined with an impervious barrier as landfills are today and received Pb contaminated dredge spoils, plating sludge, waste paints and solvents and spent sand blasting grit in addition to other hazardous materials (Table 1.1). As contaminants became a concern to the Shipyard, the JIL was capped with clay in 1975 to prevent rainwater from percolating through the landfill and transporting hazardous materials into the surrounding estuary. Although the clay barrier prevented water from seeping into the landfill's surface, there was no in-ground barrier to prevent groundwater from flowing through the landfill or to prevent tidal recharge and drainage. Groundwater and tidal water could potentially flow into the landfill, leach hazardous materials from the soil, and transport them into the estuary.

Table 1.1 Partial list of hazardous materials disposed in the JIL between 1945 - 1978 (from ERA Technical Report, 1997).

Waste	Estimated Quantity	Time Period	Comment
Paint Sludges:			
chrome	5,000-10,000 lbs	1945-1972	Mixed with refuse and disposed in unknown locations.
lead	5,000-10,000 lbs		
cadmium	5,000-10,000 lbs		
Dredge Spoils:			
	82,571 cubic yards	1978	Material from Berths 6, 11 and 13; low levels of mercury also present.
chromium	5,000 lbs		
lead	20,000 lbs		
Waste Paints and Solvents	500,000 gallons	1945-1965	Likely disposed in 55 gallon drums.
Spent Sandblasting Grit	5,000 tons/yr	1945-1975	Disposed throughout site.

The ERA determined that "stress and contamination levels measured were indications of chronic exposure". However the ERA was not designed to identify sources of the contamination (Johnston *et al.*, 1994). Although Pb was indicated as a contaminant of concern near the JIL, all biotic collections for the ERA were made by composite sampling, a technique that combines a group of individual samples collected from a large area rather than from a specific collection point. Subsequently, there were two problems with using the composite technique. First, the potential of dilution of contaminants in any sample was great since a sample with high levels of contamination might be combined with a sample of low contaminant levels. Second, because the composites came from a wide sampling area, no sample could directly indicate the JIL as a contamination source.

A potential transport of contaminants from the JIL to adjacent estuarine habitats is by the movement of groundwater through the landfill and by tidal recharge, both of which drain from the island during low tide. At low tide, groundwater seepages are visible along the shore of Seavey Island, particularly along the perimeter of the JIL. The seeps vary in volume and drain into the estuary at Clark and Jamaica Coves. During spring tides of 1994, seep samples were collected and analyzed for dissolved metal content for the ERA. Mussels were also collected at six seep sites and analyzed (ERA Technical Report, 1997). Elevated levels of dissolved Pb were reported in one seep water sample and Pb levels of mussels from several seepage locations were higher than those sampled along the coast by the Gulfwatch Program (GMCME, 1992). Specifically, Pb concentrations reported in the mussels that were collected at seep locations were much higher than concentrations found in the composite mussel samples, suggesting groundwater seepage may have been transporting leachate from the landfill into the estuary. However, the mussel data only implied contaminant sources because mussels ingest sediment and plankton as they filter water. Thus, concentration data from filter feeders cannot be used to identify sources of contamination because one cannot readily discern whether Pb found in the mussel tissue was absorbed from ingested sediment and plankton that are laden with Pb or from dissolved Pb in the water column (Davies, 1978; Phillips, 1980). Lyngby and Brix (1987) also suggested that mussels did not provide as accurate an estimate of available Pb as eelgrass because mussels accumulated Pb from particulates in the water column.

Eelgrass as a Potential Sentinel Accumulator of Lead

Plants accumulate only dissolved materials and for this reason may be more useful in reflecting pollutants in the environment than the typically used filter feeders. Dissolved materials are considered biologically available and can be readily absorbed by or adsorbed onto living organisms. Many marine plants have been studied for monitoring elevated contaminants in estuarine water (Beeftink and Nieuwenhuize, 1982; Ho, 1990; Samecka-Cymerman *et al.*, 1991; Karez *et al.*, 1994). Algae have been shown to accumulate contaminants such as metals. However, age difference between samples, differences in productivity between species and length of tidal submersion are factors that may bias algal tissue contaminant concentrations (Nickless *et al.*, 1972; Bryan and Hummerstone, 1973; Munda and Hudnik 1986; Ferreira, 1991; Tropin and Zolotukhina, 1994). Estuaries are often heavily urbanized and industrialized, while also being the primary seagrass habitat. Seagrasses growing in these environments may provide a sentinel for anthropogenically generated contaminants and their fate in an estuarine ecosystem. Seagrasses are often reported as reflecting contaminant levels present in heavily industrialized areas (Stenner and Nickless, 1975; Lyngby and Brix,1982, 1987; Nienhuis, 1986; Ward, 1987; Carter and Eriksen, 1992; Malea and Haritonidis, 1995). Many studies have contributed to what is currently known of interactions between seagrasses and metal uptake in estuarine systems. Much of the laboratory experimentation conducted by Lyngby and Brix (1984, 1987, 1989) indicates that eelgrass can be used for *in situ* experimentation; their work provided much of the background information for the present study.

Seagrass communities are among the most productive ecosystems, occurring throughout much of the world in shallow coastal waters (den Hartog, 1970; Phillips and Meñez, 1988). Along with a high level of productivity, seagrasses provide many important functions to estuaries. Many of these functions have been elucidated in studies of the north temperate eelgrass (Thayer *et al.*, 1975, 1984; Kenworthy *et al.*, 1982; Phillips and Meñez, 1988). Eelgrass meadows provide shelter for marine organisms, many of which are commercially important (Thayer and Stuart, 1975; Orth *et al.*, 1984; Hoven, 1992). Short and Short (1984) demonstrated that eelgrass is a catalyst for sediment deposition, contributing to water clarity. Eelgrass also plays an important role in nutrient and metal cycling (Burrel and Schubel, 1977; Drifmeyer *et al.*, 1980; Brix and Lyngby, 1983, Short and McRoy, 1984).

Many researchers have investigated the potential for seagrasses to reflect contaminants of the surrounding environment. It has been suggested that seagrasses, including eelgrass, could be used as an indicator organism for heavy metals, implying that they could be used to monitor changes in metal concentrations in the environment (Nienhuis, 1986; Brix and Lyngby, 1983).

However, the term "indicator organism" or " indicator species" refers to organisms that reflect a negative effect of a contaminant by a change in species abundance (Preston *et al.*, 1972; Gray and Pearson, 1982). In recent studies, seagrasses have been termed "sentinel accumulators", organisms which reflect the degree and spatial variation of contamination based on their tissue metal concentration (Ward, 1987; Carter and Eriksen, 1992). Sentinel accumulator is the most appropriate term to use for the present study because eelgrass was used to determine the spatial distribution and degree of Pb contamination relative to the JIL.

Metal Uptake by Seagrasses

Toxic metals often accumulate in near-shore sediments of embayments near industrial and urban areas (Khalid *et al.*, 1978; Delaune and Smith, 1985, Kennish, 1997). Numerous works have shown the importance of marine angiosperms in the processes of accumulating and cycling heavy metals in marine coastal systems (Wolfe *et al.*, 1976; Burrel and Schubel, 1977; Gallagher and Kibby, 1980; Giblin *et al.*, 1980; Penello and Brinkhuis, 1980; Beeftink *et al.*, 1982; Orson *et al.*, 1992) and associated sediments (Lion and Leckie, 1982; Brix and Lyngby, 1983; Alberts *et al.*, 1990).

There are two methods of ion uptake in plants: active transport and passive transport (Salisbury and Ross, 1978). Active transport involves moving ions against an electrochemical gradient across cell plasmalemmas. In the case of root cells, active ion uptake occurs in the cortical cells via electron carriers. Once inside the cortical cells, ions are transported along a network of cytoplasmic spindles into neighboring cells until entrance into the xylem is gained and upward transport to other organs begins. Passive ion uptake is driven by concentration gradients and involves apoplastic diffusion of ions across cell walls between intercellular spaces. With regard to seagrasses, studies have shown that Pb is primarily taken up by the latter process in both leaves and roots (Bond *et al.*, 1985; Everard and Denny, 1985). Crystalline or amorphous deposits of Pb can bind to the outside of cells, within cell walls as is often the case in eelgrass, or can be deposited in vesicles, all of which keep Pb from interfering with a plant's metabolic processes (Maeda *et al.*, 1966; Lyngby and Brix, 1989). Because Pb is often sequestered in plant cell walls, plants act as sinks for Pb, enabling plants such as eelgrass to tolerate levels of Pb that are highly toxic to humans and other organisms (Sharpe and Denny, 1976; Lyngby and Brix, 1984).

Lead can be translocated within some plants and phytotoxic effects have been reported (Mayes *et al.*, 1977; Welsh and Denny, 1979; Heisey and Damman, 1982; Påhlsson, 1989; Guilizzoni, 1991). Welsh and Denny (1979) found insignificant translocation of Pb in two aquatic angiosperms. It is not clear, however, whether eelgrass translocates Pb or whether eelgrass exhibits signs of Pb toxicity (Lyngby and Brix, 1984). In general, some of the reported physiological effects of Pb on terrestrial plants are decreased photosynthetic and transpiration rates with increasing Pb concentration, inhibition of chlorophyll biosynthesis, decreased biomass, and reduced root elongation (Khalid *et al.*, 1978; Heisey and Damman, 1982; Clijsters and Van Assche, 1985; Barua and Jana, 1986; Påhlsson, 1989).

Lyngby and Brix (1982, 1989) demonstrated metal partitioning within eelgrass such that various metals showed specificity for above or below ground tissue and accumulate in different quantities and locations within eelgrass (Brix and Lyngby, 1983). Specifically, the highest concentration of Pb was found in roots in comparison to rhizomes of naturally occurring eelgrass, however there was no significant difference between above and belowground Pb accumulation. Lyngby and Brix (1983) found concentration of Pb increased with age of eelgrass leaves. Significant leaf age-dependent patterns of other metals have been observed in *Zostera noltii* Hornem. (Wasserman and Lavaax, 1991). In addition to spatial variation of Pb levels within eelgrass, Pb concentration in eelgrass belowground tissue varied seasonally but was attributed to growth patterns of the plant rather than difference in Pb loading rates within the environment (Lyngby and Brix, 1982). Thus, seasonal variation in Pb accumulation has been observed in eelgrass and other seagrasses and should be considered when planning a sampling schedule (Lyngby and Brix, 1982; Ward, 1987; Malea and Haritonidis, 1992).

Lyngby and Brix (1982) found proportionality between Pb concentrations in sediments and belowground eelgrass tissue Pb of naturally growing eelgrass shoots. They also found that initial Pb uptake by eelgrass tissue was proportional to the concentration of Pb in water of hydroponically grown eelgrass (Lyngby and Brix, 1984). In a later study, comparison of tissue Pb concentrations of eelgrass and blue mussels with sediment Pb concentrations indicated that eelgrass Pb concentrations gave a more accurate representation of Pb pollution than mussels did (Lyngby and Brix, 1987).

Biotic and Abiotic Factors Affecting Lead Availability

Other biological controls may affect the availability of Pb besides growth dynamics of eelgrass, such as mobilization and cycling caused by microbial activity and bioturbation (Hines, 1981). Bioturbation creates microzones of oxidation within anoxic sediments caused by the burrowing, pumping or irrigation and flushing activities of invertebrates. Acid volatile sulfides

(AVS), that are buried within the reducing zone are re-exposed to oxic conditions by bioturbation activity where they may be readily released as hydrogen sulfide (H_2S) and free metal ions for uptake by plant roots or animals, transport within interstitial water, complexation with organic ligands such as humic acids, or re-formation of sulfides. In addition to invertebrate bioturbation as a mechanism for oxidizing reduced sediment, eelgrass itself has a mechanism that oxygenates the sediment of its rhizosphere (Sand-Jensen *et al.*, 1982). Eelgrass oxygenates its rhizosphere by providing for oxygen diffusion down its lacunae and into the aerenchyma of rhizomes and roots. The oxygen diffuses outward into the surrounding sediment, oxidizing reduced compounds so that metals associated with acid volatile sulfides (AVS) become bioavailable for root uptake.

In general, Pb is a strongly sorbing metal (McBride, 1994). Although there are biological factors that may influence bioavailability of Pb in the marine environment (as described above), the majority of factors controlling lead's availability are physicochemical in nature. When marine sediment is experimentally oxygenated, hydrogen ions are released from oxidation lowering the sediment pH and increasing the redox potential, thereby releasing Pb and other trace metals into solution (Khalid *et al.*, 1978; Delaune and Smith, 1985). Bioturbation can have the same effect (Hines, 1981). Khalid et al. (1978) found that as little as 0.1% oxygen purged through reduced marine sediment increased Pb concentration in the water column by 25 - 30 %. Lead was present as hydrated divalent cations (soluble), as complexes of chlorides and sulfides (soluble), and as hydroxides (limited solubility). Even though AVS are generally a small fraction of total sulfur in marine sediments, their importance as sinks for heavy metals can be great, particularly if spoils from dredged marine sediments are to be deposited on land where the reduced sulfides would oxidize and release metals (Delaune and Smith, 1985). However, in the microzone of the eelgrass rhizosphere, AVS may be absent or unable to retain Pb due to oxidation from the roots. Although hydroxides act as scavengers of metal ions, the decrease in pH due to oxidation may be enough to reduce hydroxides, and release Pb into solution (Khalid *et al.*, 1978; Delaune and Smith, 1985). The presence of organic matter can greatly increase the concentration of soluble Pb as well (Lindberg and Harris, 1974; Khalid *et al.*, 1978).

Physicochemical controls of Pb bioavailability and thus, ion uptake, are related to tidal flux and mixing. During the onset of flood tide, Balls et al. (1994) found high concentrations of particulate matter resuspended from bottom sediment of the Forth estuary, Scotland. The suspended particles tended to scavenge Pb from solution before salinity increased, resulting in raising the Pb concentration of suspended particles. Removal of trace metals from solution by high concentrations of particles was also important in the low salinity region of the Tamar estuary of England (Morris, 1986). After the mixing of ocean and lower saline water occurs, salinity of the estuary increases and flocculation results from increased pH and ionic strength of the water. Lead (and iron oxides / hydroxides) precipitates out of the water column (Elbaz-Poulichet *et al.*, 1984; Balls *et al.*, 1994). Thus, salinity is a major control of soluble Pb concentrations in the water column.

Trace elements are also known to concentrate on and in detrital tissue in marine environments (Ragsdale and Thorhaug, 1980; Rice and Windom, 1982). Upon degradation of intact membranes, more organic surfaces become charge unsaturated, providing sites for metal sorption. As eelgrass leaves decompose, Pb continues to adsorb to the leaf material from the surrounding water (Lyngby and Brix, 1989). Since eelgrass is a source of food for detritivores, Pb may transfer up the marine food chain. However, bioamagnification of Pb in aquatic plants and animals has not been observed (Ward *et al.*, 1986; Prosi, 1989).

Focus of the Present Study

The goal of my work was to test whether eelgrass could be used to indicate sources of anthropogenically introduced Pb in estuarine water near the Shipyard. Eelgrass is an appropriate species to use for several reasons. Portsmouth Harbor is well within its distributional range. Its continual leaf renewal makes it useful for studying effects of contaminant exposure. Because eelgrass is easily transplanted and deployed, it can be collected from uncontaminated locations and placed in potentially contaminated locations (Phillips, 1974; Short, 1987; Fonseca *et al.*, 1994, Davis and Short, 1997; Hoven, 1992). Four experiments were designed to address the main goal.

I was interested in testing whether a component of the sediment in Clark and Jamaica Coves impeded eelgrass growth. Although both Jamaica and Clark Coves have protected shallow water habitat, natural beds of eelgrass were only found at the mouths of the coves. Both coves were also adjacent to the landfill. Should eelgrass be restored within the coves, I wanted to investigate the fate of Pb in eelgrass that is transplanted into Pb polluted sediment.

Sediment was collected from locations around the perimeter of both coves and was contained in mesocosms constructed for eelgrass culture. Using mesocosms allowed control for environmental characteristics such as water quality and light that may differ among natural settings.

Eelgrass was also grown in sediment that was heavily enriched with Pb and in sediment with moderate Pb contamination that was collected near the Shipyard. I was interested in assessing whether physiological effects of Pb on eelgrass could be detected. I was also interested in determining whether eelgrass density affected the amount of Pb accumulated in eelgrass tissue. Eelgrass was grown at three densities in marine sediment of three Pb concentrations to address these questions.

Until my study, only naturally growing beds of eelgrass have been used to indicate Pb pollution in industrialized areas. No research has been done to test whether hydroponically deployed eelgrass can be used to reflect Pb in the environment. To separate and identify water-borne Pb from sediment Pb, eelgrass was deployed unrooted in the water column. By keeping eelgrass from directly contacting the sediment, the ability of eelgrass to monitor Pb in the water column could be tested. Eelgrass deployments were placed around the perimeter of the Shipyard near the JIL in both Clark and Jamaica Coves. Some of the deployments were placed directly offshore from water seepages that were suspected of Pb contamination to test the eelgrass as a sentinel accumulator for water-borne Pb inputs to the estuary.

Finally, eelgrass was tested as a sentinel accumulator of Pb inputs to the estuary. Common Pb isotopic composition was determined of the eelgrass that was deployed in Clark and Jamaica Coves near water seepages. The isotope ratio $^{206}Pb/^{207}Pb$ has been used to indicate sources of industrial Pb versus naturally occurring (ambient) Pb (Flegal *et al.*, 1987; Vernon *et al.*, 1994; Gobeil *et al.*, 1995). Lead isotope ratios were compared among water from seeps, sediments, and deployed eelgrass to determine whether sources of water-borne Pb contamination could be indicated by eelgrass and whether a pathway of Pb contamination from groundwater to the estuary could be identified.

Collectively, the experiments described in this thesis will address the use of eelgrass as a sentinel accumulator of Pb pollution in estuarine waters. The research also addresses whether eelgrass distribution in the vicinity of the Shipyard is limited by some component of marine sediment in that area and the implications of restoring eelgrass habitats near the Shipyard where sediment Pb concentration are elevated above background levels.

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PART I

EELGRASS MESOCOSM RESEARCH

CHAPTER II

GROWTH AND MORPHOLOGY OF EELGRASS (*Zostera marina* L.) IN SEDIMENT COLLECTED FROM SITES AROUND THE PORTSMOUTH NAVAL SHIPYARD

Abstract

Eelgrass was transplanted into mesocosms that contained sediment which ranged from 23 to 142 ppm Pb. The sediment was collected from sites near the Portsmouth Naval Shipyard Jamaica Island Landfill (JIL) to test viability of eelgrass in the Shipyard's marine sediment. Lead accumulated in eelgrass tissue and appeared to have stunted rhizome growth during the first month of exposure, but did not affect growth during the second month. Lead concentration of the mesocosm grown eelgrass was not significantly higher than Pb concentrations of reference shoots. Additional eelgrass growth metrics and sediment textural analysis confirmed that the mesocosm-grown eelgrass was not affected by physical or chemical characteristics of the sediment after two months' exposure. These data provide supporting evidence that eelgrass is well suited to grow within Clark and Jamaica Coves of the Shipyard.

INTRODUCTION

In New Hampshire, the seagrasses, *Zostera marina* L. and *Ruppia maritima* L., are the only submerged vascular plants that grow in estuarine waters. *Z. marina*, known as eelgrass, is prevalent in many protected coves of New Hampshire coastal waters, including areas near the Portsmouth Naval Shipyard (Shipyard), which is located on Seavey Island, Maine. Unlike algae that absorb nutrients and contaminants from the water column only, eelgrass grows rooted in the sediment, absorbing nutrients and contaminants from both sediment and water (Lyngby and Brix, 1982). Known for their ability to anchor sediment, eelgrass meadows are often found in depositional areas. As the long eelgrass leaves tend to baffle water flow and filter particulates from the water column (Short and Short, 1984), heavy metals from local industrial activities and natural sources are accumulated by live and dead eelgrass tissue (Fenchel, 1977; Drifmeyer *et al.*, 1980; Brix and Lyngby, 1983). Although some heavy metals are transported from roots to leaves and can have toxic effects on eelgrass metabolism, lead translocation has been shown to be insignificant in other aquatic angiosperms and toxic effects of Pb uptake by eelgrass have not been observed (Welsh and Denny, 1979; Lyngby and Brix, 1984).

Elevated levels of lead (Pb) have been identified in the marine sediment surrounding the Jamaica Island Landfill (JIL) at the Shipyard (Johnston *et al*, 1994). Although natural beds of eelgrass persist at the mouths of both Clark and Jamaica Coves, eelgrass was not present along any of the shorelines adjacent to the JIL. Because eelgrass is an endemic species in Portsmouth Harbor and it has been shown to tolerate elevated levels of Pb (Lyngby and Brix, 1892, 1984, 1987), I was interested in determining whether there was a component of the Shipyard sediment which precluded its growth.

METHODS

An accepted and widely used experimental method for growing eelgrass in a way that mimics its natural habitat is the use of mesocosms, which are large above ground tanks that have a flow-through seawater system (Short, 1987; Short et al, 1993, 1995; Moore et al, 1995). Eelgrass was grown in mesocosms (1.5 m^2) with marine sediment that was collected offshore of the Shipyard. The mesocosms were located at the easternmost point of Seavey Island and received a southeastern exposure of sunlight. Water was drawn from 50 m offshore and pumped into a head tank from which the water was gravity fed into the mesocosms. The average Pb concentration of Portsmouth Harbor water was 0.100 $\mu g \cdot L^{-1}$ (Cullen and Arimato, 1995).

Shoots of eelgrass were collected from a donor site near Fishing Island in Kittery, Maine, because previous collections were shown to have low levels of tissue lead (1.08 ± 0.06 ppm, Short, 1995). Sediment was collected from 17 sites in Clark and Jamaica Coves (Fig. 2.1) with modified, stainless steel oyster tongs. Six 5 L buckets were filled with approximately 2.5 L of homogenized sediment from each site and were randomly dispersed among eight mesocosms. Some of the sediment was collected directly offshore from the JIL and was suspected of having elevated Pb due to groundwater seepage from the island. Sediment from Adams Cove in Great Bay, New Hampshire, was used as control sediment (36 ppm Pb) and was prepared in the same manner. Each bucket was planted with 15 eelgrass shoots that were held in place with bent bamboo skewers (Davis and Short, 1997) and were allowed to acclimate for three weeks in the flow-through seawater. Each shoot had a wire tag that was loosely wrapped around its rhizome between the third and

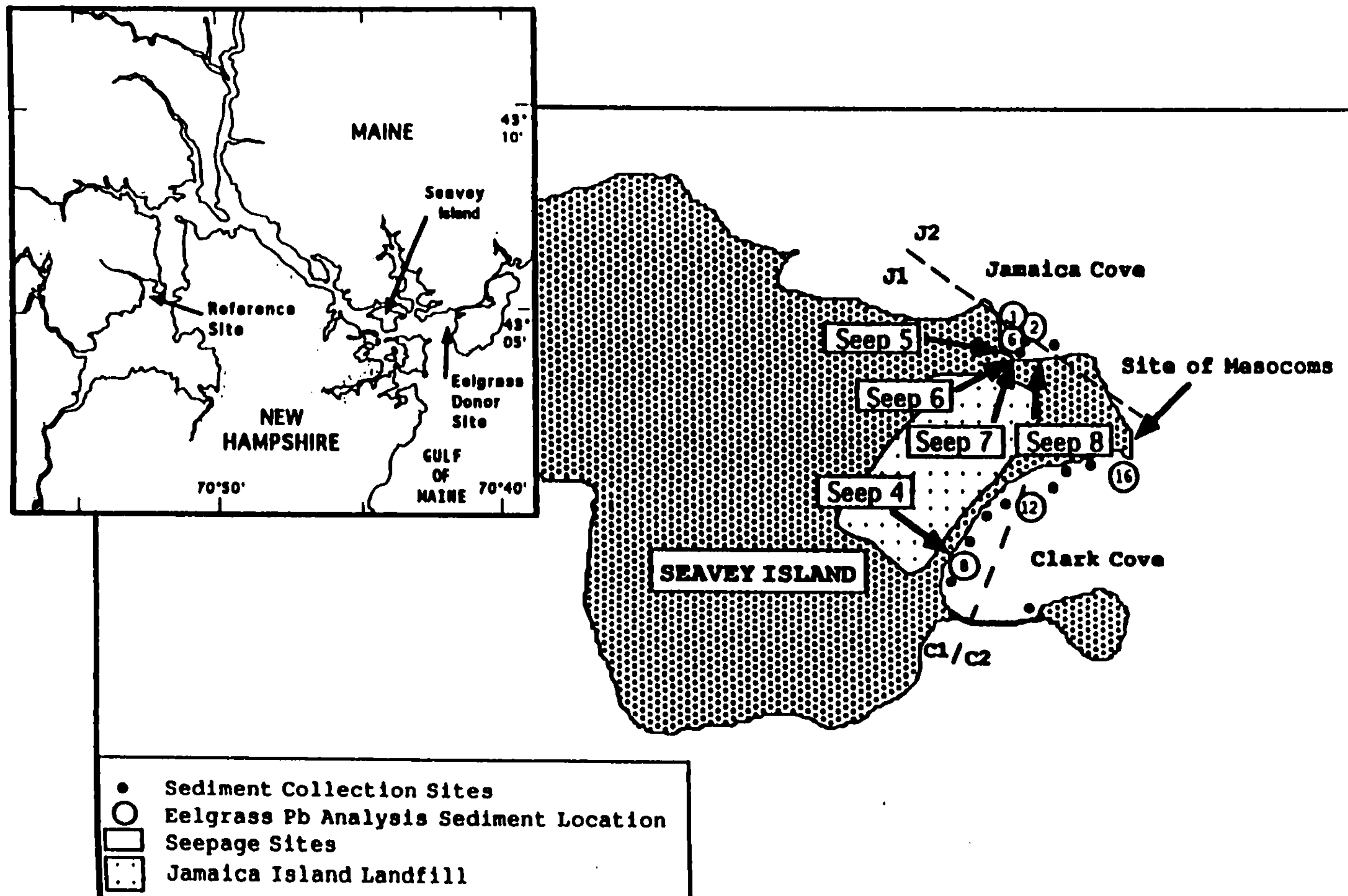

Figure 2.1. Location of sediment collection sites, water seepage and the JIL of Seavey Island, Maine. Sediment locations corresponding to eelgrass that was analyzed for Pb concentration are circled and numbered. Clark and Jamaica Cove stations are divided by inner and outer cove subgroups: C1, C2 and J1, J2, respectively.

fourth nodes. The wire served as a mark for determining new growth of the rhizome. During the fourth week, three shoots in each bucket were marked with a 20 gauge needle hole through the bundle sheath just above the meristem and left to continue growing for one week. After one week, the marked shoots were collected for growth and morphological measurements as described by Short (1987). An additional shoot per bucket was collected at the same time for later lead analysis.

Above ground growth rates were determined by first measuring the length from the meristem to the distal end of each leaf. How much new tissue grew between the meristem and the mark was measured by using the mark of the outermost, nongrowing sheath of the shoot as a reference point. The width at the mid-length of each leaf was also measured to determine the total area of leaf tissue. The number of leaves of each shoot were counted, scraped free of epiphytic algae and cleaned of sediment with distilled, deionized water and dried at 80°C to a constant weight. Rhizomes were separated from the growth analysis shoots, and roots were removed. The length of new versus old rhizome growth was measured, rinsed of sediment with distilled, deionized water and dried at 80°C to a constant weight.

Specific growth (mg new leaf tissue $\cdot$ mg total leaf tissue $^{-1}$ $\cdot$ day $^{-1}$) was calculated from the leaf measurements and weights. Also determined were the leaf turnover $\cdot$ day $^{-1}$ (inverse of specific growth) and number of days per leaf initiation (leaf turnover rate divided by number of leaves) as described by Dennison (1990). Vegetative vigor was determined from the number of nodes produced per shoot divided by the number of days growth. New rhizome growth per day was also calculated. Growth and morphological measurements were repeated a second time after another month of exposure to the sediment. At the time of each growth collection, density of terminal, lateral, and reproductive shoots in each bucket was counted.

In order to determine whether sediment types affected growth and morphology of eelgrass, particle size fractions were determined from a representative eight of the 18 sampling locations. Three of the eight sediment samples were selected from locations that were naturally vegetated with eelgrass, while the remaining five were from non-vegetated locations allowing for comparison of sediment characteristics between vegetated and non-vegetated sites. Percent gravel, sand, silt and clay were determined according to Ward (1992).

Eelgrass tissue samples were compiled from the growth analysis shoot collections and were prepared for Pb concentration analysis in the following way. Three eelgrass samples from Clark Cove sediments and three from Jamaica Cove sediments were analyzed for Pb concentration. Leaf and rhizome tissue were ground separately by hand with a clean ceramic mortar and pestle. As much as 0.5 g of each sample was weighed in acid cleaned teflon capsules and digested with 7.5 N HNO_3. Lead concentration was determined using inductively coupled plasma atomic emission spectrometry (ICP-AES) at New Hampshire Materials Lab of Somersworth, NH.

Samples of marine sediment that corresponded to eelgrass Pb samples were collected for determination of lead concentration. Sediment samples were dried at 80°C for 48 hr and disaggregated with a clean ceramic mortar and pestle. Lead was extracted from 3 g of sediment with 3N HNO_3 for 24 hr and concentrations were first determined using Flame Atomic Absorption Spectrophotometry (FAAS) according to specifications made by the Environmental Protection Agency at the Analytical Services Laboratory of

The University of New Hampshire. However, a repeated analysis using ICP-AES of the sediment revealed that FAAS was less sensitive and showed higher error. Thus, the ICP-AES data was used in the statistical analysis of the results.

Statistical comparisons among eelgrass growth, morphological data, and eelgrass tissue and sediment Pb concentrations were made to see whether Pb in the sediment could be associated with a negative impact on eelgrass growth and survival. One-way ANOVA models were used to test for differences between growth and morphological patterns of eelgrass growing in the different sediment treatments. Analysis of residuals showed that assumptions of homogeneity of variance and normal distribution were met.

In order to make it easier to identify the location of where sediment was collected, subgroups of the sediment collection sites were formed based on their distance from the JIL. Eelgrass growing in sediment from locations within Jamaica and Clark Coves was grouped as "inner" and "outer" portions of the coves and compared with shoots grown in control sediment, after initial statistical comparisons among all 18 sediment treatments showed no consistent patterns in the growth data. Subgroups of the data were as follows: inner Jamaica Cove (J1) designated stations 4 - 6; outer Jamaica Cove (J2) designated stations 1 - 3; inner Clark Cove (C1) designated stations 7 - 11; outer Clark Cove (C2) designated stations 12 - 17; and the reference sediment collected at Adams Cove (Reference) was station 18.

RESULTS

Sediments were similar in grain size based on the subgroup of textural analyses. Seven of the eight representative sediment samples were characterized as sandy mud, while the eighth was muddy sand (Table 2.1). The outer Jamaica Cove sample from station 1 was near the shore but close to the channel and may have been exposed to higher energy of water movement than the other locations, explaining the high percentage of sand. Sediment characterizations showed various ratios of sand - silt - clay and had mean phi values that ranged from 4.9 - 6.3. Additionally, sediment from two of the three marine locations that were naturally vegetated with eelgrass was characterized as sandy mud an was no different from sediment from marine locations that were non-vegetated.

The growth data showed typical correlations of eelgrass growth characteristics (Tables 2.2, 2.3). For instance, various measures of growth and expansion correlated positively with each other such as LA and leaf width; shoot density and laterals produced $\cdot$ shoot $^{-1}$ $\cdot$ day $^{-1}$; and cm $\cdot$ m $^{-2}$ $\cdot$ day $^{-1}$ and g $\cdot$ m $^{-2}$ $\cdot$ day $^{-1}$. Similar positive correlations held true during the second month. There were no significant correlations between the growth metrics measured and sediment Pb concentration.

Eelgrass generally grew well in sediment from Jamaica and Clark Coves as well as the reference site. All ANOVA growth models were first blocked on tank effect to verify no difference among the mesocosms. Vegetative vigor ranged from 0.34 - 0.48 nodes $\cdot$ shoot $^{-1}$ $\cdot$ day $^{-1}$ and did not vary much between months (Fig. 2.2). The data from the two coves combined showed no statistical difference in vegetative vigor between shoots that grew in sediment from inner versus outer cove areas or between the grouped coves versus the reference site during the first month (P-value = 0.956) or during the second month (P-value = 0.510). However, when vegetative vigor in Jamaica Cove was compared to the reference site, shoots were found to be more vigorous in

Table 2.1. Characteristics of Shipyard marine sediment. Location descriptions are Jamaica Cove (JC), Clark Cove (CC), and vegetated with eelgrass (E).

Location, Description	Mean Phi	Sort	% Sand	% Silt (z)	% Clay	Description
1, Outer JC, E	4.94	1.42	58	24	18	mS
3, Outer JC, E	5.79	1.02	36	41	23	sM
5, Inner JC	6.27	1.08	27	47	26	sM
7, Inner CC	5.88	2.69	22	51	27	sM
10, Inner CC	5.98	1.86	32	40	28	sM
12, Inner CC	5.83	1.40	42	36	22	sM
15, Outer CC	6.18	1.1	33	42	25	sM
16, Outer CC, E	5.75	1.18	43	34	23	sM

Table 2.2. Correlation matrix of eelgrass growth and morphology with sediment Pb concentration during month one. Significant correlations are shown (n = 12).

Variable	Width mm	Lats/sht/day	Nodes/sht/day	Rhiz cm/day	Density/m2	mg/shoot/day	mg/mg/day	cm/shoot/day	cm/cm/day
Width mm	1.000								
Lats/sht/day		1.000							
Nodes/sht/day			1.000						
Rhiz cm/day				1.000					
Density/m2		0.809			1.000				
mg/shoot/day						1.000			
mg/mg/day						0.642	1.000		
cm/shoot/day								1.000	
cm/cm/day							0.929		1.000
Longest Leaf									
cm/m2/day					0.679	0.708	0.677		0.724
gram/m2/day					0.706	0.867	0.604		
tot. wt/tot. L									
PI(days/node)			-0.873						
Leaf turnover						-0.609	-0.884		-0.802
Leaf initiation						-0.591	-0.722		-0.679
Leaf Area	0.684								
Sed. Pb (ppm)									

Table 2.2.

Variable	Longest Leaf	cm/m2/day	gram/m2/day	tot. wt/tot. L	Plast. Int.	Leaf turnover	Leaf initiation	Leaf Area	Sed. Pb (ppm)
Width mm									
Lats/sht/day									
Nodes/sht/day									
Rhiz cm/day									
Density/m2									
mg/shoot/day									
mg/mg/day									
cm/shoot/day									
cm/cm/day									
Longest Leaf	1.000								
cm/m2/day		1.000							
gram/m2/day		0.849	1.000						
tot. wt/tot. L				1.000					
PI(days/node)					1.000				
Leaf turnover						1.000			
Leaf initiation						0.880	1.000		
Leaf Area								1.000	
Sed. Pb (ppm)									1.000

Table 2.3. Correlation matrix of eelgrass growth and morphology with sediment Pb concentration during month two. Significant correlations are shown. (n = 12)

Variable	Width mm	Lats/sht/day	Nodes/sht/day	Rhiz cm/day	Density/m2	mg/shoot/day	mg/mg/day	cm/shoot/day	cm/cm/day
Width mm	1.000								
Lats/sht/day		1.000							
Nodes/sht/day			1.000						
Rhiz cm/day			0.635	1.000					
Density/m2		0.589			1.000				
mg/shoot/day						1.000			
mg/mg/day							1.000		
cm/shoot/day						0.867	0.675	1.000	
cm/cm/day							1.000	0.674	1.000
Longest Leaf									
cm/m2/day					0.593				
gram/m2/day		0.642			0.694	0.851		0.669	
tot. wt/tot. L									
Plast. Int.			-0.645						
Leaf turnover							-0.863	-0.602	-0.863
Leaf initiation						-0.629	-0.704	-0.778	-0.702
Leaf Area									
Sed. Pb (ppm)									

Table 2.3.

Variable	Longest Leaf	cm/m2/day	gram/m2/day	tot. wt/tot. L	Plast. Int.	Leaf turnover	Leaf initiation	Leaf Area	Sed. Pb (ppm)
Width mm									
Lats/sht/day									
Nodes/sht/day									
Rhiz cm/day									
Density/m2									
mg/shoot/day									
mg/mg/day									
cm/shoot/day									
cm/cm/day									
Longest Leaf	1.000								
cm/m2/day		1.000							
gram/m2/day		0.664	1.000						
tot. wt/tot. L				1.000					
Plast. Int.					1.000				
Leaf turnover						1.000			
Leaf initiation						0.791	1.000		
Leaf Area	0.615							1.000	
Sed. Pb (ppm)									1.000

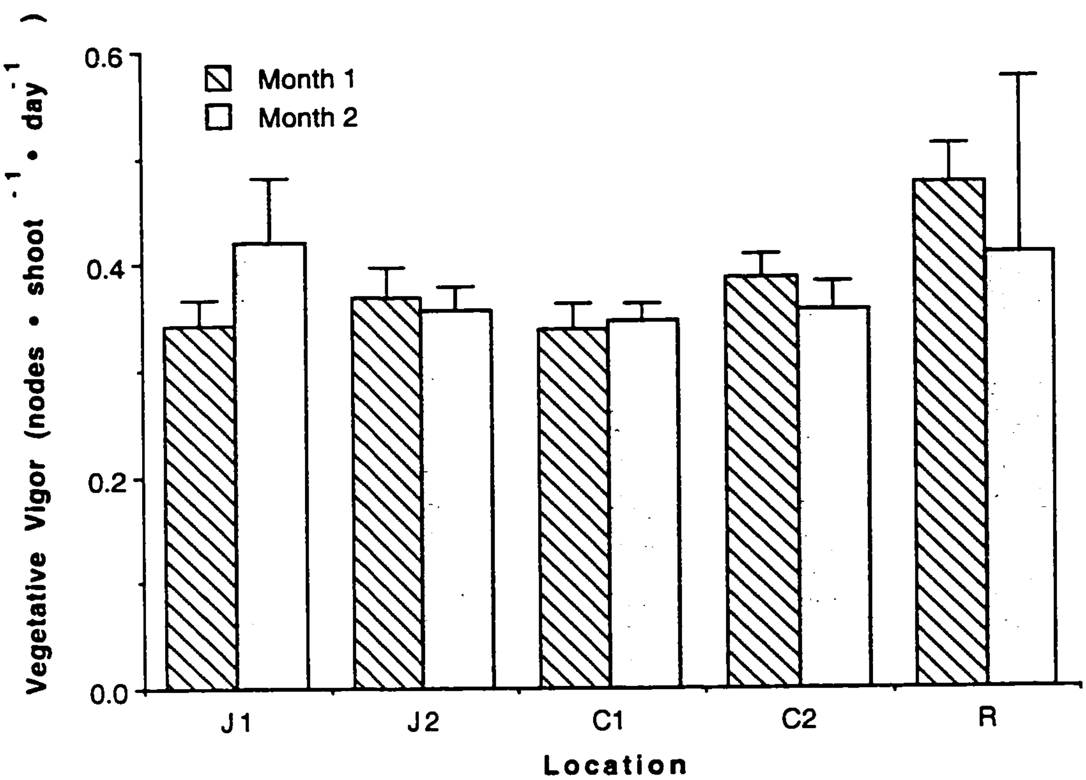

Figure 2.2. Vegetative vigor of shoots growing in mesocosm sediment from inner and outer Jamaica Cove (J1, J2), inner and outer Clark Cove (C1, C2) and the reference site (R). Mean ± se.

the reference sediment during the first month (Table 2.4). The same trend occurred between shoots growing in Clark Cove and reference sediment.

Below ground growth of rhizomes was slightly higher during the first month (0.21 - 0.44 cm · shoot $^{-1}$ · day $^{-1}$) than during the second month (0.22 - 0.30 cm · day $^{-1}$; Fig. 2.3). However, there was no statistical difference between the two coves and the reference sediment (P-value = 0.58) both months. During the first month, however, rhizomes of shoots growing in the reference sediment grew faster than rhizomes of shoots growing in inner Jamaica Cove (Table 2.4).

Above ground growth (as cm · shoot $^{-1}$ · day $^{-1}$) ranged from 2.47 - 3.8 with no statistical differences among the shoots during the first month (P-value = 0.524, Fig. 2.4). During the second month, above ground growth was slightly higher, ranging from 3.49 - 5.05 but again showed no significant difference among treatments (P-value = 0.619). Shoots from both Jamaica and Clark Coves grew comparably with reference shoots regardless of the location from which the sediment was collected.

Specific growth remained similar between months, ranging from 0.012 - 0.015 mg · mg $^{-1}$ · day $^{-1}$ during the first month and 0.016 - 0.021 mg · mg $^{-1}$ · day $^{-1}$ during the second month (Fig. 2.5). Specific growth did not vary among shoots sampled during the first (P-value = 0.100) or second (P-value = 0.535) month. There were no significant differences among reference treatments and both cove treatments during both months.

New leaves were produced every 15 to 22 days during the first month and production increased to generate leaves every 12 to 17 days during the second month (Fig. 2.6). There were no statistical differences among sediment treatments during month one (P-value = 0.132) or month two (P-value = 0.470).

Table 2.4. ANOVA of growth paramters of eelgrass rhizomes (vegetative vigor, nodes · shoot $^{-1}$ · day $^{-1}$; and growth, cm · shoot $^{-1}$ · day $^{-1}$) growing in Jamaica Cove sediment during the first month.

Vegetative Vigor	df	SS	MS	F-value	P-value
JC x Reference	2	0.110	0.055	6.386	0.005
Residual	31	0.267	0.009		

Rhizome Growth	df	SS	MS	F-value	P-value
JC x Reference	2	0.339	0.267	4.832	0.015
Residual	31	1.087	0.035		

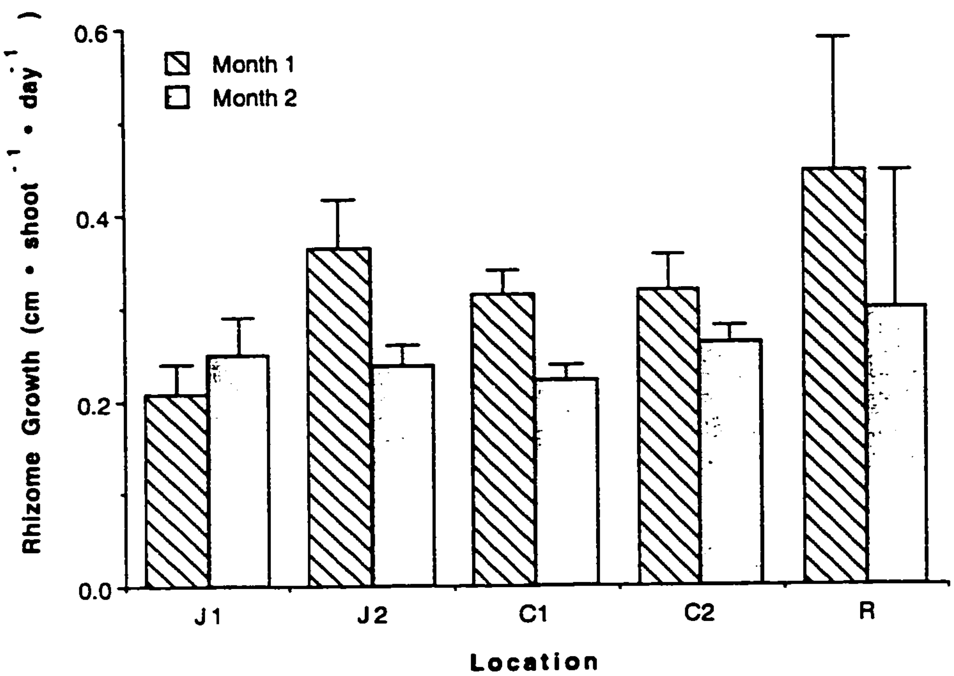

Figure 2.3. Below ground growth rate of shoots growing in mesocosm sediment from inner and outer Jamaica Cove (J1, J2), inner and outer Clark Cove (C1, C2) and reference site (R). Mean ± se.

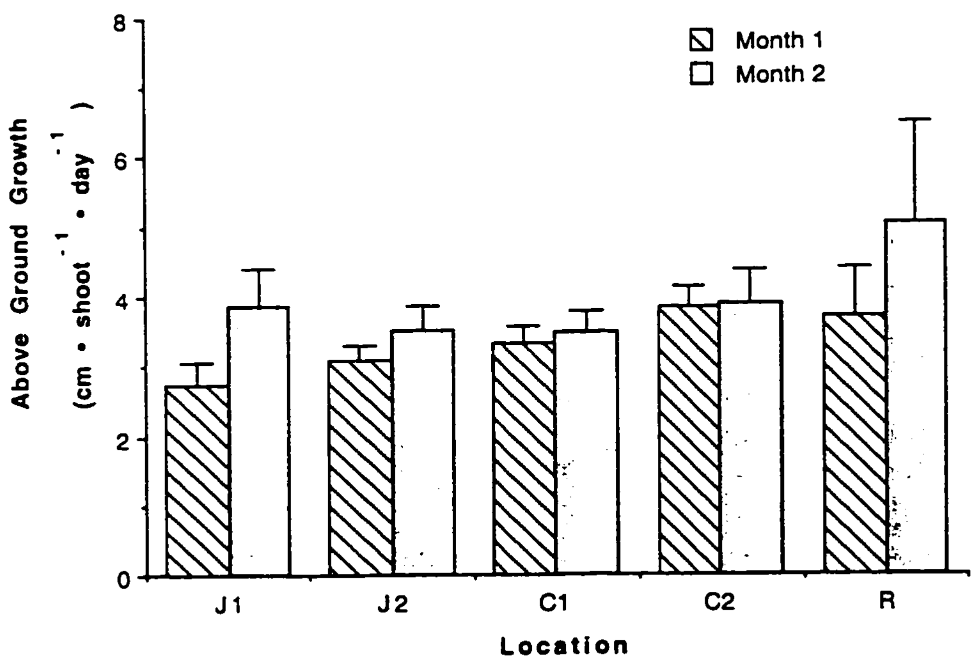

Figure 2.4. Above ground growth of shoots growing in mesocosm sediment from inner and outer Jamaica Cove (J1, J2), inner and outer Clark Cove (C1, C2) and reference site (R). Mean ± se.

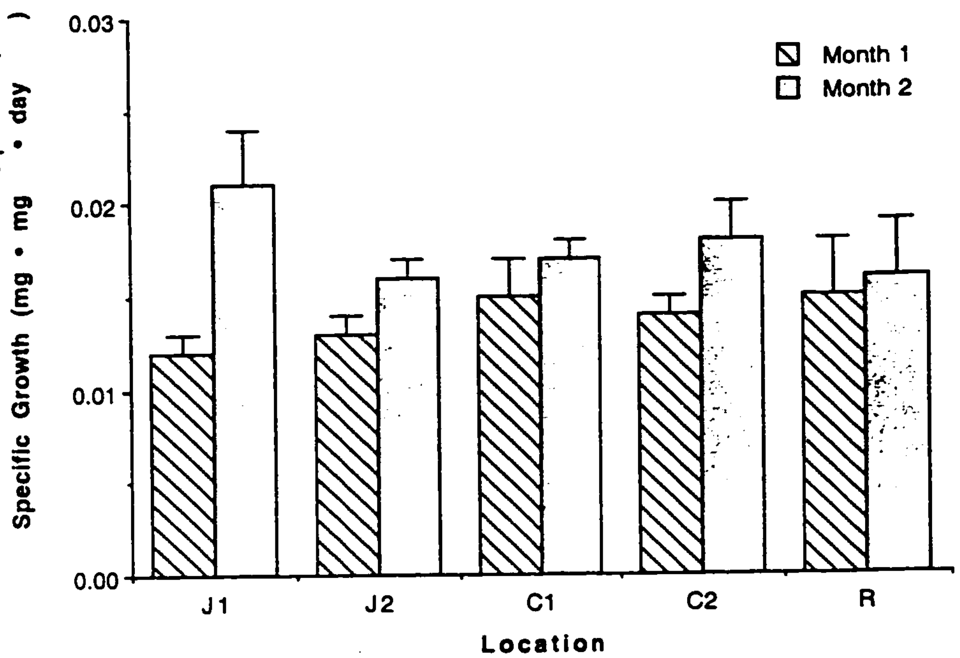

Figure 2.5. Specific growth of shoots growing in mesocosm sediment from inner and outer Jamaica Cove (J1, J2), inner and outer Clark Cove (C1, C2) and reference site (R). Mean ± se.

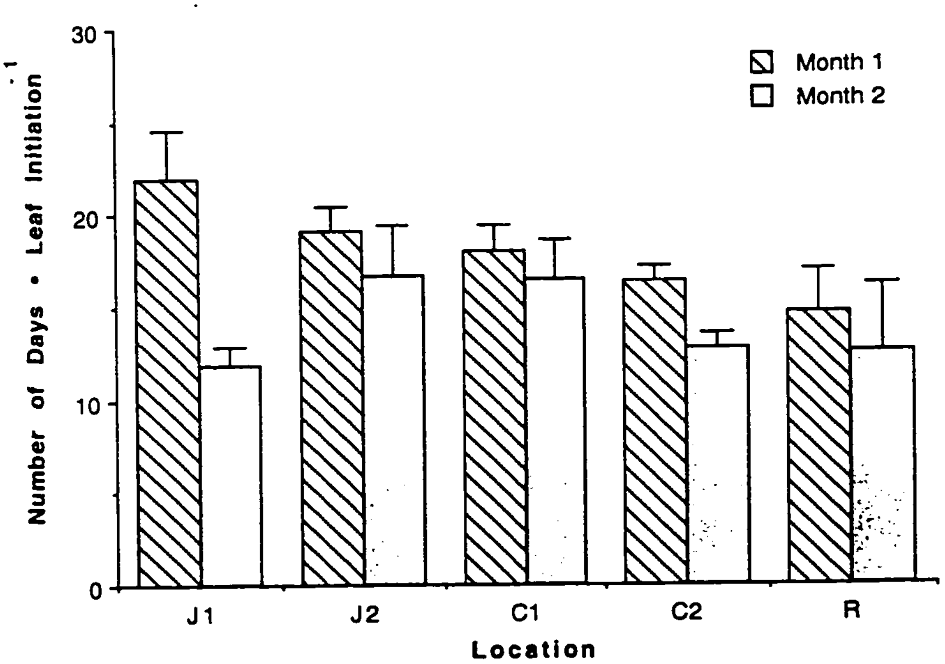

Figure 2.6. Leaf initiation rates of shoots growing in mesocosm sediment from inner and outer Jamaica Cove (J1, J2), inner and outer Clark Cove (C1, C2) and reference site (R). Mean ± se.

Leaf turnover rates ranged from 81 to 99 days during the first month and from 56 to 73 days during the second month (Fig. 2.7). There was no statistical difference in variation during the first month (P-value = 0.15) or the second month (P-value = 0.704).

Leaf area (length * width) remained fairly constant between months except leaves of shoots in reference sediment during month 2 (Fig. 2.8). There were no significant differences among any of the treatments, which ranged from 18.64 - 21.36 cm^2 during the first month (P-value = 0.904) and 17.34 - 28.64 during the second month (P-value 0.544).

At the termination of the experiment, the remaining plant material within the buckets was compared. The expansion of shoots was comparable among treatments and there were no significant differences among all treatment densities, which ranged from 6.78 - 10.24 shoots $\cdot$ m^{-2} (P-value = 0.48). The other metrics also showed no significant differences: live rhizome length (51.69 - 78.27 cm, P-value = 0.202); below ground biomass (0.63 - 1.16 g dry weight, P-value = 0.155); and above ground biomass (0.46 - 0.68 g dry weight, P-value = 0.117).

After two months, leaf Pb concentrations were similar to the reference concentration, which was 1.4 ppm Pb (Table 2.5). Additionally, Pb concentrations of leaves from shoots transplanted into both Clark and Jamaica Cove sediment were similar to Pb concentrations of leaves from shoots collected from natural beds (Johnston *et al.*, 1994). Rhizome Pb concentrations of plants in the present study were close in range to the leaf Pb concentrations.

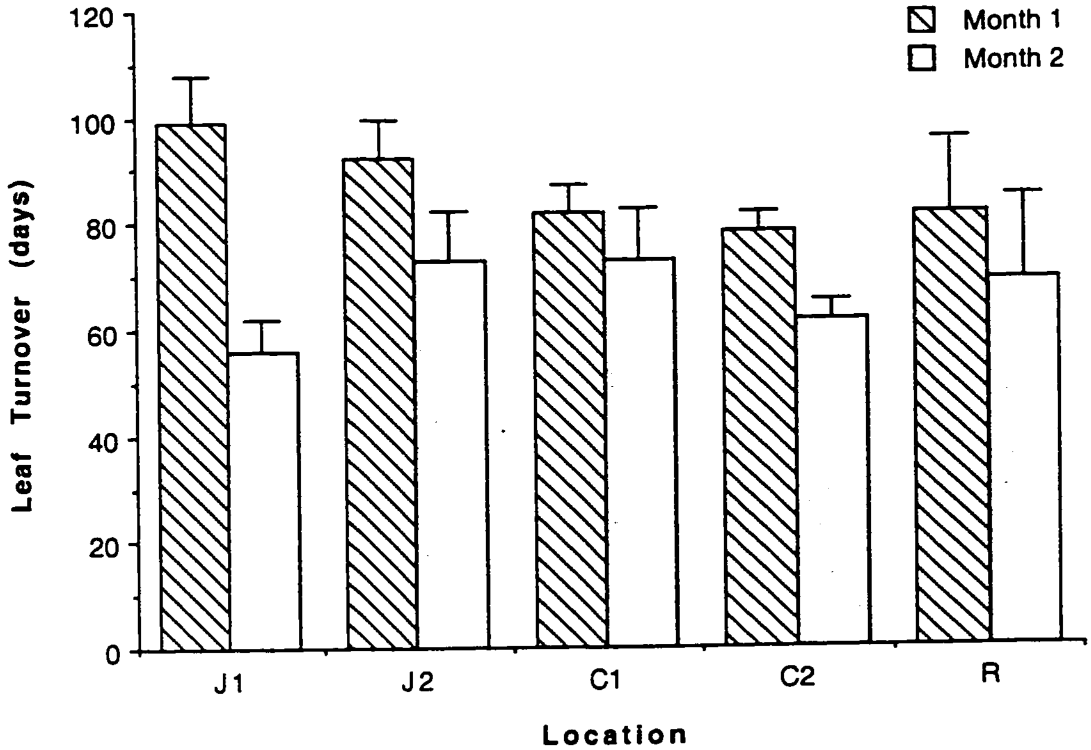

Figure 2.7. Leaf turnover rate of shoots growing in mesocosm sediment from inner and outer Jamaica Cove (J1, J2), inner and outer Clark Cove (C1, C2) and reference site (R). Mean ± se.

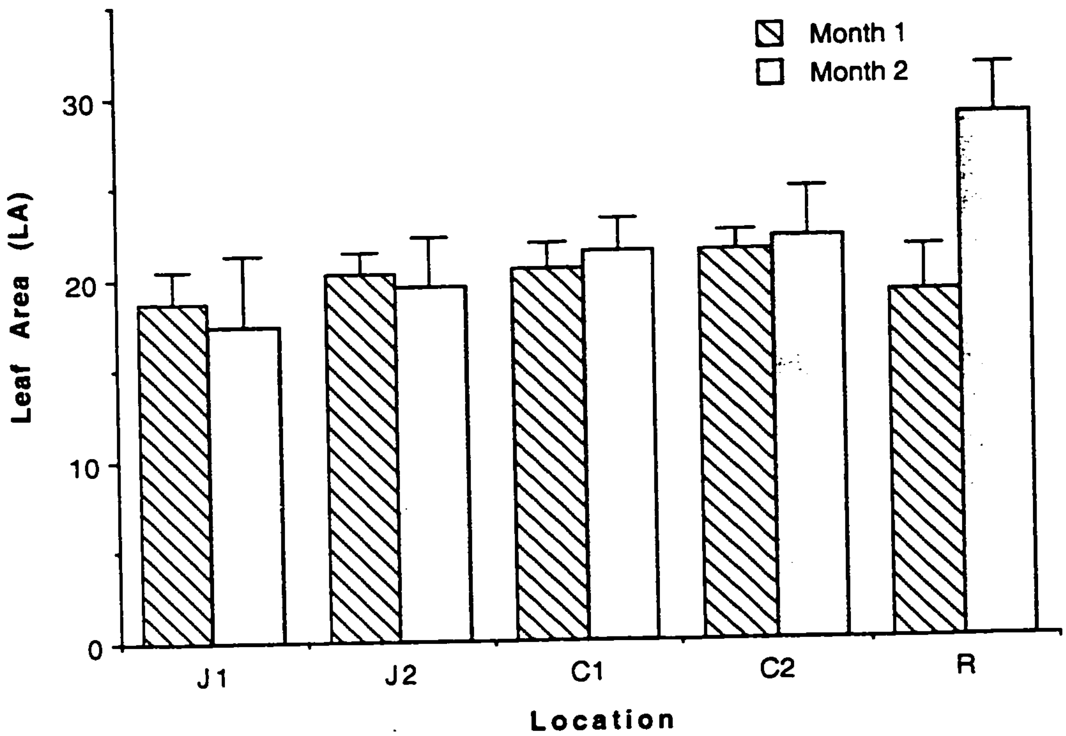

Figure 2.8. Leaf area of shoots growing in mesocosm sediment from inner and outer Jamaica Cove (J1, J2), inner and outer Clark Cove (C1, C2) and reference site (R). Mean ± se.

Table 2.5. Mean Pb concentration (ppm) of eelgrass leaves and rhizomes after two months of exposure to marine sediment of varying concentrations (this study) and tissue Pb concentrations of naturally growing eelgrass in other studies. JC = Jamaica Cove; CC = Clark Cove; -- indicates no data; * washed in chelating agent, EDTA.

Location	Leaf	Rhizome	Root + Rhizome	Sediment	Reference
JC	1.5	2.0	--	64	This Study
JC	1.9	--	14.9	44	Johnston et al., 1994
CC	1.7	1.8	--	90	This Study
CC	1.8	--	8.0	46	Johnston et al., 1994
Reference	1.4	0.0	--	20	This Study
Denmark	4.8*	--	4.0*	91	Brix & Lyngby, 1983
Denmark	17*	--	--	2600	Lyngby & Brix, 1987

DISCUSSION

Viability of eelgrass in Shipyard marine sediment collected from Jamaica and Clark Coves was tested because of the lack of natural eelgrass beds growing in close proximity to the JIL. Since most of the shallow water surrounding the Shipyard was unvegetated by eelgrass, it was important to determine whether some component of the sediment might cause stress on eelgrass growth. Certain heavy metals have shown toxic effects on terrestrial plants (Clijsters and Van Assche, 1985; Påhlsson, 1989). Lyngby and Brix (1984) demonstrated that eelgrass growth was significantly inhibited by Cd, Cu, Hg, and Zn. Analysis of sediment and seep water in Jamaica and Clark Coves showed elevated levels of lead, chromium, nickel, copper, mercury and zinc.

The sediment was primarily composed of sandy mud, regardless of whether it was collected from an area that was vegetated by eelgrass or not. All eelgrass treatments were growing in sediment of similar grain size. Since eelgrass grew well in sediment collected from both vegetated and non-vegetated areas near the Shipyard, there may be factors affecting the distribution of eelgrass around the Shipyard other than sediment composition. Such factors may consist of, but are not limited to, light and water quality and are not within the scope of the present study.

There was some evidence during the first month of exposure that eelgrass growth was inhibited in Jamaica Cove sediment (Table 2.2). Stunting of root and rhizome growth as a result of exposure to Pb has been observed in other plants and could explain the decreased vegetative vigor and rhizome growth by shoots growing in Jamaica Cove sediment (Påhlsson, 1989). However, none of the transplanted shoots had rhizome Pb concentrations elevated above reference shoots. The pattern did not persist during the second month and was not explained by Pb levels in the sediment either month (Tables 2.3 and 2.4). No sediment treatments showed any significant effects on eelgrass growth or morphology after two months of exposure and indicated that eelgrass growth was not impaired by short-term exposure to Shipyard sediment.

After two months of growth in Shipyard sediment, leaf tissue Pb concentrations were not much higher than the reference concentration and did not differ from leaf tissue Pb concentration in naturally growing shoots that were collected at the mouths of Clark and Jamaica Coves. Rhizomes, on the other hand, accumulated low levels of Pb that were comparable to leaf Pb concentrations of mesocosm-grown shoots.

Lead concentrations measured in eelgrass growing in Portsmouth Harbor sediment were not much lower than Pb concentrations of eelgrass that grew in sediment with similar Pb concentration in Denmark (Brix and Lyngby, 1983). Plants that grew in sediment with high Pb concentration in Denmark (2600 ppm) had a leaf Pb concentration of 17 ppm (Lyngby and Brix, 1987).

Brix and Lyngby (1983) found the same Pb concentrations in leaf and below ground tissue but their below ground sample included root tissue. Below ground tissue Pb concentrations determined in the ERA study (Johnston *et al.*, 1994) also included root tissue and were higher than rhizome Pb concentrations of the present study. However, the samples were not rinsed in a chelating agent prior to analyses and are therefore not comparable to concentrations of samples in Denmark (Lyngby and Brix, 1987). Root Pb concentrations of ERA samples may have been elevated by Pb and sediment that was sorbed to the exterior of the roots.

Conclusions

Eelgrass transplanted into Shipyard sediment was not affected by physical or chemical characteristics of the sediment after two months of growth. Although there may have been something in Jamaica Cove sediment that hindered rhizome growth during the first month, the effect was not persistent. Therefore, distribution of eelgrass within Clark and Jamaica Coves is likely affected by factors other than physical or chemical characteristics of the sediment, such as water quality.

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CHAPTER III

EXPOSURE OF EELGRASS (*Zostera marina* L.) TO MARINE SEDIMENT CONTAMINATED WITH LEAD: IMPLICATIONS OF HABITAT RESTORATION IN LEAD POLLUTED SEDIMENT

Abstract

Eelgrass, *Zostera marina* L., was planted in mesocosms at three different densities in marine sediment of three different lead (Pb) concentrations to determine to what extent Pb affects eelgrass growth and whether Pb uptake by eelgrass is affected by plant density. Plant uptake rates and accumulation of Pb were determined from eelgrass collected after one and two months of exposure to 1x sediment (ambient, 90 ppm Pb) and 6x sediment (600 ppm Pb) concentrations. Morphology, growth rate, oxygen evolution and chlorophyll fluorescence of eelgrass were monitored to determine whether plant morphological and physiological effects occurred. Within the micro-environment of the mesocosms, eelgrass responded to available light according to competition imposed by increasing densities. There was no evidence from the plant growth metrics measured that Pb impacted eelgrass growth per se. However, two lines of evidence indicate eelgrass was affected physiologically by increased exposure to Pb. Although photosynthetic rates (measured by oxygen evolution) were similar among

treatments, F_v/F_m ratios were low. Furthermore, chlorophyll biosynthesis was impaired at elevated Pb exposure. Eelgrass experiences some level of toxicity with exposure to high levels of Pb.

Consistent with the literature, leaves and rhizomes both showed evidence of rapid initial Pb uptake followed by a gradual leveling off, indicating the Pb attached to plant binding sites achieved equilibrium with Pb concentrations in the water and sediment. Eelgrass leaves of tethered, unrooted shoots in mesocosms accumulated as much or more Pb than leaves of shoots rooted in high Pb sediments, indicating that little or no translocation occurred. Lead uptake by eelgrass was affected by shoot density. As shoot density increased, Pb uptake decreased in both rhizomes and leaves for shoots growing in sediment enriched to 600 ppm Pb. Therefore, if eelgrass is to be used as a sentinel accumulator for Pb contamination, comparisons should be made among eelgrass growing at similar densities. Additionally, where eelgrass habitats are to be restored in areas of known Pb contamination, the data implies that planting eelgrass at higher densities would decrease the plant tissue burden of Pb.

INTRODUCTION

Seagrasses are distributed in many coastal waters and form meadows that are among the most productive ecosystems in the world (den Hartog, 1970; Thayer *et al.*, 1975). Eelgrass (*Zostera marina* L.) grows in estuaries of temperate regions and is subject to anthropogenic impacts in heavily developed watersheds. In addition to atmospheric inputs, discharge of municipal and industrial wastes, runoff and other point and nonpoint sources transport pollutants from residential, industrial, and agricultural practices into estuaries (Kennish, 1997). Because eelgrass and its analogues are endemic to many polluted estuaries and are tolerant of elevated levels of many heavy metals associated with industrial activity, seagrasses have been useful in reflecting pollutant levels in estuarine water (Lyngby and Brix, 1984, 1987; Bond *et al.*, 1985; Ward *et al.*, 1986; Tiller *et al.*, 1989).

Similar to other aquatic angiosperms, eelgrass plays an important role in trace metal cycling by accumulating essential and non-essential elements (Drifmeyer, *et al.*, 1980; Ragsdale and Burchett, 1980; Lyngby and Brix, 1989; Williams *et al.*, 1994). Although the distribution of metals in seagrasses has been widely studied (Lyngby and Brix, 1982, 1989; Nienhuis, 1986; Ward *et al.*, 1986; Ward, 1987; Pulich, 1980; Malea and Haritonidis, 1994) eelgrass Pb uptake and effect of Pb on growth of eelgrass require additional research in order to develop the use of eelgrass as a sentinel accumulator (Lyngby and Brix, 1984, 1987; Bond *et al.*, 1985). Because of its high productivity and rapid turnover of biomass, and because some metals remain in association with eelgrass tissue once bound, eelgrass is of interest in investigating the fate of Pb in the estuarine environment (Drifmeyer, *et al.*, 1980; Lyngby and Brix, 1989).

During 1991 - 1993, an Ecological Risk Assessment (ERA) for the Portsmouth Naval Shipyard identified elevated levels of Pb in eelgrass and marine sediment surrounding the Shipyard (Johnston *et al.*, 1994). The Shipyard is located on Seavey Island in the Portsmouth Harbor, which borders the states of Maine and New Hampshire. During the 1940's, a landfill was created on a mudflat between two adjacent islands, Clark and Jamaica Islands, which connected Seavey Island to Jamaica Island. The unlined landfill was only recently capped with a clay barrier and has been used for disposal of non-hazardous and hazardous material, including lead batteries, dredge spoils, plating sludges, spent sandblasting grit and waste paints and solvents (ERA Technical Report, Vol. I, 1997). Although there were natural beds of eelgrass around the Shipyard, there were no beds growing near the perimeter of the Jamaica Island Landfill (JIL) at the time of the ERA case study nor is the historical extent of the beds around the Shipyard known. In the event that eelgrass meadows would be restored or used for remediative purposes in areas of Pb contaminated sediment, I cultured eelgrass in marine sediment that was collected from the perimeter of JIL. I also cultured eelgrass in marine sediment that was enriched with Pb to better understand interactions between Pb and eelgrass.

Shoots of eelgrass were grown in sediment enriched with Pb in above ground mesocosms. Eelgrass has been successfully grown in mesocosms for many different studies and is an appropriate candidate for mesocosm experiments (Short, 1987; Short *et al.*, 1995). Lead has been shown to have minimal effects on eelgrass growth when grown in hydroponic Pb enrichments (Lyngby and Brix, 1984) , although the effects of Pb uptake on eelgrass physiology has not been assessed. Mesocosms were used to grow eelgrass in sediment with ambient levels of Pb near the Shipyard and sediment amended with lead to better understand interactions between eelgrass and lead. The purpose of the study was to verify that sediment enriched with Pb causes no adverse effects on eelgrass growth, to see if more detailed physiological measures could detect adverse effects of Pb exposure, and to determine the effects of shoot density on Pb accumulation in eelgrass.

METHODS

Mesocosm Assembly

Six large above ground mesocosms with flow-through sea water were assembled at the eastern end of Seavey Island of the Portsmouth Naval Shipyard. Eelgrass was grown within the mesocosms in marine sediment of three known Pb concentrations. The sediment Pb concentrations were referred to as control, 1x and 6x sediment and were approximately 2, 90 (ambient sediment levels around the Portsmouth Naval Shipyard), and 600 ppm Pb, respectively, as determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). The control sediment was collected from Brave Boat Harbor, Maine. Sediment was also collected from various locations around Seavey Island and homogenized for the 1x sediment treatment. The enriched, 6x sediment was collected offshore from Seavey Island as well and was elevated to approximately 600 ppm using marine sediment enriched with aqueous lead nitrate ($PbNO_3$) prepared by Nacci et al. (1994). Time zero samples of each sediment type were frozen for later lead analyses and archive purposes.

Approximately 2.5 L of sediment was contained in each 5 L bucket. There were 12 buckets for each sediment Pb treatment. In order to prohibit cross-contamination among Pb treatment samples, only one Pb treatment per mesocosm was permitted. There were two mesocosms per Pb treatment. The mesocosms stood above ground in a row with southeastern exposure to the sun. Each mesocosm received seawater that was contained in a head tank and drawn from 50 m offshore. Outflow water drained from the surface of the mesocosms, allowing any sediment that was resuspended during manipulation of the plants to settle to the bottom of the tank rather than drain into the estuary. All sediment with elevated Pb levels was collected at the end of the experiment and disposed of using procedures approved by the Environmental Protection Agency.

Eelgrass shoots were collected from a site at Fishing Island, Kittery, Maine, that had known low levels of Pb in the plant tissues and sediment (Johnston *et al.*, 1994) and were transplanted into the buckets at varying densities. Eelgrass density treatments were included in the experimental design to investigate whether plant uptake of Pb was affected by eelgrass density.

The three densities were 1, 4, and 40 shoots per bucket representing 16, 64, and 640 shoots $\cdot$ m^{-2} of eelgrass, and were referred to as low, medium, and high eelgrass density, respectively. Two replicates of each density treatment were dispersed randomly in each mesocosm. Additionally, as an unrooted control, twenty shoots of eelgrass were tethered to the outside of a plastic coated wire frame and submersed in each mesocosm. The unrooted, tethered eelgrass was used to monitor Pb levels in the mesocosm water. Each mesocosm was inoculated with 200 herbivorous snails (*Littorina littorea* L.) to control algal overgrowth by their grazing activity.

Time zero plant tissue samples were scraped free of epiphytic algae, washed in distilled, deionized water, sorted into above and below ground parts and frozen for later tissue Pb analysis. All roots were removed from rhizomes prior to freezing.

Eelgrass Growth and Morphology

The eelgrass shoots were allowed to acclimate in the mesocosms for three weeks. During the fourth week, one shoot in each treatment was marked with a 20 gauge needle hole through its bundle sheath just above the meristem and left to continue growing for one week. After one week, the marked shoots were collected from the medium and high density treatments for growth and morphological measurements as described by Short (1987). Measurements were taken *in situ* from the low density shoots since there was only one shoot per bucket. Additional shoots from the high density treatments were collected at the same time for later Pb analysis. Rhizomes, which had been trimmed to three nodes initially to mark for new node production and growth, were only collected from the high density treatments during the first month for new growth assessment because low and medium density shoots had to remain rooted for the duration of the experiment. During the second and final month of the experiment, all plants that were marked for growth were collected for dry weight and growth analysis.

Shoots were separated into above ground and below ground parts. Roots were removed from all rhizomes and the remaining eelgrass tissue was cleaned of sediment with distilled-deionized water. Above ground growth rates were determined by first measuring the length from the meristem to the distal end of each leaf. New tissue growth between the meristem and the mark was measured by using the mark of the outermost, non-growing sheath of the shoot bundle as a reference point. Growth ($g \cdot m^{-2}$ eelgrass $\cdot$ day^{-1}) and specific growth (cm new leaf growth $\cdot$ cm leaf $length^{-1}$ $\cdot$ day^{-1}) were calculated and compared among treatments and during time. The width at the mid-length of each leaf was also measured to determine the total area of leaf tissue, referred to as leaf area (LA, modified from Dennison, 1990). One sided

LA was calculated by multiplying leaf width times total leaf length per shoot of eelgrass, rather than on a m^2 basis.

Below ground growth rates were determined by measuring new rhizome length, new rhizome weight and the number of newly produced nodes and laterals during time, giving an indication of the plant's vegetative vigor. Rhizomes were dried with their respective above ground tissue at 80°C in clean glass jars for two days and weighed individually. At the time of each growth collection, density of terminal, lateral, and reproductive shoots in each bucket were counted.

Eelgrass Physiology

Physiological assessments of the eelgrass were conducted to determine if Pb affected the plant's physiological functioning. Two assays of the photosynthetic systems were carried out on the high density treatments: photosynthetic rates, as determined from oxygen evolution, and chlorophyll fluorescence. The third oldest leaf was cut from eelgrass shoots of each sediment Pb treatment, placed in seawater, and transported to the laboratory in a cooler. Samples were stored in a 15°C growth chamber.

At the lab, leaves were dark adapted for 10 minutes before measuring F_v/F_m with a pulse modulated flourimeter. The ratio F_v (variable fluorescence) / F_m (maximum fluorescence) is used as a quick assessment of the functioning of the initial photochemistry of photosystem II (Bolhàr-Nordenkampf *et al.*, 1989). After the relative health of photosystem II was assessed, three 3.0 cm^2 pieces of a leaf were cut from 20 cm below the apex of the leaf. The pieces of leaf varied in length depending on their width. One piece was kept for chlorophyll analysis. Wet and dry weights were determined from the second piece. The third piece was used for measuring oxygen evolution using a Clark O_2 electrode.

Chlorophyll was extracted with 100% acetone for 24h in a dark 5°C chamber using procedures described by Dennison (1990). The eelgrass tissue samples were thoroughly macerated in ice baths and then diluted to 80% acetone. Absorbance was read at 663 and 645 nm with a Beckman DU 640 spectrophotometer. Determination of mg chl a + b $\cdot$ ml $^{-1}$ was calculated using equations described for terrestrial plants by Dennison (1990). Chlorophyll concentrations were also used in oxygen rate calculations.

Oxygen evolution was measured in µmol $O_2 \cdot$ mg chl $^{-1} \cdot$ hr $^{-1}$ at six light intensities (50, 100, 250, 500, 1000, and 2000 µE $\cdot$cm $^{-2}$ $\cdot$sec $^{-1}$) and two temperatures (10 and 20°C). A 3.0 cm^2 piece of eelgrass was clipped into smaller pieces (2 - 3 mm in length) and added to 4.0 ml filtered, autoclaved seawater in the Clark O_2 electrode chamber. It was necessary to replace dissolved inorganic carbon (DIC) in the seawater with 50 µl 1M $NaHCO_3$ (final concentration was 0.0125M $NaHCO_3$) because the DIC was lost when the water was autoclaved.

Lead Analysis

Samples of eelgrass tissue were compiled from the growth analysis shoot collections and prepared for Pb concentration analysis. Because eelgrass tissue samples had to be combined to obtain enough material for extraction, there were no replicate tissue samples for Pb concentration determination. However, each sample was analyzed four times to determine analytical error for each composite sample. Dried leaf and rhizome tissues were ground separately by hand with a clean ceramic mortar and pestle. Once tissue was ground into a fine powder, 0.5 g of each sample were digested with ultra pure 7.5N HNO_3 in acid cleaned teflon capsules. Filtrates were analyzed for total Pb concentration using ICP-AES at the New Hampshire Materials Lab of Somersworth, NH.

Sediment samples from time zero and the end of the experiment were dried at 80°C for several days and disaggregated. Precisely 3.0 g of each sample were weighed and extracted with 10 ml 3N HNO_3. The extracts were gently agitated several times during the 24 hr extraction period and then filtered. Total Pb concentration was analyzed using ICP-AES.

Eelgrass tissue Pb concentration was plotted versus time to determine Pb accumulation patterns. Additionally, SEM (simultaneously extracted metal) values were determined for the sediment used in this study (ERA Technical Report, Vol. I, 1997). SEM data were used to calculate the expected SEM for the bulk Pb concentrations measured in 1x and 6x sediment. Di Toro *et al.*, (1990) theorized that where SEM in in equilibrium with acid volatile sulphides (AVS, or sediment sulfides that are soluble in cold acid), no metals are available for uptake. SEM:AVS ratios greater than 1.0 imply that metals are in excess of AVS and available for uptake.

Statistical Analysis

One and two way ANOVA models followed by Fisher's protected LSD (least significant difference) means comparisons were used to test for growth and morphological differences between patterns of eelgrass growing in the different sediment Pb concentrations and density treatments. Data were log transformed to reduce the error heterogeneity of residuals.

RESULTS

Eelgrass Growth and Morphology

Growth trends of eelgrass were evident among the density treatments and were characteristic of density effects. The effects were specifically associated with shoot density and showed no significant correlation to sediment Pb treatments.

At the two greater density treatments, leaves were noticeably wider during the first month (P-value = 0.001, Fig. 3.1). After two months of growth however, leaves in high density treatments narrowed, resulting in no clear differences in leaf width among density treatments. Tethered shoots had significantly more narrow leaves than any of the rooted density treatments after the first month but had widths that were equivalent to the low density leaves after two months. Longest leaf or canopy height was greater with increasing density than low density shoots during both months (Fig. 3.2). The differences were most pronounced between the low density and the medium and high density shoots during the second month (P-value = 0.0002). Canopy height of rooted shoots decreased for all density treatments during the second month. Tethered shoots were similar in height to low density shoots both months. Shoot density did not change during the experiment for tethered, low or medium density shoots, but shoot density of the high density treatments decreased by one fourth after the first month of growth (Fig. 3.3).

Vegetative vigor, measured by the number of nodes produced per shoot per day, is used here as a surrogate measure of leaf initiation. The vegetative vigor of tethered shoots was lower during the first month than that of shoots in medium and high densities but were equivalent to low density shoots

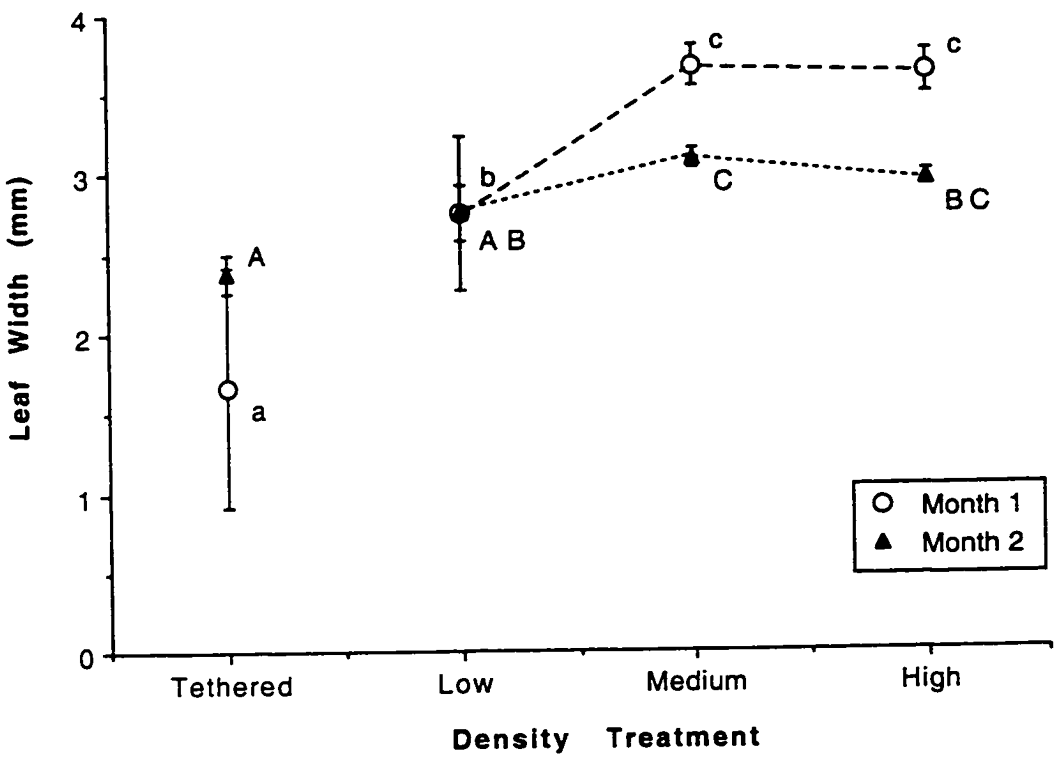


Figure 3.1. Eelgrass leaf widths of shoots grown tethered and rooted at different densities. Letters (lower case for month 1, upper case for month 2) represent determined statistical differences among density treatments. n = 12 ± se.

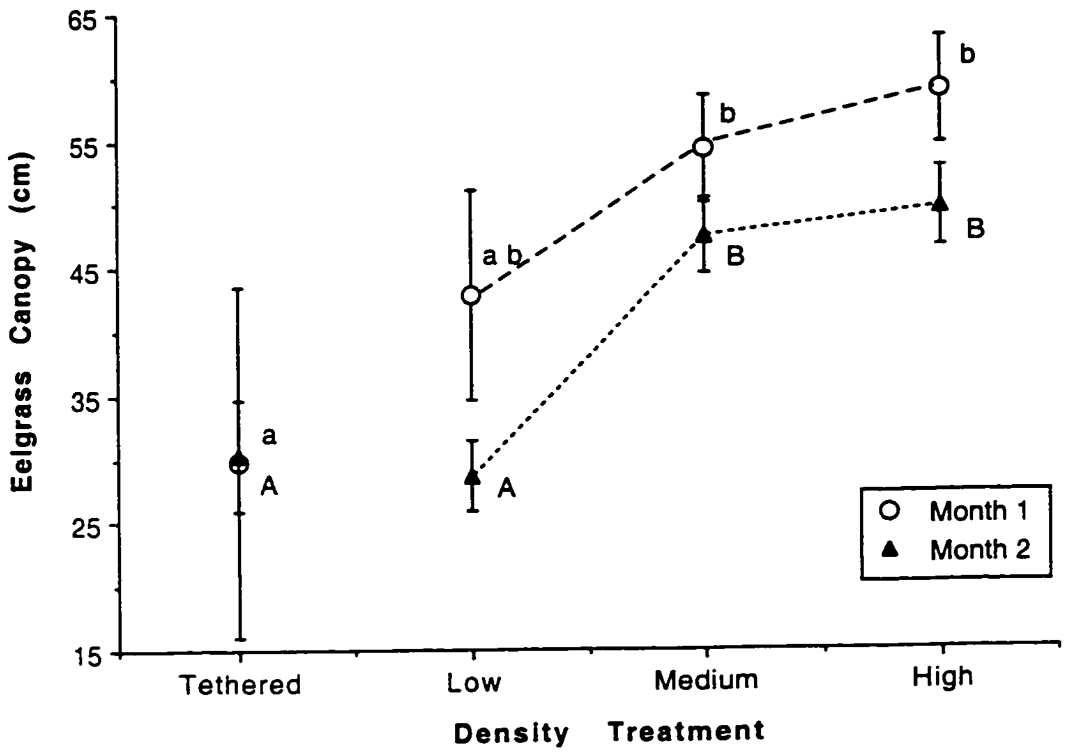


Figure 3.2. Canopy height of eelgrass grown tethered and rooted at different densities. Letters (lower case for month 1, upper case for month 2) represent determined statistical differences among density treatments. n = 12 ± se.

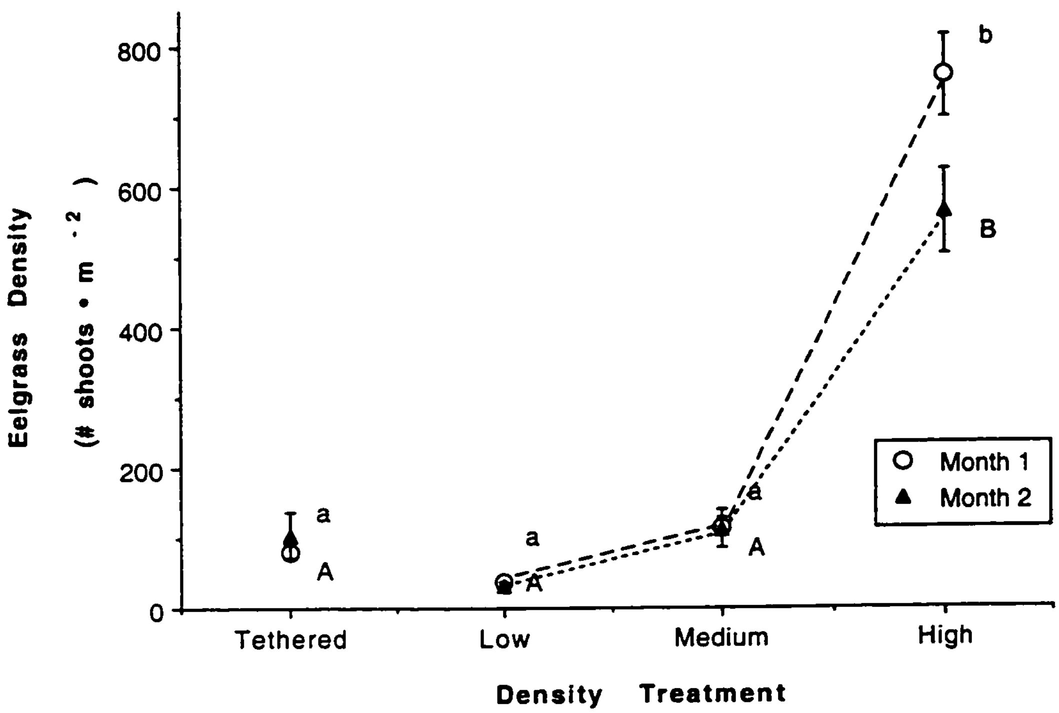


Figure 3.3. Measured eelgrass density of assigned density treatments. Letters (lower case for month 1, upper case for month 2) represent determined statistical differences among treatments. n = 12 ± se.

(Fig. 3.4). After the second month of growth, tethered shoots and shoots from all density treatments had equivalent new leaf production.

New rhizome growth per shoot per day increased with increasing density during the first month (P-value = 0.0007, Fig. 3.5). Tethered shoot rhizome growth was similar to low density shoot rhizome growth both months. After the second month, however, rhizome growth was not significantly different among density treatments and leveled off near 0.2 cm per day. Rhizome growth of low density shoots increased during the second month, while rhizomes of low density shoots decreased in growth.

Above ground growth rates increased on an areal basis during both months with increasing density (P-value ≤ 0.0002, Fig. 3.6). Productivity on an areal basis is specifically related to shoot density and I expected to find higher productivity in the high density treatments. During the second month, however, growth rates of high density shoots decreased by two thirds those of the first month. Growth rates of tethered shoots were comparable with growth rates of low density shoots both months.

Although tethered shoots had significantly lower specific growth than rooted shoots during the first month (P-value = 0.0025), rooted shoots from all three densities had equivalent specific growth (Fig. 3.7). After two months, specific growth of the high density shoots had decreased significantly and was equivalent to that of the tethered shoots (P-value = 0.05).

Leaf area (LA) also changed during time with respect to density. Leaf area was the same for tethered and rooted shoots of all densities during the first month of growth (Fig. 3.8). Shoots from all treatments decreased in LA after two months. Leaves of both tethered and low density shoots had

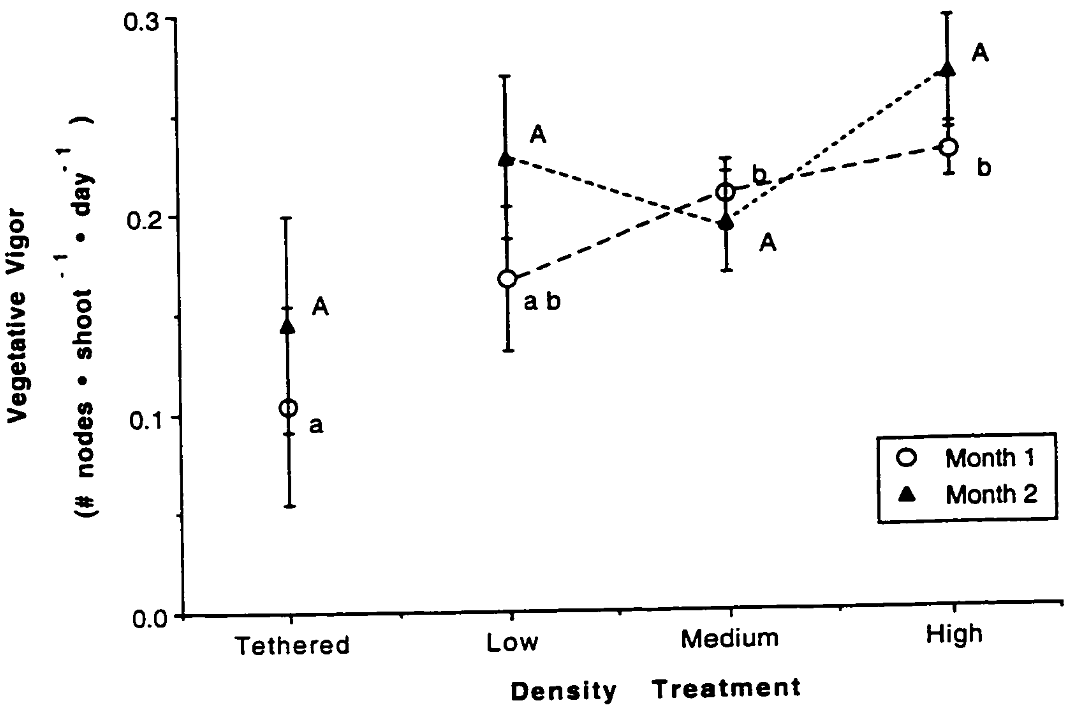


Figure 3.4. Vegetative vigor of eelgrass grown tethered and rooted at different densities. Letters (lower case for month 1, upper case for month 2) represent determined statistical differences among density treatments. n = 12 ± se.

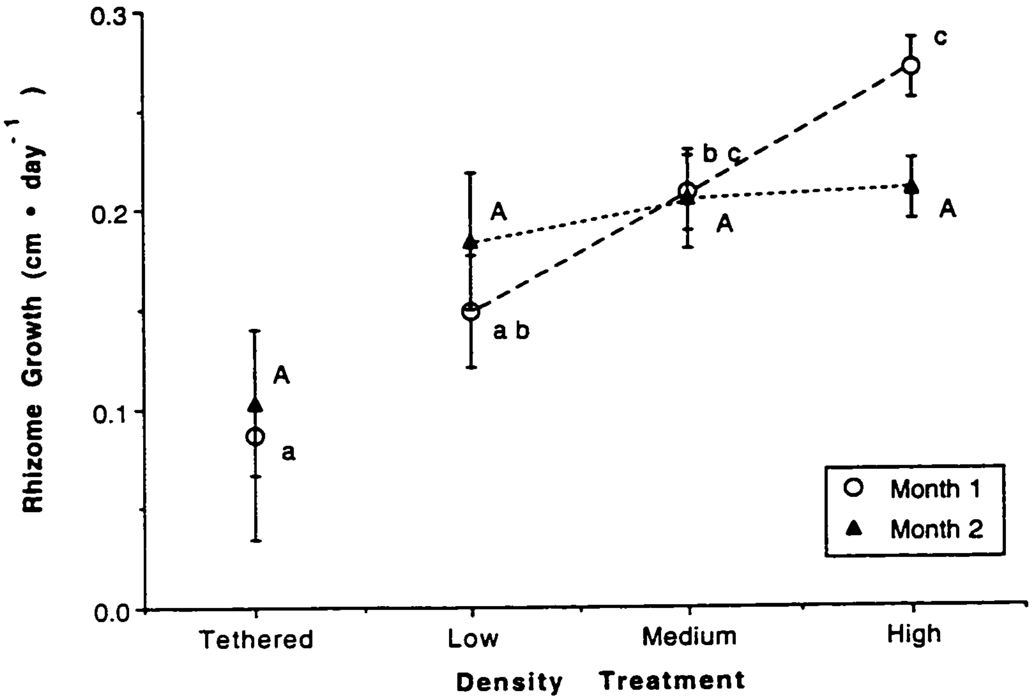


Figure 3.5. Rhizome growth of eelgrass grown tethered and rooted at different densities. Letters (lower case for month 1, upper case for month 2) represent determined statistical differences among density treatments. n = 12 ± se.

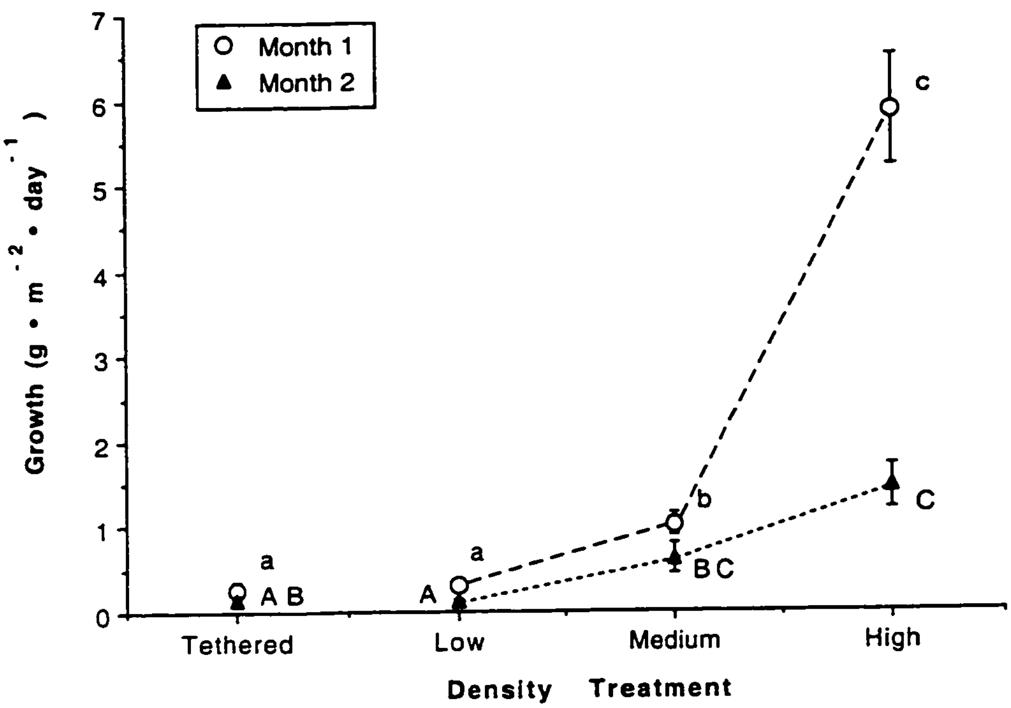


Figure 3.6. Growth of eelgrass grown tethered and rooted at different densities. Letters (lower case for month 1, upper case for month 2) represent determined statistical differences among density treatments. n = 12 ± se.

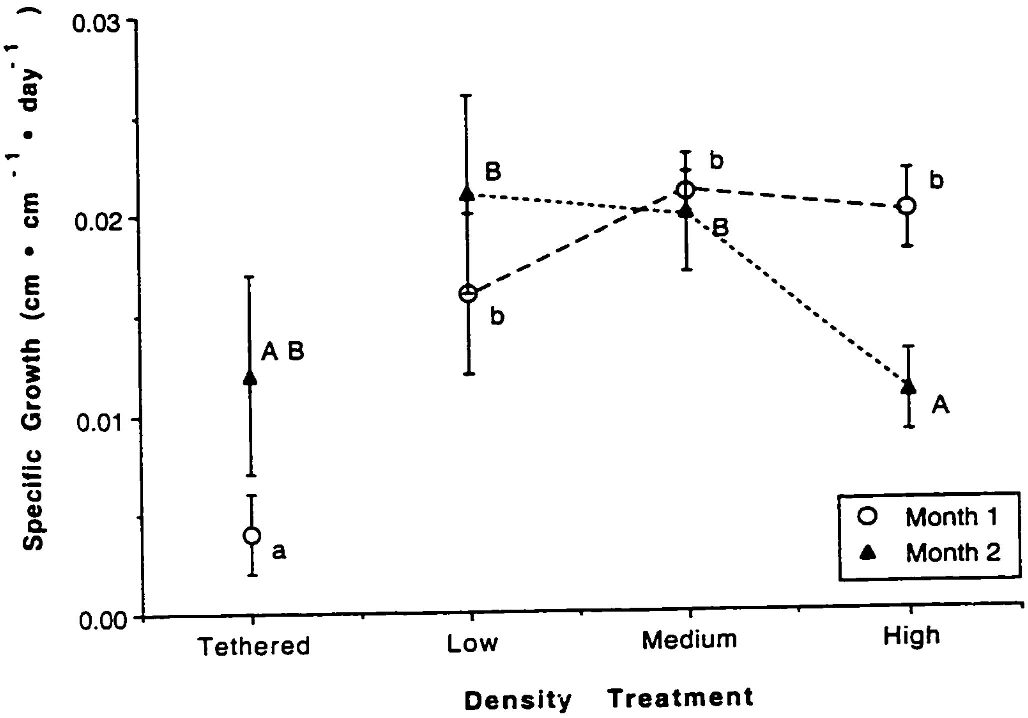


Figure 3.7. Specific growth of eelgrass grown tethered and rooted at different densities. Letters (lower case for month 1, upper case for month 2) represent determined statistical differences among density treatments. n = 12 ± se.

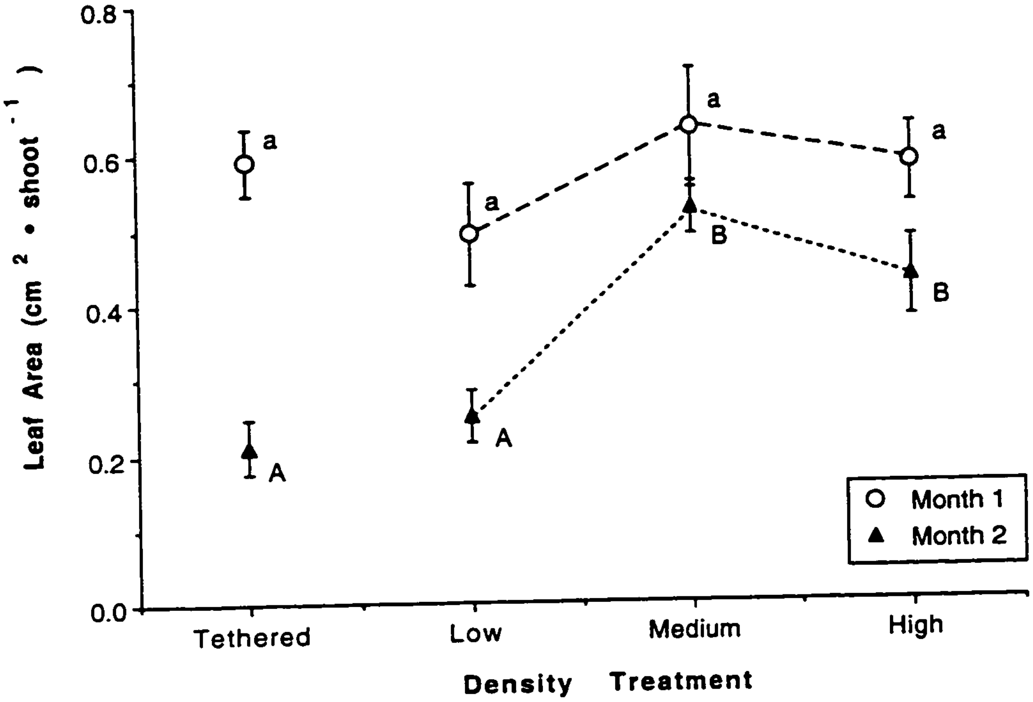


Figure 3.8. Leaf area per shoot of eelgrass grown tethered and rooted at different densities. Letters (lower case for month 1, upper case for month 2) represent determined statistical differences among density treatments. n = 12 ± se.

significantly smaller LA than leaves of medium and high density shoots (P-value < 0.001). After two months of growth, high density shoots also had twice the weight to length ratio as low and medium density and tethered shoots (P-value = 0.047, Fig. 3.9).

Shoots from each treatment were monitored during the course of the experiment for signs of stress that might be attributed to elevated levels of Pb. After one month of exposure, bundle sheaths of shoots in 6x sediment appeared slightly yellow compared to shoots in the 1x and control treatments and had a white residue on the base of the shoots at the interface of the water and sediment. Several shoots from all treatments and all mesocosms appeared to have a rotted meristem and died. There was also a noticeable amount of wasting disease on many of the leaves during the first month but there was not enough to warrant quantification (Burdick *et al.*, 1993) and it did not appear to threaten the survival of the shoots. There was no evident trend for the survival of shoots in one mesocosm treatment versus another (P-value = 0.3973).

There was a small amount of algal growth in each of the mesocosms. The control mesocosms had blooms of blue-green algae, which were floating on the water surface. The other mesocosms supported minor amounts of ephemeral green algae and diatoms that were attached to the walls of the mesocosms. Snails were effective in controlling algal overgrowth with their grazing activity.

Eelgrass Physiology

The F_v/F_m ratios of the eelgrass in the control sediment was 0.71 and in the 6x sediment was 0.67 as shown in Table 3.1. Although the ratio decreased

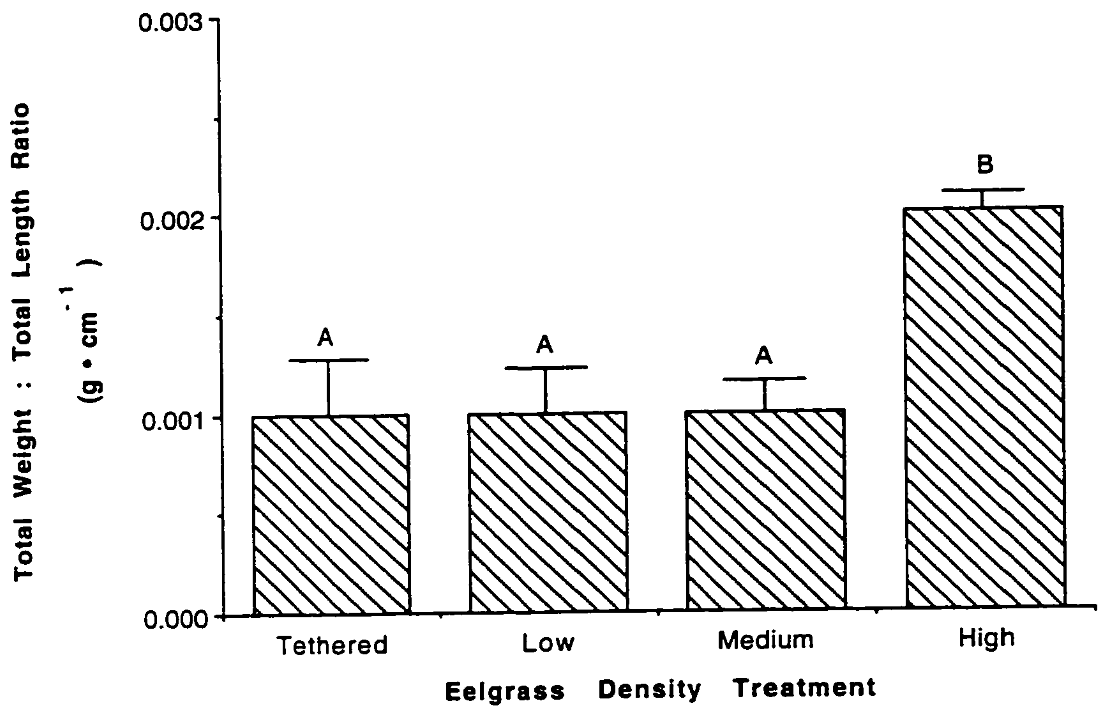


Figure 3.9. Total weight to total length ratios of eelgrass grown tethered and rooted at different densities. Letters represent determined statistical differences among densities treatments. n = 12 ± se.

Table 3.1. Chlorophyll fluorescence of eelgrass that was grown in three different Pb sediment treatments. Control, 1x, and 6x = <2, 90, and 600 ppm Pb, respectively. F_v/F_m is the ratio of variable (or the rise in) fluorescence to maximum fluorescence of fluorescence induction curves. (n = 4 ± se).

Pb TREATMENT	F_v/F_m
Control	0.71 ± 0.09
1x	0.69 ± 0.02
6x	0.67 ± 0.02

with increasing sediment Pb concentration, the trend was not significant (P-value = 0.273).

Photosynthesis rates (as oxygen evolution) were comparable among Pb treatments within each temperature regime (Fig. 3.10). At 10°C, leaves from all Pb treatments grouped tightly as the light intensity varied (Fig. 3.10a). Respiration rates, alpha slopes (initial slope of the P/I curve) and P_{max} were very similar. At 20°C, P_{max} increased for eelgrass from all treatments (Fig. 3.10b). There was a slightly lower alpha for all treatments, indicating a slower initial rise of O_2 production. Leaves from all Pb treatments were tightly grouped, showed higher respiration rates and reached P_{max} at higher light intensity than leaves in 10°C water. There were no significant differences in photosynthesis rates among Pb treatments for each temperature regime (Table 3.2).

Chlorophyll levels of eelgrass (mg chl · cm^{-2}) varied significantly with Pb concentration of the sediment. Eelgrass grown in 6x Pb sediment had significantly lower chlorophyll than eelgrass grown in 1x or control sediment (P-value = 0.0043, Fig. 3.11).

Pb Analysis

Eelgrass tissue Pb concentration was determined for leaves and rhizomes of shoots growing in 1x and 6x Pb enriched sediment. After the first month of exposure to 1x sediment, leaves in low and medium density treatments had absorbed 4.0 and 7.4 ppm Pb, respectively (Fig. 3.12a). By the end of the second month of exposure, leaves from low and medium densities had 3.2 and 1.94 ppm Pb, respectively, and rhizomes had 1.7 and 1.3 ppm Pb, respectively (Fig. 3.12b). Lead concentrations of rhizomes were slightly lower than leaf Pb concentrations.

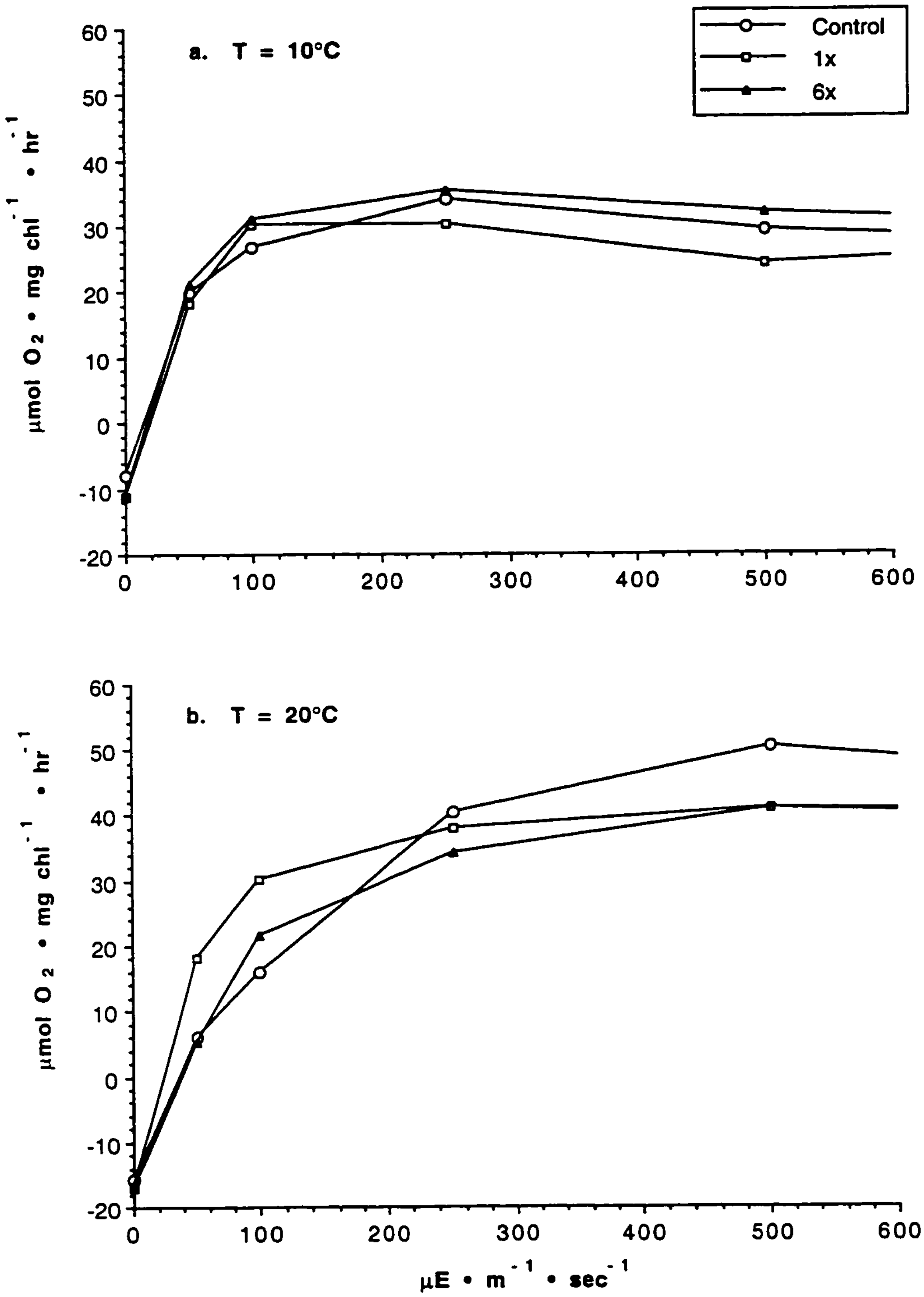


Figure 3.10. Photosynthesis / irradiance curves of eelgrass after one month exposure to control (< 2 ppm), 1x (90 ppm), and 6x (600 ppm) sediment Pb at 10° (a) and 20°C (b); n = 4.

Table 3.2. Two-way ANOVA for O_2 evolution of eelgrass grown in varying concentrations of Pb.

10°C

Source	df	Sums of Squares	Mean Square	F-value	P-value
Pb trmt	2	71.07	35.53	0.305	0.74
µE	7	15558.05	2222.58	19.079	< 0.001
Pb trmt * µE	12	220.94	18.41	0.158	1.00
Residual	62	7222.59	116.49		

20°C

Source	df	Sums of Squares	Mean Square	F-value	P-value
Pb trmt	2	128.80	64.40	0.39	0.682
µE	7	32138.98	4591.28	27.43	< 0.001
Pb trmt * µE	12	890.96	74.25	0.44	0.938
Residual	56	9373.79	167.39		

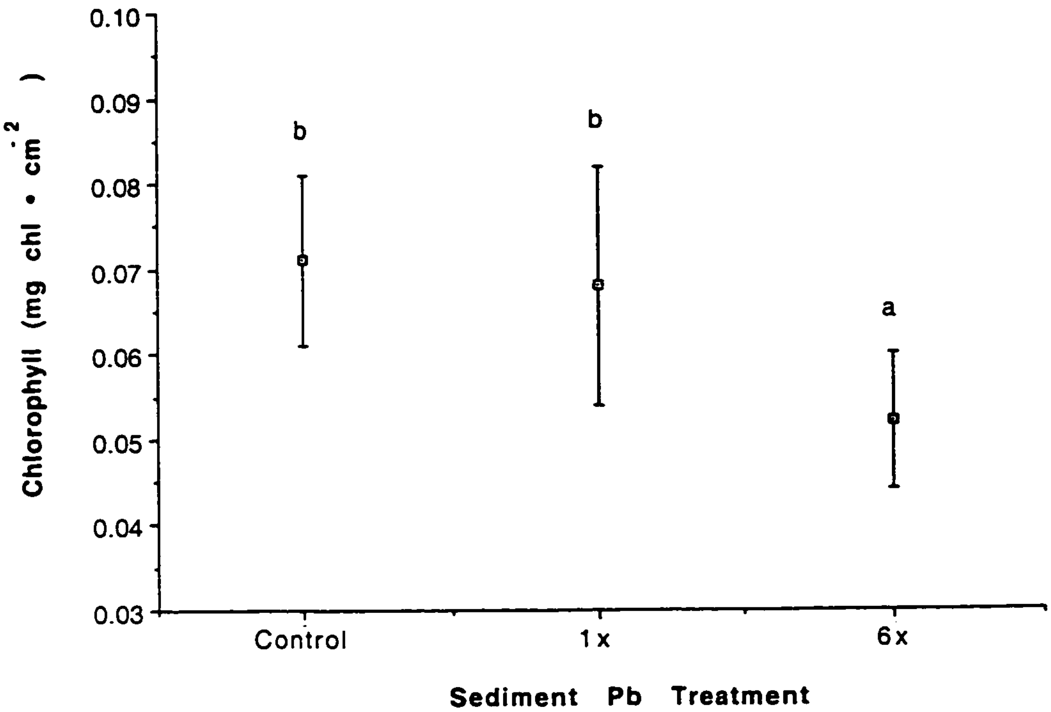


Figure 3.11. Chlorophyll levels of eelgrass grown in control, 1x, and 6x sediment (< 2, 90, 600 ppm Pb, repectively). Letters represent determined statistical differences. n = 4 ± se.

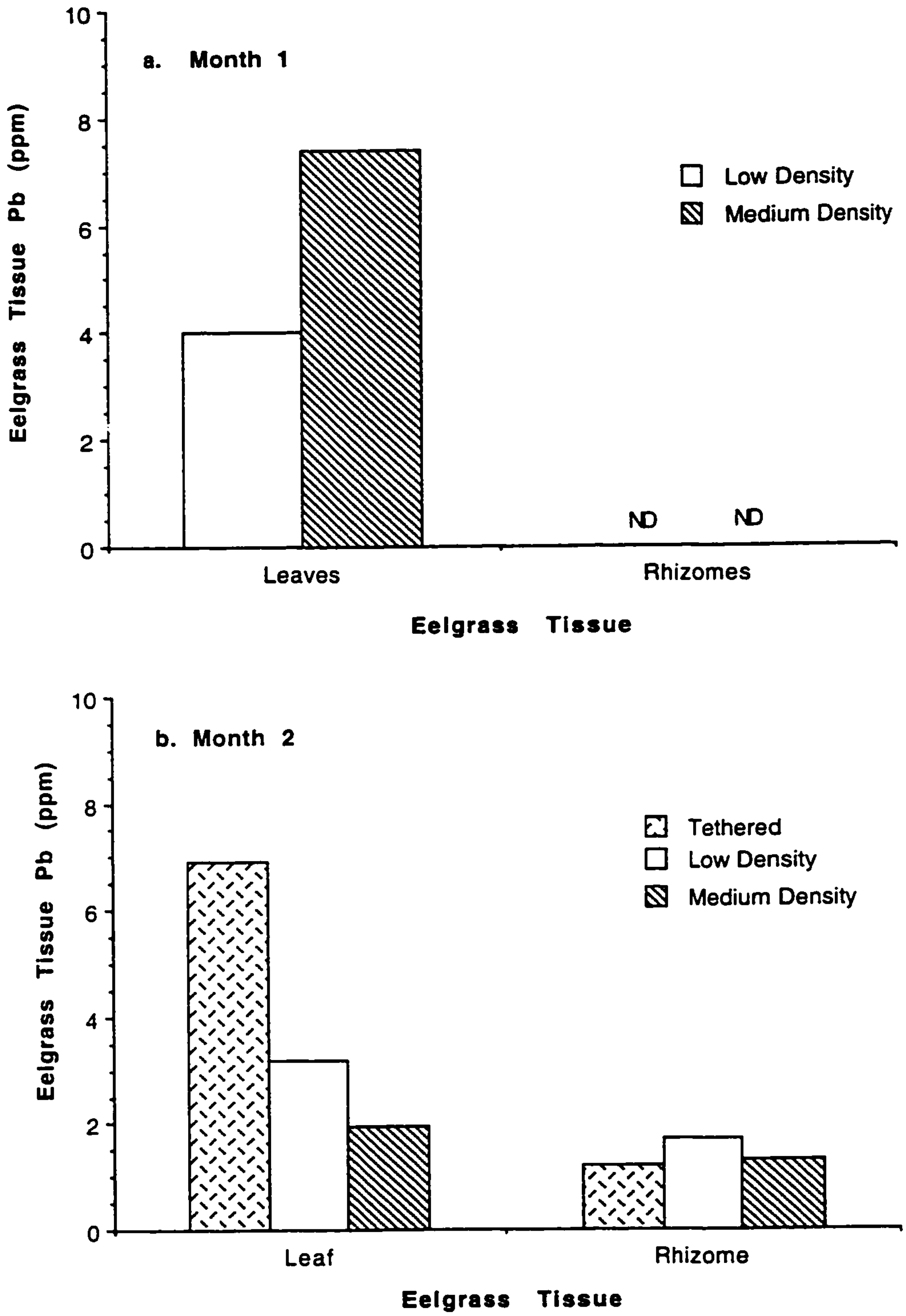
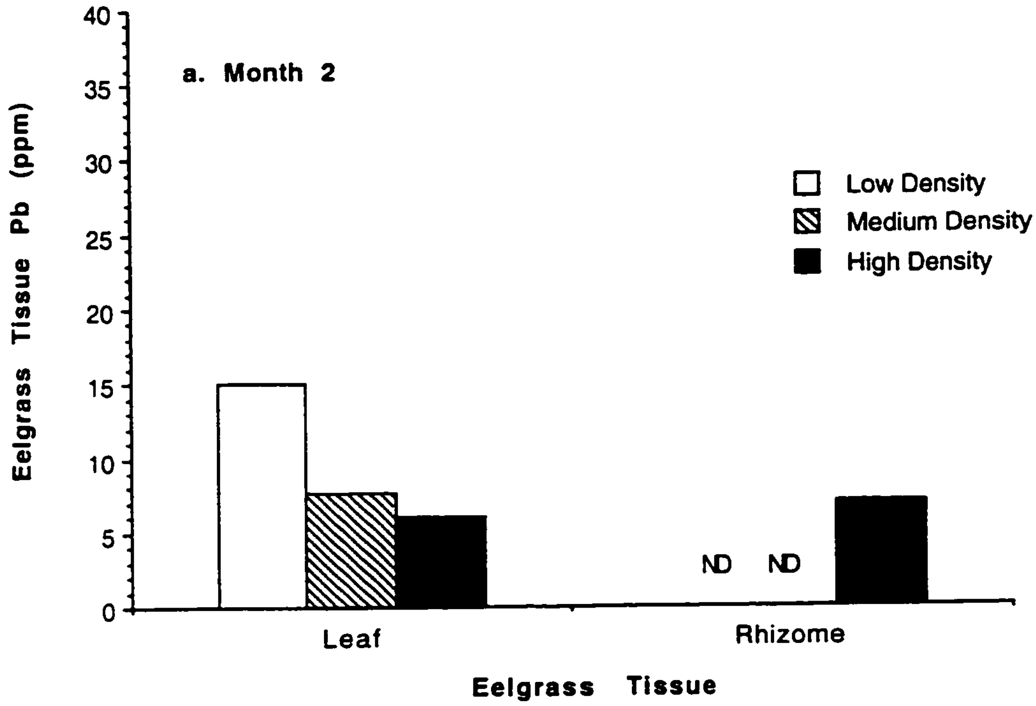


Figure 3.12. Eelgrass tissue Pb of shoots rooted at different densities and tethered in the water column of mesocosms after one (a) and two (b) months of exposure to 90 ppm Pb (1x) sediment. High density not available (ND = no data; n of 1).

Leaves from shoots that were tethered in the mesocosms of 1x sediment treatments absorbed twice the concentration of Pb of leaves from both low and medium densities by the end of the experiment (Fig. 3.12b). Rhizomes of tethered shoots, on the other hand, had nearly the same Pb concentration as the rhizomes of shoots from low and medium densities.

After one month of growth in 6x sediment, Pb was detected in both rhizome and leaf tissue (Fig. 3.13a). Leaves from the low eelgrass density treatments had nearly twice the concentration of Pb as leaves from medium and high density treatments. Rhizomes of the high density treatment had slightly higher concentrations of Pb than leaves of the same treatment. After the second month of exposure to 6x sediment, leaves from tethered, low and medium density shoots all had at least twice the Pb concentration of the high density treatment (Fig. 3.13b). Rhizomes of tethered shoots had low Pb concentration, while rhizomes of all the density treatment shoots had higher Pb concentrations than leaves of the same densities. Low density rhizomes had absorbed at least three times the Pb concentration of low density leaves. High density rhizomes also had three times the Pb concentrations of high density leaves.

Leaf Pb concentration after short term exposure to 1x sediment for two months was comparable to Pb concentrations that occurred in eelgrass leaves of naturally growing beds (Fig. 3.12b, Table 3.3). Leaf Pb concentration of low density shoots growing in 6x sediment, however, was three times that observed in natural beds, while leaves of high density shoots accumulated levels of Pb similar to those observed in natural beds (Fig. 3.13, Table 3.3). Rhizome Pb concentrations of the ERA samples included roots and were therefore not comparable the rhizomes of the present study. Roots have been



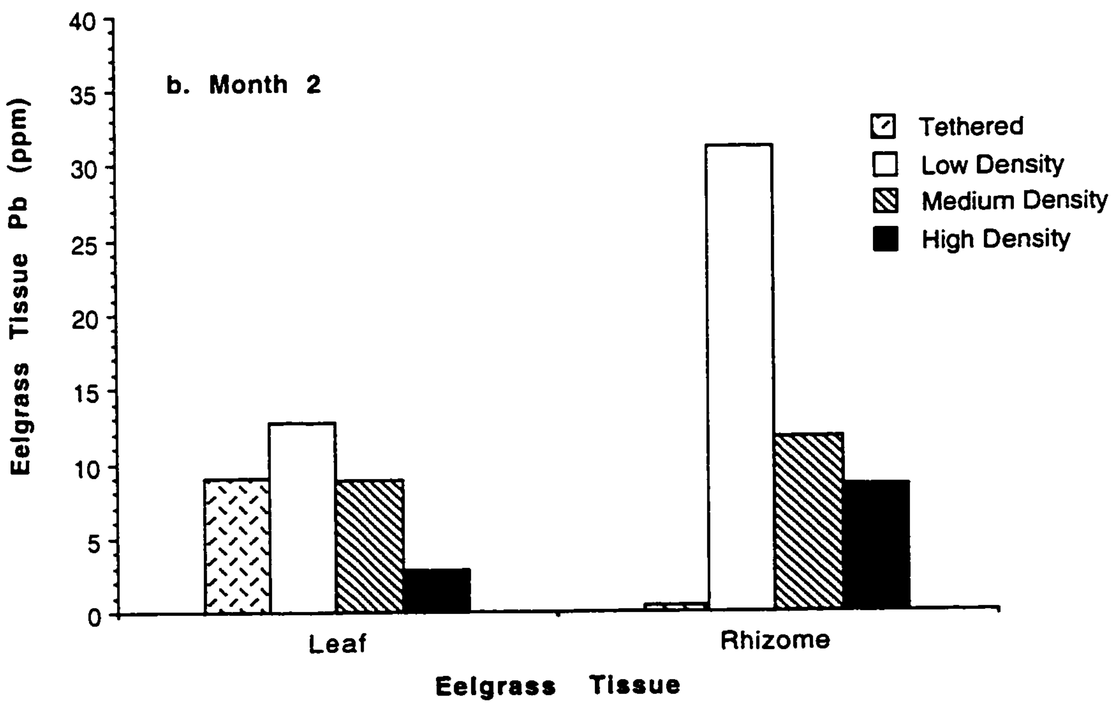


Figure 3.13. Eelgrass tissue Pb of shoots rooted at different densities and tethered in the water column of mesocosms after one (a) and two (b) months of exposure to 600 ppm Pb (6x) sediment; n of 1.

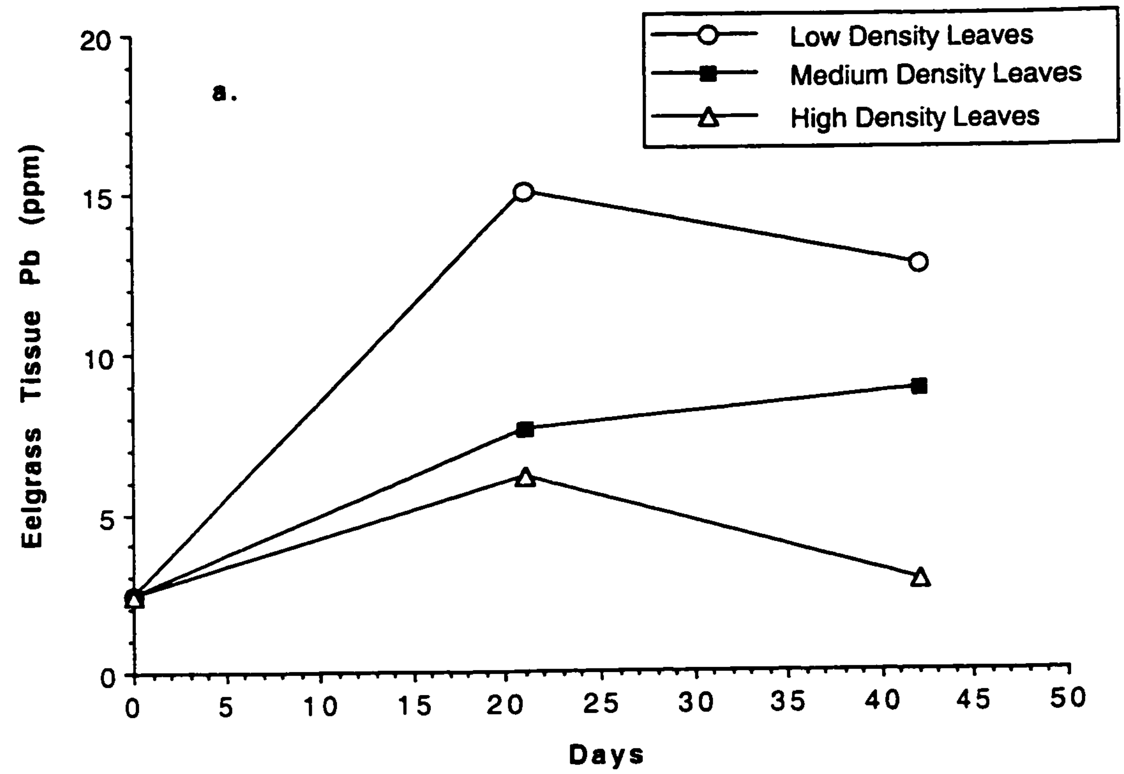
Table 3.3. Comparison of ranges of Pb concentrations (ppm) found in eelgrass leaf and rhizome tissues of shoots grown in 1x, and 6x sediment with shoots and sediment collected for the Ecological Risk Assessment (ERA, Johnston *et al.*, 1994). * Includes root tissue. ‡ Values correspond to the ERA sediment Pb concentrations from two natural eelgrass beds in the mouths of Clark and Jamaica Coves.

SAMPLE	LEAF [Pb]	RHIZOME [Pb]	SEDIMENT [Pb]
ERA‡	0.8 - 5.1	0.4 - 28.2 *	34.7 - 81.9
1x	3.2 - 7.4	1.6 - 1.7	90
6x	2.8 - 15.0	7.0 - 31.1	600

shown to accumulate much higher levels of Pb than rhizomes (Brix and Lyngby, 1983; Lyngby and Brix, 1984) Despite this, rhizome Pb concentrations in the high Pb treatment exceeded those observed in natural beds around the Portsmouth Naval Shipyard (Table 3.3).

Accumulation of Pb by leaves of all three density treatments and rhizomes of the high densities were plotted versus time in order to determine Pb uptake rates of plants grown in sediment with high Pb levels (Fig. 3.14 a,b). Time zero tissue concentrations (reference levels) were used as initial concentration levels. Leaves from low density treatments had the fastest uptake of the three density treatments and accumulated nearly twice the concentration of Pb as leaves of medium and high density during the first month of exposure. After the second month, leaves and rhizomes of all treatments showed a leveling off of uptake though Pb concentration still increased slightly with decreasing shoot density.

Sediment SEM (simultaneously extracted metals) for Pb was compared with eelgrass rhizome tissue Pb accumulated after two months of exposure to 1x and 6x sediment (Fig. 3.15). Although sediment SEM for Pb was below the SEM:AVS ratio of 1.0, rhizome Pb concentrations of low, medium, and high density shoots all showed some Pb accumulation. When Pb concentrations of rhizomes in the 6x Pb treatment were converted to Pb accumulated by the below ground biomass · top five cm of sediment in each bucket $^{-1}$, it was evident that more Pb was removed from the sediment as eelgrass density increased (Fig. 3.16).



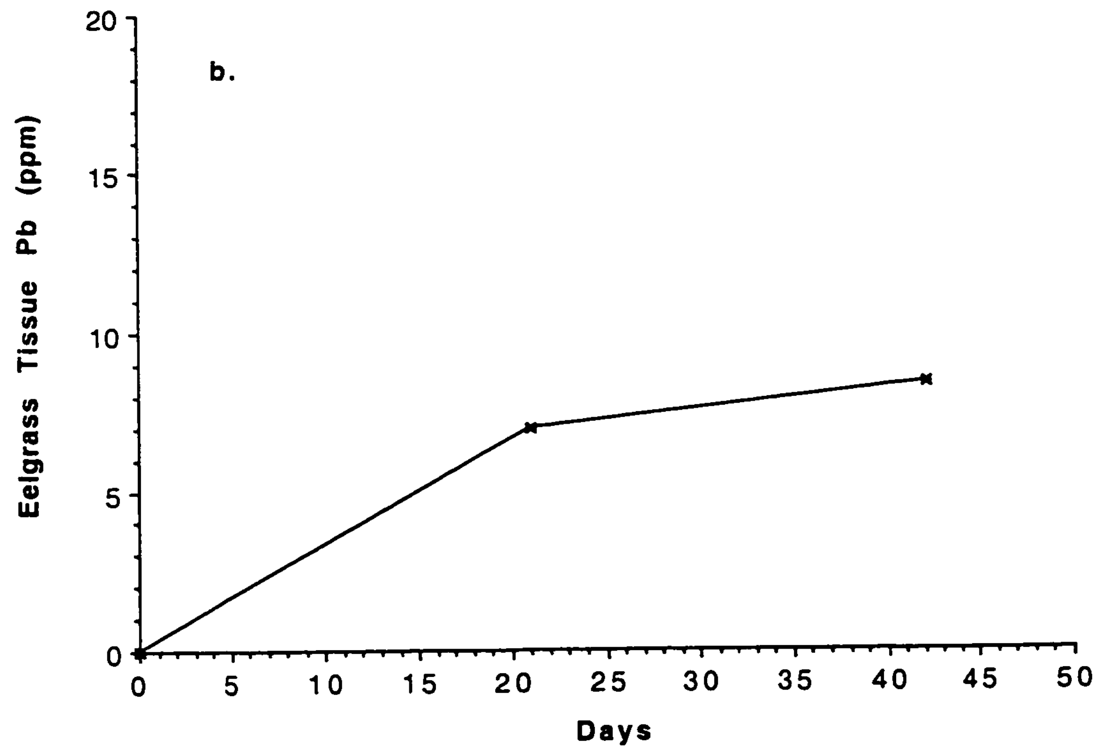


Figure 3.14. Concentration of Pb taken up by leaves of shoots grown at different densities (a) and rhizomes of plants grown at high density (b) in 6x (600 ppm Pb) sediment.

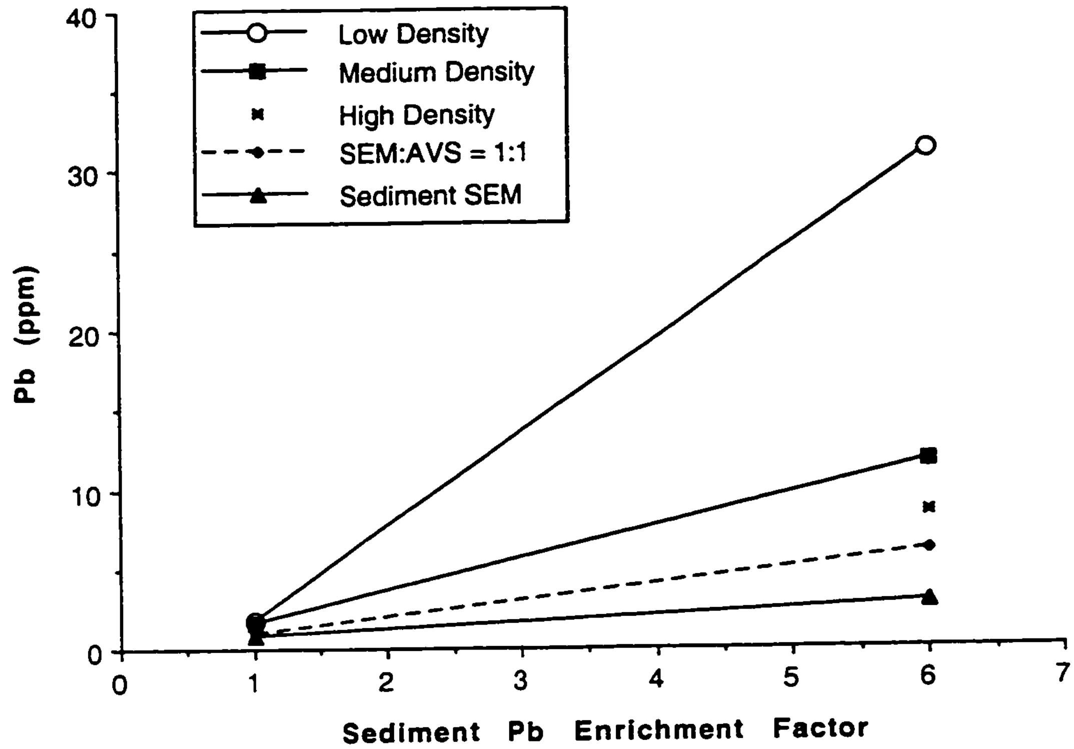


Figure 3.15. Comparison of expected sediment SEM and accumulated Pb in rhizomes planted at low, medium, and high densities in 1x and 6x Pb enriched sediment. The dashed line indicates the SEM:AVS ratio of 1.

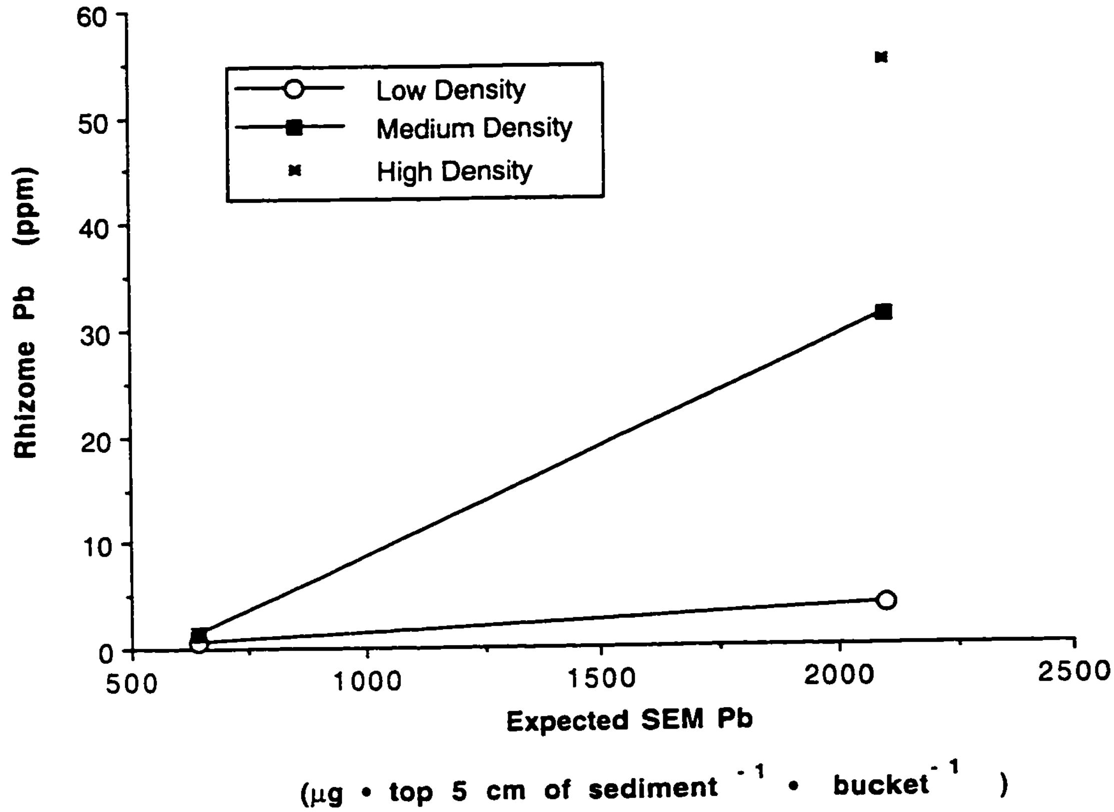


Figure 3.16. Amount of Pb removed by eelgrass rhizomes from top the 5 cm of 1x and 6x sediment treatments after two months of exposure.

DISCUSSION

Eelgrass Morphology

Eelgrass shoots that were grown under experimental conditions responded to environmental parameters in a predictable way (Dennison and Alberte, 1985; Dawes and Tomasko, 1988; Short *et al.*, 1995). As the shoot density of eelgrass increased, competition for light increased due to increased shading by neighboring plants. Response to increased shading was reflected in typical morphological changes that enabled the plants to better utilize the light available to them. For example, leaf length and width were greater at higher density after the first month, extending and widening the leaf tissue in the higher density treatments for more exposure to light (Figs. 3.1, 3.2). With an expanded leaf area, shoots were capable of intercepting more photons of light and carrying out more photosynthesis to supply their greater respiratory needs. Tethered shoots survived growing hydroponically and were statistically comparable in morphology to the low density shoot treatment.

During the first month, rhizomes of high density shoots expanded faster than rhizomes of shoots of lower density treatments perhaps to spread to less vegetated areas and increase exposure of the photosynthetic tissue to light (Fig. 3.5). Because the shoots were growing in a confined area however, shoots planted at high density were unable to spread to unvegetated areas. As a result, significant morphological changes occurred that altered the structure of the eelgrass growing in the high density buckets. For instance, shoots growing in high the density treatment thinned out by decreasing shoot density during the second month (Fig. 3.3). Although leaf canopy was highest for high density shoots (Fig. 3.2), leaves of medium and high density plants were more narrow than during the previous month (Fig. 3.1). Leaf area was also significantly greater for medium and high density shoots than low density shoots during the second month (Fig. 3.8). Thus, shoots were responding to shading by decreasing their density and leaf width to diminish shading while increasing their leaf length to intercept more light. The morphological response to reduced light availability in the densely vegetated treatments indicates that the plants were acclimating to their mesocosm environment.

Eelgrass Growth

Eelgrass growth was not affected by enriched levels of Pb in the sediment. However, there were significant differences in growth among the density treatments. When growth was determined on a per unit area, the highest density shoots produced the most above ground material during both months (Fig. 3.6). However, shoots that grew in high density treatment decreased their above ground production by over two thirds during the second month, indicating the shoots were responding to reduced light and / or nutrients.

There was no significant difference in specific growth among density treatments until the second month when high density shoots grew less vigorously than medium and low density shoots and grew as slowly as the tethered shoots (Fig. 3.7). The decrease in specific growth could indicate insufficient nutrients and / or light. Shoots growing at high density could have been overcrowded and could have used up the nutrient supply in the rhizoshpere and phylosphere (Short and McRoy, 1984). However, high density shoots had weight-to-length ratios that were twice that of all other shoots (Fig. 3.9) in addition to having greater leaf area and length than low density shoots, suggesting that nutrients as well as light were limiting factors for shoots growing in high densities. The greater weight to length ratios of shoots in high density treatments could be explained by nutrient limitation of production of organic compounds (Raven *et al.*, 1986).

Eelgrass Physiology

Although Lyngby and Brix (1984) found no significant effect of Pb on eelgrass growth, they did not assess physiological functioning of the plants or chlorophyll biosynthesis. Chlorophyll fluorescence ratios (F_v/F_m) in conjunction with rates of oxygen evolution provide better insight into the photosynthetic activity of the plants than oxygen evolution alone (Bolhàr-Nordenkampf *et al.*, 1989). Although the typical range of F_v/F_m ratio of healthy *Z. marina* has not been established, terrestrial plants range from 0.75 - 0.85 (Bolhàr-Nordenkampf *et al.*, 1989). Due to the terrestrial ancestry of eelgrass, it is likely that eelgrass has similar photochemistry to terrestrial plants. In other studies, F_v/F_m ratios of control shoots of the seagrasses *Ruppia cirrhosa* (Petagna) Grande and *Zostera capensis* Setchell were 0.77 (Adams and Bate, 1994) and those of *Halophila ovalis* (R. Br.) Hook. f. were an average of 0.73, suggesting that *Z. marina* ratios of my study may be low especially those growing in high levels of Pb (0.67 - 0.71, Table 3.1).

Lead has been shown to affect photosystem II (PS2) of terrestrial plants (Clijsters and Van Assche, 1985). There was a slight decrease in the F_v/F_m ratio with increasing levels of Pb, but the differences were not significant, nor were lower values of the range low enough to suggest damage to the PS2 reaction centers. However, the chlorophyll data suggest that the chlorophyll biosynthesis of plants grown in 6x sediment was affected by Pb (Fig. 3.11).

Photosynthesis irradiance curves show that eelgrass from the high Pb treatments was not photoinhibited (Fig. 3.10). Eelgrass responded to increasing light intensity just as medium and low Pb treatments did at both 10 and 20°C, which are within the normal to high temperature range for eelgrass in New Hampshire (Riggs and Fralick, 1975). The elevated P_{max} rates observed in plants that were held at 20°C versus 10°C were due to the dependency of the dark reaction on temperature (Bulthuis, 1987) and was not related to exposure to Pb. Although there was some evidence of physiological disruption in eelgrass growing in 6x sediment Pb treatments, it was concluded that low tissue Pb concentration (≤ 15 ppm) has no deleterious effect on eelgrass physiology.

Eelgrass Tissue Lead Concentration

The range of Pb concentrations within eelgrass leaves exposed to 1x Pb levels (90 ppm) was similar to plants collected for the ERA (Table 3.3). The similarity in leaf Pb concentration was probably related to sediment Pb concentration because background seawater Pb levels were low in the Portsmouth Harbor (0.100 $\mu g \cdot L^{-1}$, Cullen and Arimoto, 1995). That is not to imply that translocation of Pb from the roots and rhizomes occurred; in fact, subsequent discussion reviews evidence that translocation did not occur. Leaves of shoots in both 1x and 6x sediment were exposed to Pb when the sediment was disturbed during plant manipulation. The resuspended sediment became oxidized as it entered the water column, thereby freeing up Pb for the leaves to uptake. Everard and Denny (1985) make the same inference with a freshwater angiosperm. Diffusion of Pb from the sediment to the water column could also have occurred. Leaves of shoots in 6x sediment accumulated higher Pb levels than leaves of shoots in 1x sediment because the experiment was designed so that each mesocosm was restricted to either 1x or 6x sediment.

Comparisons of leaf and rhizome Pb concentration among shoot densities indicated that shoot density affected the amount of Pb taken up by each shoot over a short term exposure. After two months of exposure to the 6x sediment, rhizomes of low density shoots had more than twice the concentration of Pb compared with rhizomes of medium and high density shoots (Fig. 3.13b). As eelgrass density increased, rhizome tissue Pb concentration decreased. In the high density treatment, rhizospheres overlap, reducing the amount of available Pb for each rhizome. Availability of Pb can be affected by changes of soil properties that are imposed by roots and rhizomes in the rhizosphere (Dunbabin *et al.*, 1988, Williams *et al.*, 1994). Because of the density of roots in the rhizosphere of the high density treatments, there was more competition for uptake of the available cations. Therefore, individual rhizomes of high density shoots accumulated lower concentrations of Pb than medium or low density shoots.

The same process could have occurred in the phylosphere of shoots growing in 6x sediment where available Pb cations competed for sorption sites among the leaves of the high shoot density treatments. Leaves of high density shoots had the lowest Pb concentration of the three density treatments during both months (Fig. 3.13).

Uptake and Fate of Lead in Eelgrass

Comparison of Pb accumulation in rooted versus tethered shoots indicates that little or no translocation of Pb from the roots to the leaves occurred. Eelgrass contains pectic substances in its cell walls which play a role in ion absorption (Maeda *et al.*, 1966) and eelgrass has been shown to absorb metals directly from the water column in all plant fractions (*i.e.*, leaves, rhizomes, and roots; Lyngby and Brix, 1984). Tethered shoots often accumulated as much or more Pb than the rooted eelgrass shoots (Figs. 3.12, 3.13). However, the tethered shoots were not rooted in sediment and therefore must have accumulated their Pb from the water column. Although unintentional, Pb from resuspended, oxidized sediment came into direct contact with the leaves of all rooted and tethered shoots and was a source of Pb for the leaves as described by Everard and Denny (1985). Dissolved Pb could have been absorbed by the leaves or adsorbed to the surface of the leaves (Bond *et al.*, 1985). Therefore, concentrations of Pb found in the leaves of rooted shoots probably did not result from translocation since leaves of unrooted shoots accumulated the same or higher Pb concentrations. If translocation was occurring, more Pb should have accumulated in the rooted shoots than the tethered shoots since the rooted shoots had a constant source of Pb.

Accumulation of Lead by Eelgrass

Although shoots growing in 6x sediment (600 ppm Pb) had higher accumulation of Pb than shoots growing in 1x sediment (90 ppm Pb), the amount of Pb taken up by eelgrass was not proportional to the amount of Pb in the sediment treatments and therefore not linear (Figs 3.12 and 3.13). It was thought that some proportionality would be observed between sediment and tissue Pb concentration based on the proportionality of comparable Pb concentrations observed under laboratory conditions by others (Lyngby and Brix, 1982, 1984; Bond *et al.*, 1985). Since sediment and associated organic material have high binding capacities for Pb, Pb uptake by eelgrass is strongly influenced and perhaps dependent upon biotic and physicochemical processes (such as bioturbation, sediment resuspension, and rhizoshpere oxidation) that free up Pb from AVS. Fluxes of Pb that occur in the natural environment and are related to these processes can be reflected by aquatic plants. For example, Everard and Denny (1985) suggested that resuspension of sediment was integrally related to the amount of free Pb^{++} that is available for plant uptake. In this way, physicochemical processes may limit Pb availability. Therefore, proportionality between sediment and tissue Pb concentration may not be predictable in the natural environment.

Eelgrass from all three densities in 6x sediments showed evidence of a rapid initial uptake followed by a slower accumulation of Pb during the second month (Fig. 3.14), which was consistent with the literature (Lyngby and Brix 1984; Bond *et al.*, 1985; Everard and Denny, 1985). The data show that Pb was sorbed rapidly at the onset of the experiment and may have reached equilibrium with free Pb cations in the environment.

Under the experimental conditions, there was a finite amount of Pb in the sediment and no additional Pb was added to the system over time. Although the expected SEM for both 1x and 6x sediment indicated that no Pb should have been available for uptake because it was below the SEM:AVS ratio of 1, Pb accumulation occurred in all density treatments (Fig. 3.15). As the rhizome grew, new roots were produced and exposed to new portions of the sediment. As the rhizosphere was extended, the sediment was oxidized by the roots, thereby increasing the availability of cations (Williams *et al.*, 1994). As reduced metal sulphides are oxidized, cations such as Pb^{++} are released. Lead that was previously unavailable may become available for uptake during oxidation. The data suggest that Pb uptake by rhizomes may be intimately associated with the expansion and oxidation of the rhizosphere. Additionally, the amount of Pb accumulated by individual shoots of eelgrass was related to shoot density. At high eelgrass density, free Pb cations had more available sorption sites and Pb concentration per shoot was decreased, indicating a density effect. Decreased Pb concentration with increasing shoot density is illustrated by rhizome concentration in Figure 3.15.

When the amount of Pb removed from the sediment by total rhizome biomass was calculated, treatments with the highest plant densities removed the most Pb per unit volume of sediment (Fig. 3.16). Such information is useful in cases where remediation in areas of known Pb pollution is mandated. Higher densities of eelgrass accumulate more Pb from the sediment than lower densities but concentration per gram of tissue is lower. The situation is ideal for remediation purposes since the greatest amount of Pb is removed without creating a burden of high tissue Pb concentration. Additionally, Pb that becomes associated with the rhizomes is likely to remain bound and buried in sediments, which would prevent further cycling of the Pb (Ragsdale and Thorhaug, 1980; Lyngby and Brix, 1989).

Conclusions

Under these experiments, eelgrass responded to environmental conditions in the mesocosms as imposed by the experimental design in predictable ways. For example, as shoot densities increased, competition for light increased and shoots responded by thinning out and growing longer leaves to allow more light to reach photosynthetic tissue. Additionally, shoots in high density treatments may have been limited in growth by nutrient availability in the mesocosms. High density shoots had twice the weight to length ratio at the end of two months of growth indicating that organic carbon was primarily being stored as complex sugars and fats, while protein and nucleic acid production was limited.

Although there was no significant evidence of damage to the photosystem II reaction centers of the leaves, there was a decrease in F_v/F_m values with increased exposure to Pb. Values of shoots growing in 1x and 6x sediment were lower than the range of healthy terrestrial plants and a seagrass relatives (Adams and Bate, 1994; Ralph and Burchett, 1995; Bolhàr-Nordenkampf *et al.*, 1989). Chlorophyll decreased significantly with increased exposure to Pb suggesting there was some effect on the plants' ability to manufacture chlorophyll, although during these experiments the ability of the leaves to photosynthesize was not affected.

Disruption of chlorophyll biosynthesis indicates that active transport of Pb occurred in the leaves. The average Pb concentration in Portsmouth Harbor was 0.100 $\mu g \cdot L^{-1}$ (Cullen and Arimato, 1995), which is lower than that accumulated in the leaves. Lead had to have been concentrated internally to affect chlorophyll biosynthesis, indicating that eelgrass leaves transported Pb against a concentration gradient.

These data suggest that at high Pb concentration, eelgrass physiology may be somewhat adversely impacted. Only shoots from the high density treatments were assessed in the present study, however, and more insight would be gained by investigating F_v/F_m values and chlorophyll levels of leaves with higher Pb concentrations such as those from the low density shoots.

Density of shoots grown in 6x sediment Pb appeared to affect the amount of Pb that accumulated in above and below ground eelgrass tissue.

There are two implications of the effect of shoot density on Pb uptake by the plants. In order to compare the extent of Pb contamination among different sites, it would be important to compare Pb accumulation of eelgrass that is growing at the same plant density or choose low density sites to be conservative. Furthermore, data from the present study imply that if eelgrass is to be used as a remediative tool where coastal marine sediment has been contaminated with Pb, it should be planted in high densities to reduce Pb tissue burden per shoot. An example of this kind of remediation would occur where shallow coastal habitats have been dredged for industrial activity and displaced or impacted eelgrass habitats are to be restored.

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PART II

EELGRASS DEPLOYMENT RESEARCH

CHAPTER IV

EELGRASS DEPLOYMENT NEAR SUSPECTED SITES OF WATER-BORNE SOURCES OF LEAD

Abstract

Eelgrass, *Zostera marina* L., was deployed hydroponically in the water column for 2.5 months to see if lead (Pb) contamination could be detected in estuarine water adjacent to water seepages along the Jamaica Island Landfill of the Portsmouth Naval Shipyard of Kittery, Maine. The hypothesis was that eelgrass tissue would reflect the Pb levels of water seepage draining from this identified source of Pb contamination. Growth metrics were monitored and light conditions were compared among deployment locations to determine whether the deployed eelgrass experienced environmental stress. Light attenuation coefficient values (K_d) indicated that differences in growth parameters were due to differences in light condition among the deployment stations (P-value $\leq$ 0.0001). Lead accumulation occurred in eelgrass leaves at five of the six stations sampled, but there was no evidence of Pb affecting eelgrass growth. Lead that accumulated in deployed eelgrass was necessarily from a water-borne source since the eelgrass was growing hydroponically. There was a higher accumulation of Pb in shoots that were deployed closest to the seepages, suggesting that eelgrass tissue Pb reflects Pb exposure in water. Hydroponically deployed eelgrass may serve as a sentinel accumulator of water-borne sources of Pb.

INTRODUCTION

Estuaries have often been influenced by industrial development because they offer protected harbors for the transport and delivery of industrial goods. Many major cities border estuaries today because of access to ocean shipping and the protective nature of estuaries. Unfortunately, concomitant with the industrial age came pollution from industrial waste. It was common practice to dispose of waste through drainage that lead directly into waterways and estuaries. By the 1970s, many U.S. waters were so polluted that the Clean Water Act, 1972, was established to regulate levels of pollutants entering waterways with the aim of restoring aquatic systems to healthier conditions. Today, there are areas from past waste disposal practices which may still have negative impacts on the surrounding estuarine environment. An example is the Jamaica Island Landfill (JIL) on Seavey Island of the Portsmouth Naval Shipyard.

When the JIL was created during the 1940s, there was no impervious lining placed under and around the landfill. Without an impervious barrier, there was nothing to prevent rain, tidal water and groundwater from flowing through the landfill and leaching toxic materials into the surrounding estuary. A clay cap was placed on the surface of the JIL in 1975 to keep rainwater from leaching through the landfill. It was not evident that groundwater was the major transport of toxic material into the estuary until an Environmental Risk Assessment (ERA) was carried out in 1991 (Johnston *et al.*, 1994), when elevated levels of Pb were found in water seepage and sediment offshore from the JIL. The primary goal of my study was to determine whether water borne Pb was leaching from the JIL and whether eelgrass could be used to detect it.

Providing one of the most productive ecosystems in New England estuaries, eelgrass, *Zostera marina* L., has the potential to act as a sentinel accumulator for heavy metal contamination of estuarine communities (Lyngby and Brix, 1982, 1984, 1987; Brix and Lyngby, 1983; Ward *et al.*, 1986; Tiller *et al.*, 1989). As opposed to bioindicators, which reflect the presence of contamination by decreased species abundance (Gray and Pearson, 1982), seagrasses have been suggested as sentinel accumulators, which reflect the degree and spatial distribution of metal pollution in estuaries (Ward, 1987; Carter and Eriksen, 1992). Eelgrass has a broad distribution in the temperate regions of the Northern Hemisphere (den Hartog, 1970) and has several growth characteristics that make it potentially useful as a sentinel indicator. For instance, the life span of an eelgrass leaf is 56 days on average (Jacobs, 1979) and thus exposure time can be controlled, particularly if the contaminant does not translocate within the plant. The evidence found to date strongly suggests Pb is not translocated in eelgrass (Welsh and Denny, 1979; Lyngby *et al.*, 1982; Chapter III). Additionally, shoots of eelgrass can be successfully uprooted and transplanted or grown hydroponically (Lyngby and Brix, 1984; Short *et al.*, 1993; Davis and Short, 1997) so that eelgrass can be exposed to water and / or sediment where the plants do not naturally occur. Because eelgrass leaves are continually replaced, and because of the ease of transplanting shoots from non-contaminated locations to suspected contaminated sites, eelgrass is particularly suitable as a sentinel accumulator.

Aquatic plants and animals have often been used to give a representative indication of pollution in estuaries (Phillips, 1976; Viarengo and Canesi, 1991; Widdows and Donkin, 1992; Widdows *et al.*, 1995). The filter feeder, *Mytilus edulis* (the blue mussel) has commonly been used in monitoring programs such as Mussel Watch (GMCME, 1996) to identify and monitor areas of concern. Although adult mussels do not migrate as fish and crustaceans do, they filter water for food and may ingest sediment and plankton that have contaminants, *e.g.* Pb, sorbed to their surface (Phillips, 1976). Therefore, contaminant concentrations that are measured in mussel tissue may not accurately reflect the levels of dissolved contaminants in the water.

Lyngby and Brix (1987) compared the uptake of metals by eelgrass and blue mussels and found that tissue metal concentrations (particularly Pb) fell off rapidly in eelgrass with increasing distance from the source but mussel metal concentrations were less responsive. They suggested that mussels did not provide as accurate a representation of metal availability as eelgrass due to ingestion of sediment and plankton, illustrating the point that tissue metal concentrations in filter feeders have an inherent bias.

Eelgrass is often found in and perpetuates depositional areas (Kemp *et al.*, 1984; Short and Short, 1984), which could have important implications for the numerous commercial species that use seagrass beds as nursery and feeding grounds if there are contaminants in the water column. Because estuaries act as filters (Kemp *et al.*, 1984; Nixon and Pilson, 1984), depositional areas within estuaries may be subject to increased contamination, putting organisms that dwell in those areas, and perhaps their predators, at risk. Metal concentrations of eelgrass and other seagrasses have been shown to reflect available metals that are dissolved in the estuarine water column (Lyngby and Brix, 1982; Tiller *et al.*, 1987; Ward, 1989; Malea and Haritonidis, 1995).

In order to test whether eelgrass tissue Pb reflected elevated Pb concentrations in the water column, eelgrass was tethered to cages and deployed in water adjacent to ground water seepages along the perimeter of the JIL. Eelgrass was also deployed at locations away from identifiable seepages to determine whether there was a gradient of Pb in the estuary that was associated with the Shipyard. Eelgrass growth parameters of the deployed plants and light conditions at the deployment sites were assessed to eliminate sources of physiological stress.

METHODS

In order to detect water borne sources of Pb independent from sediment influences, eelgrass was deployed hydroponically in the water column. Wire frames were constructed to lift the eelgrass 15 cm off the bottom by tethering the plants to the upper surface of the frames (Fig. 4.1). The wire was coated with an inert plastic, preventing the eelgrass from being exposed to the underlying metal of the frames.

Eelgrass was collected from the Fishing Island donor site, an area with known low levels of lead in the plant tissues (Johnston et al., 1994). Shoots were first prepared by clipping the rhizome between the fourth and fifth node, and the leaves were trimmed to a length of 46 cm. By cutting the rhizomes and leaves, assessments of growth could later be made by measuring additional node production and leaf length. The eelgrass was prepared by tethering 45 pairs of shoots to the frames in tanks of seawater to prevent dessication. Reclosable plastic ties wrapped with cloth tape were used

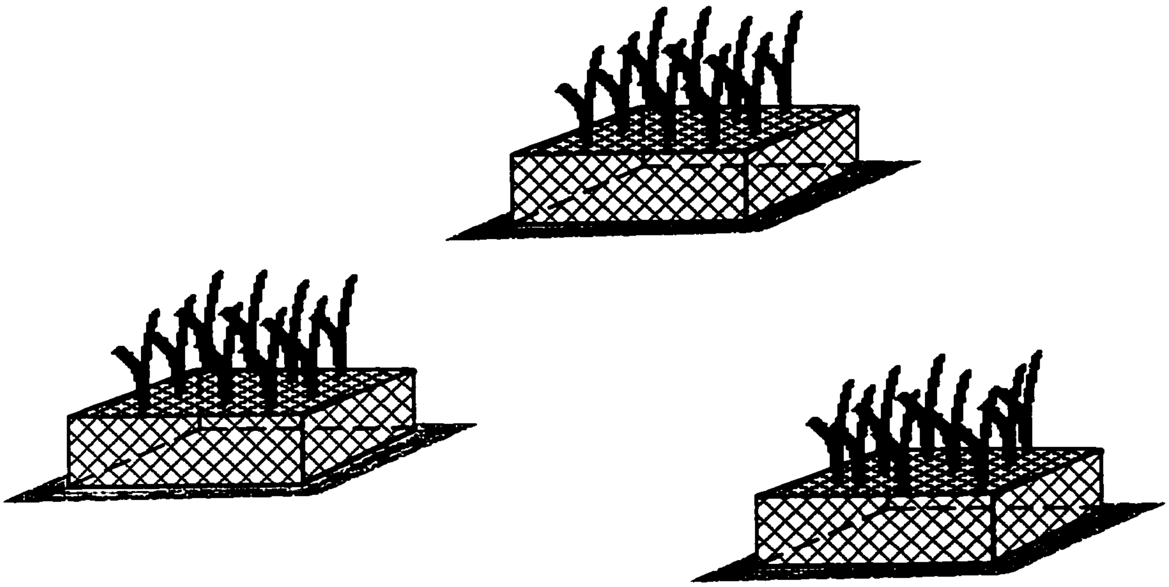

Figure 4.1. Shoots of eelgrass from the reference site were tethered to three plastic coated wire frames at each deployment site and placed on the bottom for growth while suspended, unrooted, in the water column.

to attach the rhizomes to the frames. The cloth tape provided protective padding between the plastic tie and the rhizome.

At mean low tide, there are points along the modified shore of Seavey Island where water seepage is evident. Eelgrass deployment sampling locations were chosen with reference to the seepage points and distance from the perimeter of the JIL (Fig. 4.2). Three frames were deployed at an average depth of 1.5 m mean low water (MLW) at each sampling location.

After one month of exposure in the deployment sites, six shoots were collected from each cage. The length from the meristem to the distal end and the width at midlength of the leaves was measured. Above ground growth rates were determined by measuring the new leaf material that exceeded 46 cm and dividing by the number of days since the leaves were initially cut. Specific growth (mg new growth · mg tissue $^{-1}$ · day $^{-1}$) was calculated and compared between sampling locations. Vegetative vigor measured as rhizome node production was determined by counting the number of new nodes produced.

Once measurements were made, rhizomes were separated from the shoots. The aboveground tissue of each shoot was scraped free of epiphytic algae, washed with distilled, deionized water and dried in a clean glass jar at 80°C for 48 hr and weighed. Growth measurements were taken a second time one month later. All dried plant tissue was stored in clean plastic bags for later Pb analysis.

It was necessary to determine whether the plants at the various deployment sites were exposed to similar environmental conditions for growth. Probably the most important criteria for plants is whether the place of growth has adequate light (Dennison, 1987). During the middle of September, 1994, a Licor light sensor was used to determine the amount of

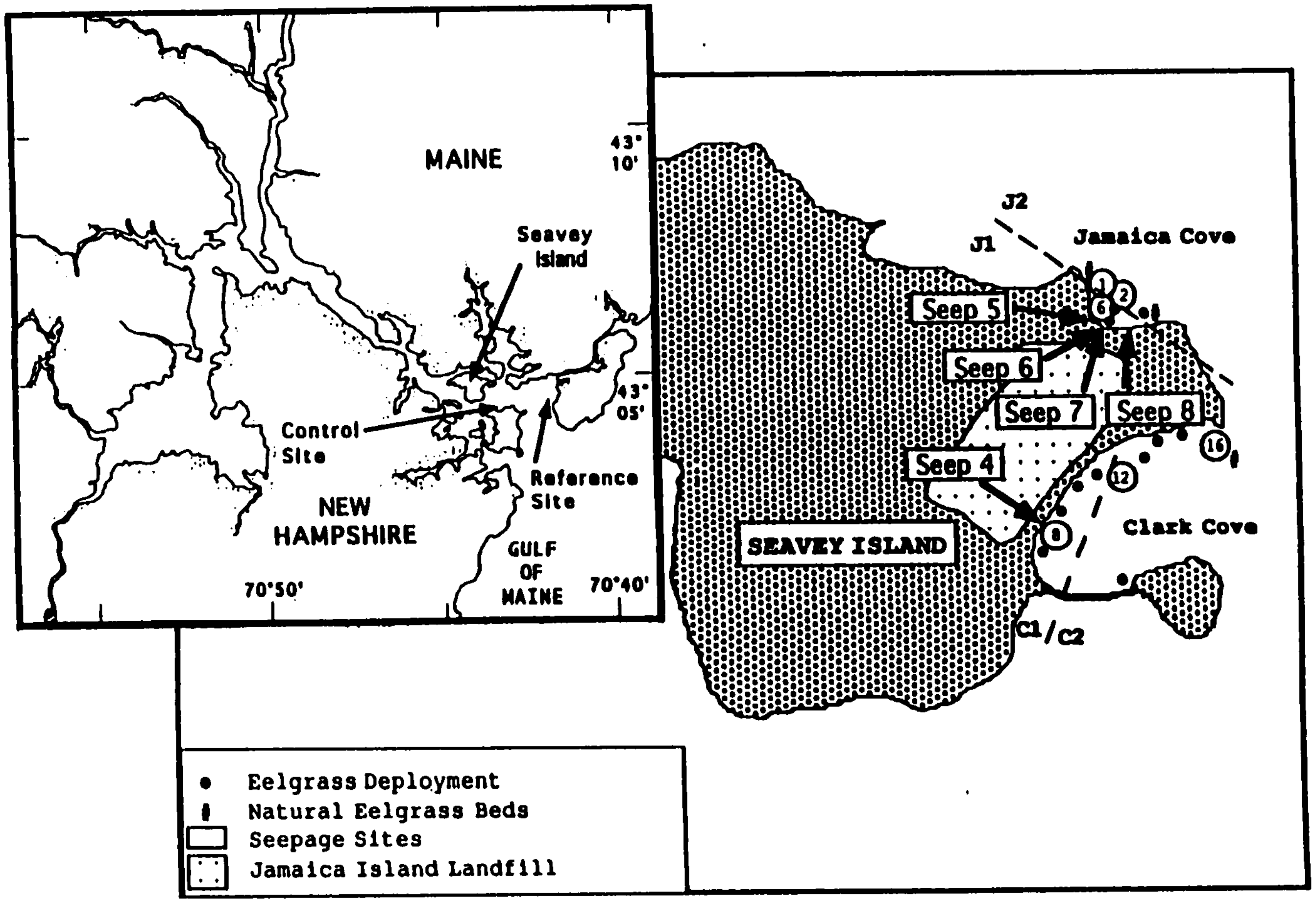

Figure 4.2. Eelgrass deployments and seepage sites around Seavey Island, Maine. Station numbers of eelgrass sampled for Pb analysis are circled. Clark and Jamaica Cove stations are divided by inner and outer cove subgroups: C1, C2 and J1, J2, respectively.

insolation reaching the deployed eelgrass canopy at each sampling station from one hour before to one hour after low tide. A profile was read from the surface down to 0.5 m above the bottom, which was considered the canopy height. Readings from all stations were completed within two consecutive days, both of which were sunny with a slight haze in the atmosphere. On average, the percentage of surface light recorded at the canopy of the deployed eelgrass was measured at 2.3 m below the surface. Light attenuation coefficients were calculated according to the Lambert-Beer equation: $I_z = I_o \cdot e^{-K_d \cdot z}$ where I_z is the incident light at depth z, I_o is the incident light just beneath the water surface, and K_d is the light attenuation coefficient.

Eelgrass tissue samples were compiled from the growth analysis shoot collections and were prepared for lead concentration analysis in the following way. Leaf tissue was ground by hand with a clean ceramic mortar and pestle. As much as 0.5 g of each sample were weighed in acid cleaned 23 ml teflon capsules and digested with ultra pure 7.5N HNO_3. Lead concentrations were analyzed at the New Hampshire Materials Lab of Somersworth, NH, by inductively coupled plasma atomic emission spectrometry (ICP-AES).

One way ANOVA models were used to test for differences between growth and morphological patterns of eelgrass deployed at different locations. Data were transformed where variance was unequal. Means were compared using Fisher's Protected LSD (least significant difference), and linear relationships were assessed between the light attenuation coefficiant and growth parameters.

In keeping with testing for a gradient of Pb accumulation relative to the level of exposure to the seepage sites and proximity to the JIL, subgroups of the data were formed in order to make identification of deployment locations easier. Although there were many differences among growth parameters of the entire data set, there were no consistent trends among stations. Subgroups of the data were designated by their distance from the JIL as follows: inner Jamaica Cove (J1) designated stations 4 - 6; outer Jamaica Cove (J2) designated stations 1 - 3; inner Clark Cove (C1) designated stations 7 - 11; outer Clark Cove (C2) designated stations 12 - 17; and the control site at Salamander Cove (Control) was station 19; all subgroups are identified on Figure 4.2. The control station was located approximately 1000 m southwest of Clark Cove in a natural bed of eelgrass in Salamander Cove, NH.

RESULTS

Average light conditions at the eelgrass canopy (0.5 m above bottom at MLW) ranged from 12.60 to 24.19 % surface light at the Seavey Island locations and one control station (Fig. 4.3). Light conditions at all Jamaica Cove stations were above the critical zone. The critical zone was designated as % surface light reaching the eelgrass canopy that falls between the range of minimal light requirements established by Duarte (1991) and that reported by Dennison et al. (1993), 11 and 20.6 % surface light, respectively. Two stations within outer Jamaica Cove were in natural beds of eelgrass (indicated by circled point, Fig. 4.3). Light conditions in Clark Cove fell within the critical zone for both inner and outer locations, however, there was a natural bed of eelgrass in outer Clark Cove, indicating sufficient light for growth at that location. The control station was also within a natural eelgrass bed and had % surface light measurements that were in the critical zone.

Light attenuation coefficients (K_d) were determined for all sampling stations (Table 4.1). Comparison of location means isolated outer Clark Cove

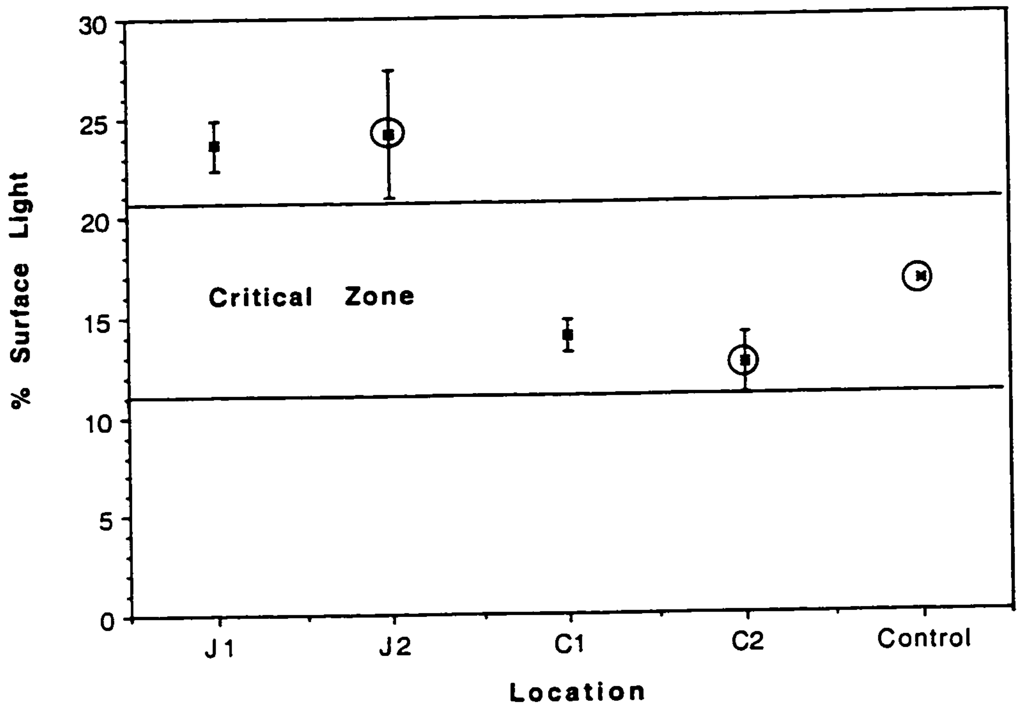

Figure 4.3. Percent surface light at the eelgrass canopy (0.5 m above bottom) at MLW. Critical zone is defined as lower light limits established for eelgrass by Duarte (1991) and Dennison et al., (1993). Circled points contain data from natural beds. Means ± se.

Table 4.1. Light attenuation coefficient (Kd) at deployment locations around Seavey Island (stations 1 - 17) and at Salamander Cove (control, station 19). Values with different letters are significantly different.

	Station	Kd	mean			
	1	0.250				
J2	2	0.123	0.241	a		
	3	0.349				
	4	0.292				
J1	5	0.276	0.284	a - b		
	6	0.284				
	7	0.394				
	8	0.340				
C1	9	0.223	0.307		b - c	
	10	0.338				
	11	0.591				
	12	0.167				
C2	13	0.316	0.342			c
	16	0.351				
	17	0.254				
Control	19	0.347	0.347			c

(C2) and the control as having the highest K_d and outer Jamaica Cove (J2) as having the lowest K_d. Inner Clark and Jamaica Coves (C1, and J1, respectively) had comparable K_d means.

The deployed shoots varied in vegetative vigor due to location both months (P-value = 0.054, month 1; ≤ 0.0001, month 2; Fig. 4.4). During the first month, shoots ranged from 0.067 - 0.080, and during the second month from 0.058 - 0.085 (nodes · shoot $^{-1}$ · day $^{-1}$). Shoots at both inner and outer Clark Cove had the lowest vegetative vigor both months. Shoots in Jamaica Cove had values equivalent to the control shoots during the first month but were intermediate between Clark Cove and control shoots during the second month. Vegetative vigor all deployed shoots decreased from month one to month two except shoots at the control site.

Measurements of growth parameters during the first month indicated that all treatments were acclimating to their specific environment and growing well (Fig. 4.5). During the second month, there were significant differences among the tethering locations that were consistent with the light data (P-value ≤ 0.0001). Specifically, growth per shoot was lowest for outer Clark Cove shoots where light levels were lowest.

By the end of the second month, the shoots had already shed and replaced most of their leaves that were clipped for growth, so all the clipped leaf marks were lost. It was assumed that all leaves of the collected shoots that were not marked were new growth since initiation of the experiment, making calculated growth rates conservative estimates. Leaves produced during the experiment were only exposed to the environment and water where they were deployed. The exception was leaves that were in the bundle sheath but less than 46 cm when clipping occurred.

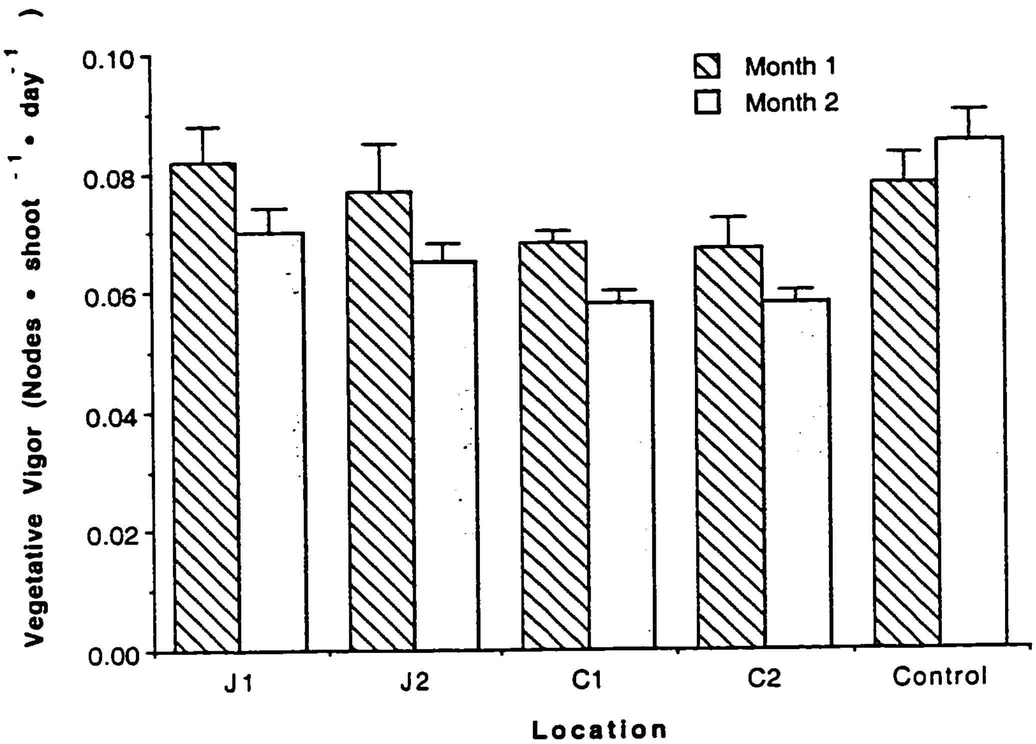

Figure 4.4. Vegetative vigor of shoots tethered at inner and outer locations of Jamaica Cove (J1, J2), Clark Cove (C1, C2) and a control site in Salamander Cove. Mean ± se.

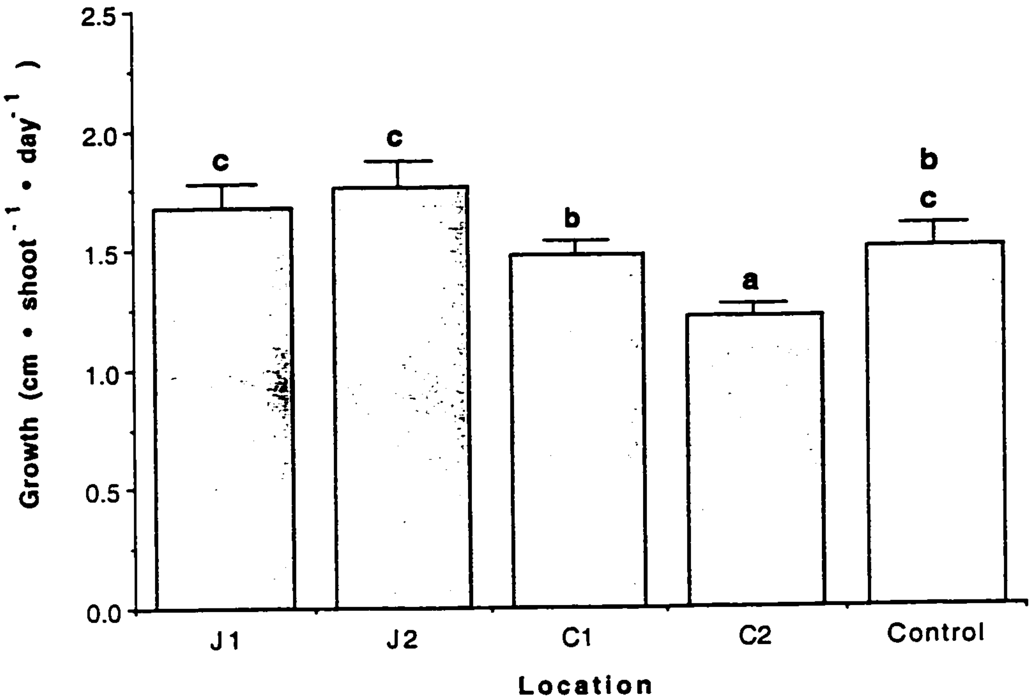

Figure 4.5. Above ground growth of shoots tethered at inner and outer locations of Jamaica Cove (J1, J2), Clark Cove (C1, C2) and a control site in Salamander Cove during month 2. Means with different letters are significantly different. Means ± se.

Specific growth values ranged from 0.012 - 0.014 mg · mg $^{-1}$ · day $^{-1}$ with shoots deployed at the control and outer Clark Cove sites having the slowest rates (P-value ≤ 0.0001, Fig. 4.6). Shoots deployed at outer Jamaica Cove stations had the fastest growth rates.

Leaf initiation rates varied significantly (P-value ≤ 0.0001, Fig. 4.7), with shoots deployed in Clark Cove having the slowest rates. The rates ranged from 19.57 - 27.13 days. Leaf initiation rates of shoots deployed at the control site were similar to shoots deployed in Jamaica Cove. Leaf turnover rates were fairly slow at all locations and ranged from 70.0 - 80.0 days (Fig. 4.8). Shoots deployed at outer Clark Cove and the control site had the highest (*i.e.* slowest) turnover rates (P-value ≤ 0.0001).

The growth data showed typical correlations of eelgrass growth characteristics (Table 4.2). For instance, various measures of growth and expansion correlated positively with each other, such as leaf area and cm · m $^{-2}$ · day $^{-1}$; shoot density and laterals produced · shoot $^{-1}$ · day $^{-1}$; and cm · m $^{-2}$ · day $^{-1}$ and g · m $^{-2}$ · day $^{-1}$.

Correlations between means of growth parameters of the station subgroups and their respective K_d values showed two significant trends (Figs. 4.9, 4.10). At high K_d values (decreased available light), specific growth rates decreased ($r^2 = 0.55$) and leaf turnover rates increased ($r^2 = 0.61$). Analyses of the entire data set also showed that as K_d increased, specific growth decreased and leaves lasted longer on the shoots (P-value ≤ 0.0001 for both models, Table 4.3).

By the end of October (the second month), the deployed plants were not producing enough leaf material to yield replicate samples for Pb concentration analysis. In order to collect enough plant material for Pb

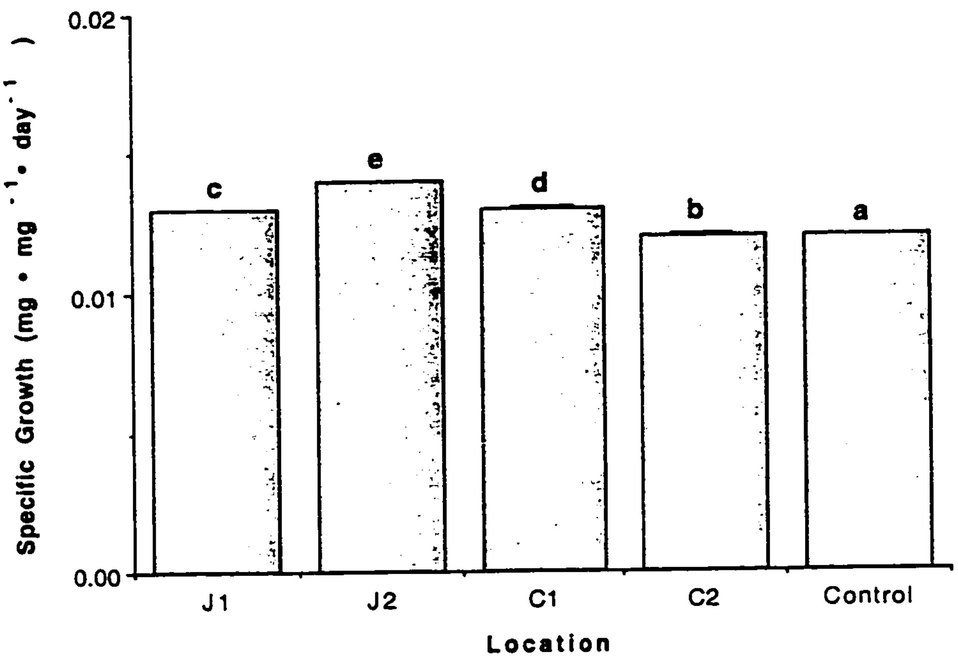

Figure 4.6. Specific growth of shoots tethered at inner and outer locations of Jamaica Cove (J1, J2), Clark Cove (C1, C2) and a control site in Salamander Cove during month 2. Means with different letters are significantly different. Means ± se.

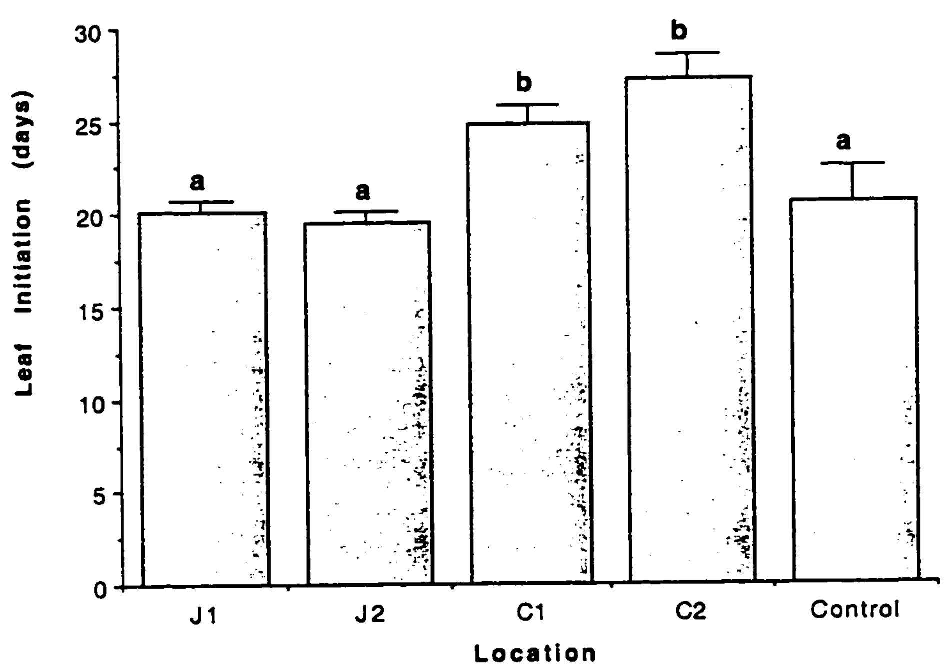

Figure 4.7. Leaf initiation rate of shoots tethered at inner and outer locations of Jamaica Cove (J1, J2), Clark Cove (C1, C2) and a control site in Salamander Cove during month 2. Means with different letters are significantly different. Means ± se.

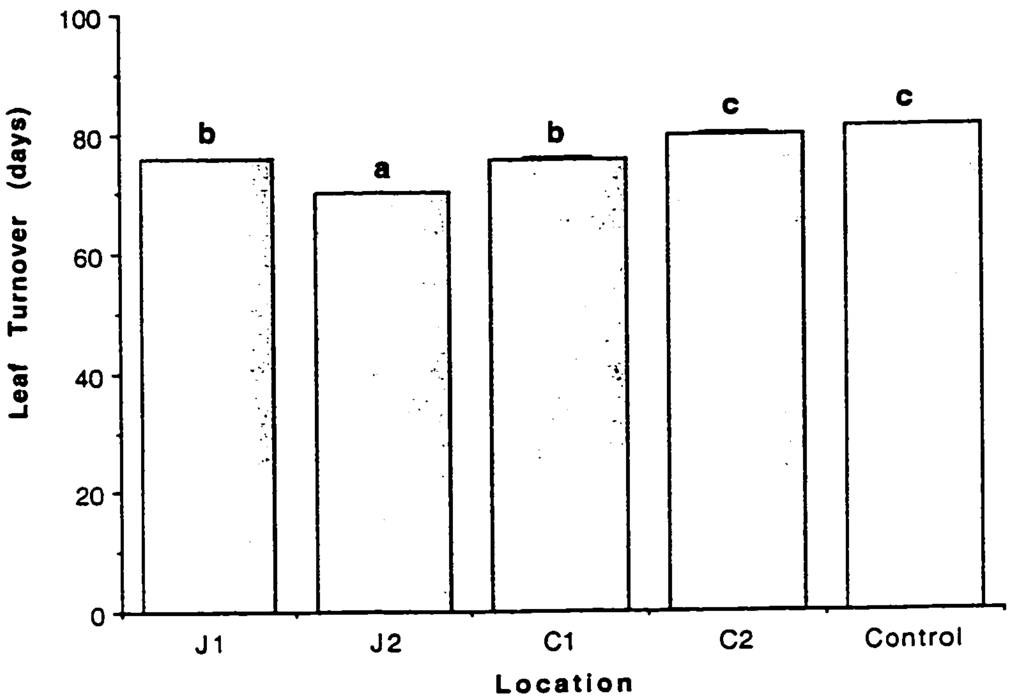

Figure 4.8. Leaf turnover rate of shoots tethered at inner and outer locations of Jamaica Cove (J1, J2), Clark Cove (C1, C2) and a control site in Salamander Cove during month 2. Means with different letters are significantly different. Means ± se.

Table 4.2. Correlations of tethered eelgrass growth parameters measured during month two. Significant correlations are shown, n = 12.

Variable	width	laterals/sh/da	nodes/sh/da	rhiz. cm/da	density/m2	mg/shoot/da	mg/mg/da	cm/shoot/da	cm/cm/da	Tot Length/sht	Longest Leaf	cm/m2/day
width	1											
laterals/sh/da		1										
nodes/sh/da			1									
rhiz. cm/da				1								
density/m2		0.6152			1							
mg/shoot/da						1						
mg/mg/da							1					
cm/shoot/da						0.8251		1				
cm/cm/da							1		1			
Tot Length/sht						0.7961		0.9891		1		
Longest Leaf						0.6782		0.8335		0.8254	1	
cm/m2/day						0.587		0.7793		0.8005		1
g/m2/day				0.66		0.8016		0.6638		0.6614		0.8347
tot. g/tot. cm												
plastocrone in.			-0.8303									
Leaf turnover							-0.9988		-0.9988			
Leaf initiation												
leaf area								0.7289		0.7648		0.9618
% surf. light												

Table 4.2.

Variable	g/m2/day	tot. g/tot. cm	plastocrone in.	Leaf turnover	Leaf initiation	leaf area	% surf. light
width							
laterals/sh/da							
nodes/sh/da							
rhiz. cm/da							
density/m2							
mg/shoot/da							
mg/mg/da							
cm/shoot/da							
cm/cm/da							
Tot Length/sht							
Longest Leaf							
cm/m2/day							
g/m2/day	1						
tot. g/tot. cm		1					
plastocrone in.			1				
Leaf turnover				1			
Leaf initiation					1		
leaf area	0.7647					1	
% surf. light							1

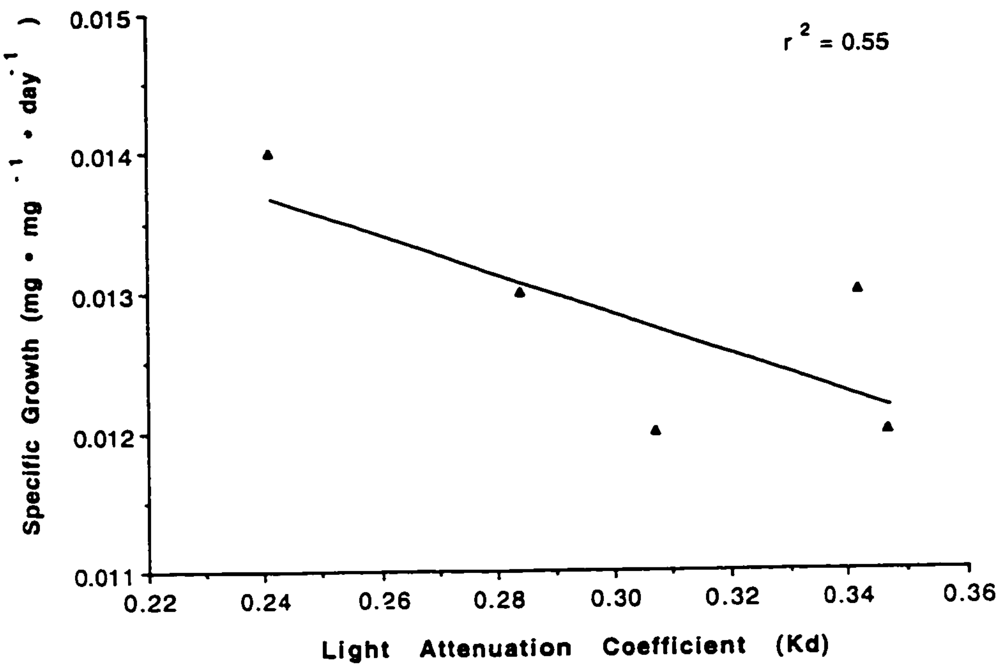

Figure 4.9. Relationship between light attenuation coefficient and specific growth for shoots tethered at Seavey Island and the control site.

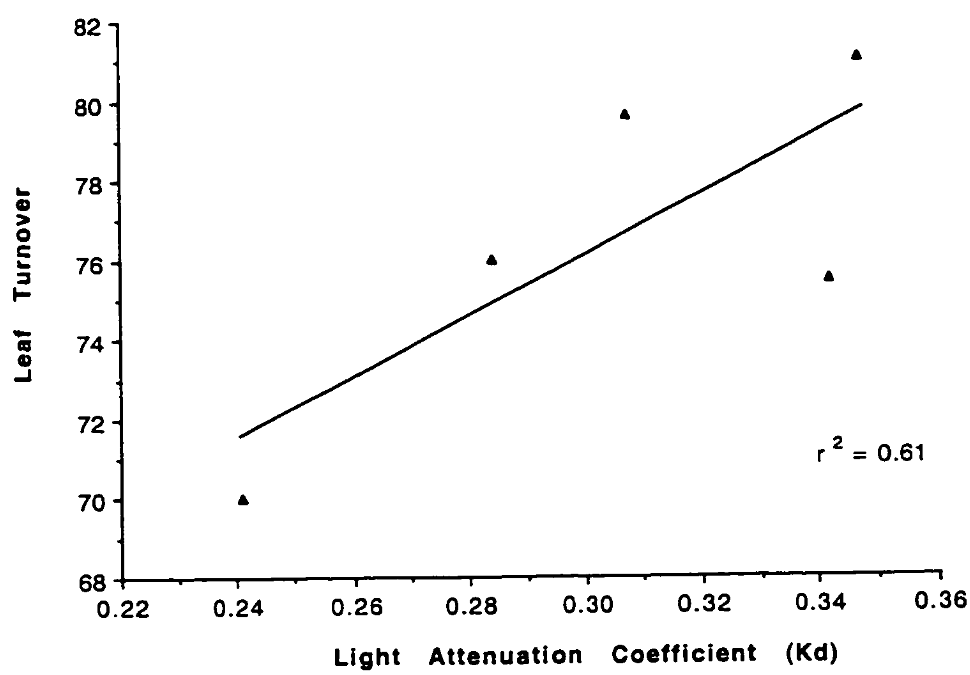

Figure 4.10. Relationship between light attenuation coefficient and leaf turnover rate for shoots tethered at Seavey Island and the control site.

Table 4.3. One-way ANOVA models of Kd x specific growth and leaf turnover.

Kd x Specific Growth

Model	**DF**	**SS**	**MS**	**F-value**	**P-value**
Error	1	2.00E-05	2.00E-05	46.364	0.0001
Total	184	1.00E-04	5.00E-07		
Residual	185	1.20E-04			

Kd x Leaf Turnover

Model	**DF**	**SS**	**MS**	**F-value**	**P-value**
Error	1	561.734	561.734	44.614	0.0001
Total	184	2316.718	12.591		
Residual	185	2878.452			

analysis at some of the stations, leaf samples were composited from the growth samples. Eelgrass tissue Pb concentration showed elevated levels of Pb at 5 of the 6 locations sampled relative to reference tissue Pb concentration (Fig. 4.11). Lead levels in the deployed leaf tissue ranged from 1.0 - 5.3 ppm Pb. Control leaf Pb concentrations were also elevated above reference eelgrass (2.9 ppm) and were located downriver from the Defense Reutilization and Marketing Office (DRMO), which was another source of Pb contamination from the Shipyard. Originally, the control site was selected at the reference site, however, the reference site is exposed at spring tides and contiguous to a public beach. To avoid disturbance of the deployments, an alternative site across the main channel and west of the Shipyard was selected. Shoots deployed at station 8 at inner Clark Cove and station 6 at inner Jamaica Cove had the highest Pb concentration of samples within each respective cove. Both stations were closest to the JIL within their respective coves. Eelgrass from station 2 had Pb levels below the reference datum suggesting no Pb was accumulated. Analysis revealed a correlation between leaf Pb concentration of both deployed and natural shoots (data of both shoot types used for line generation) in relation to distance from the JIL of $r^2 = 0.56$, P-value = 0.002 (Fig. 4.12). Distance from the DRMO was used for Pb concentrations of the control shoots in this analysis.

DISCUSSION

Hydroponic uptake of metals by eelgrass has been studied under laboratory conditions (Brix and Lyngby, 1984) but eelgrass plants have not been deployed under *in situ* conditions for the indication of heavy metals as

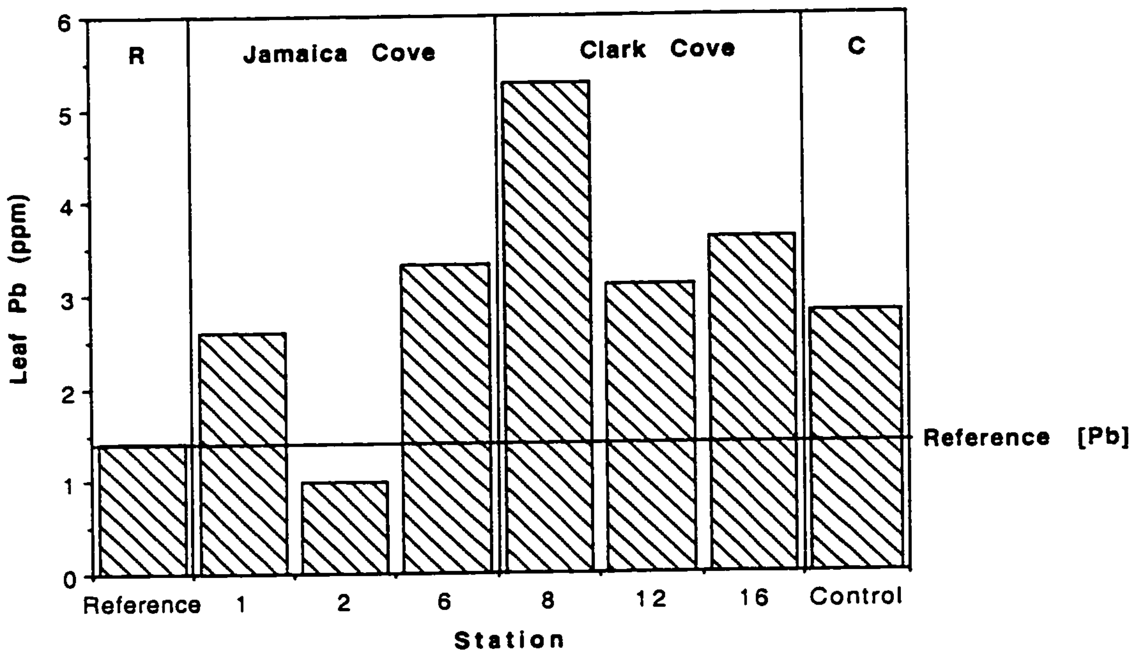

Figure 4.11. Lead concetration of shoots deployed in Jamaica and Clark Coves of Seavey Island and the control site. Reference shoots were collected at the eelgrass donor site.

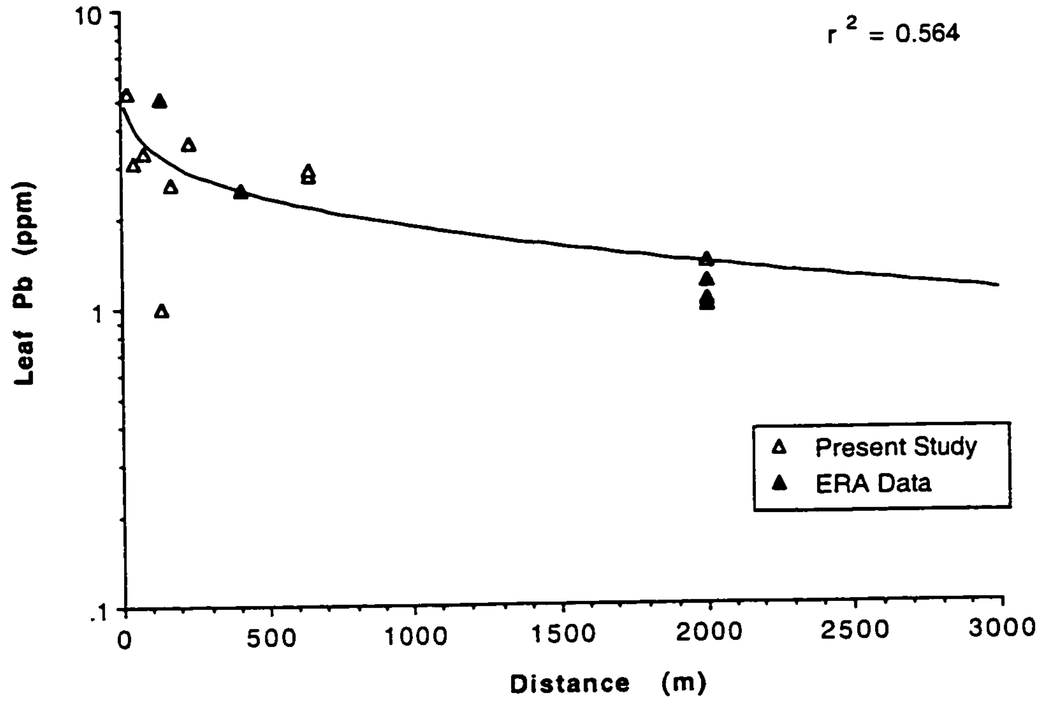

Figure 4.12. Eelgrass leaf Pb concentration with increasing distance from the JIL. Closed triangles are ERA data from natural eelgrass beds and are included in the line generation (n = 5, Johnston et al., 1994).

blue mussels have been (Widdows and Donkin, 1992; Widdows *et al.*, 1995). In the current study, water-borne Pb was detected using a short-term, hydroponic technique that exposed eelgrass to estuarine water within an industrial harbor.

The growth of deployed eelgrass was not affected by Pb concentration in plant tissue, as confirmed under laboratory conditions by Brix and Lyngby (1984) and Chapter III. Even though I observed differences in growth parameters (Figs. 4.4 - 4.8), shoots at the control site generally did not show better growth than shoots deployed near the seeps emanating from the JIL. Although the shoots were deployed at a similar depth, light condition varied greatly among deployment locations, explaining the observed variance in the growth data (Fig. 4.3, Table 4.1). The light data suggests that Clark Cove and the control site, having the highest K_d values, may be somewhat light limited even though natural beds of eelgrass exist in both locations. Both Clark Cove and the control site had light conditions within the critical zone (above the minimum surface light requirement established by Duarte, 1991, but below that reported by Dennison et al., 1993). K_d values correlated significantly with specific growth and leaf turnover (Figs. 4.9, 4.10). The relationship between K_d values and the growth parameters supports the evidence that light conditions were most suitable for eelgrass growth within Jamaica Cove. Deployments growing in the other locations experienced physiological stress related to light availability.

The data demonstrated that deployed eelgrass accumulated Pb during a short term exposure to the water column. Lead levels in eelgrass leaves increased at five of the six stations sampled in Jamaica and Clark Coves relative to the reference datum (Fig. 4.11). Additionally, the Pb accumulation by the deployments increased with proximity to the seepages. In Clark Cove, shoots that were deployed at station 8 were closest to the JIL (and seepage site) and had the highest Pb concentration of the deployments in the cove. Shoots deployed nearest the seepages in Jamaica Cove also accumulated the highest Pb of the deployments in Jamaica Cove. Leaves of deployed and natural shoots showed decreased Pb concentration with distance from the JIL suggesting a Pb source associated with the JIL (Fig. 4.12).

There was an inconsistency in Pb concentrations between the two deployments in outer Jamaica Cove. Although deployed shoots at stations 1 and 2 were similar distances from the JIL, only shoots deployed at station 1 accumulated Pb (Fig. 4.11). The difference in accumulation may be explained by the location of each station. Station 2 was out in the center of the cove, while station 1 was near the shore. Although shoots were deployed at similar depths at all stations, they were not exposed to the same water circulation patterns, which may explain the differences in Pb accumulation at the outer stations. Water circulation patterns along the shore may differ from those in the center of the cove and thus exposure to the seepage may not be the same (Bowden, 1984; Cannon *et al.*, 1984). Thus, care must be taken if *Z. marina* is to be used as a sentinel accumulator of Pb to ensure correct placement with respect to light and water movement.

Conclusions

After two months of deployment, shoots of eelgrass demonstrated that they could grow unrooted in the natural environment and act as sentinel accumulators of Pb in the water column. Additionally, the observed highest accumulation of Pb by deployments that were closest to the groundwater seepages confirmed a source of Pb that is associated with the JIL. Lead concentration of control deployments were also elevated above reference shoots and indicated the DRMO as another source of Pb from the Shipyard.

Hydroponic suspension of eelgrass proved to be an ideal technique that may be useful in monitoring sources suspected of Pb contamination in industrially impacted estuaries. However, water circulation within the coves affected Pb accumulation by the various deployments and should be considered in future work. Because eelgrass can be placed anywhere within the photic zone using this technique, inputs of industrial-based Pb and perhaps other contaminants can be identified and better understood using *Z. marina* as a sentinel accumulator.

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CHAPTER V

ISOTOPE RATIOS OF $^{206}Pb/^{207}Pb$ IN EELGRASS, *Zostera marina* L., INDICATE SOURCES OF LEAD IN AN ESTUARY

Abstract

We deployed hydroponic eelgrass, *Zostera marina* L., to test its use as a sentinel accumulator of water-borne lead (Pb) contamination in the marine environment. Eelgrass was deployed unrooted in the water column in the vicinity of the Portsmouth Naval Shipyard, located on Seavey Island in Portsmouth Harbor of New Hampshire and Maine, offshore from seepage sites near the Jamaica Island Landfill (JIL). Deployed eelgrass, seepage water, and sediment from the deployment sites were analyzed for Pb concentration and stable Pb isotopic composition. Isotopic composition was used to distinguish anthropogenic from ambient sources of Pb in the estuary. Isotope ratios indicated that two seepages were a source of anthropogenic Pb to Jamaica Cove. The eelgrass that showed the strongest presence of industrial Pb (lowest $^{206}Pb/^{207}Pb$) was closest to a high volume seepage that drained from the JIL and contained a high percentage of industrial Pb. These data confirm a source of water-borne industrial Pb in the estuary and indicate a vector of transport of industrial Pb from groundwater to the marine ecosystem.

INTRODUCTION

Eelgrass, *Zostera marina* L., is the dominant seagrass of the northeastern coast of the U.S.A. and is common throughout the Great Bay Estuary on the border of New Hampshire and Maine. Eelgrass has been shown to respond to anthropogenic stresses in estuaries such as nutrient enrichment and heavy metal contamination (Lyngby and Brix, 1982, 1984; Short *et al.*, 1993, 1996). Eelgrass is important for the general health of an estuary, offering protection from predation for juveniles of many commercially important species and, when abundant, contributing to water clarity (Thayer *et al.*, 1984; Orth *et al.*, 1984; Phillips and Meñez, 1988).

Eelgrass was monitored as part of an Ecological Risk Assessment (ERA) associated with the Portsmouth Naval Shipyard to identify whether hazardous materials from an inactive landfill at the Shipyard were impacting the surrounding estuarine environment (Johnston *et al.*, 1994). Plants from two natural eelgrass beds near the Jamaica Island Landfill (JIL) located at the mouths of Clark and Jamaica Coves on the eastern end of Seavey Island (Fig. 5.1), were sampled and showed elevated levels of lead (Pb) ranging from 2.0 - 5.1 ppm (Short, 1995). Lead was detected in the leaves, roots and rhizomes of eelgrass.

Lead for industrial use is mined from Pb ore deposits. There are four stable isotopes of Pb (^{204}Pb, ^{206}Pb, ^{207}Pb, ^{208}Pb) and, depending on the isotopic composition of Pb present at a given ore deposit, the isotope ratios present in the Pb ore will differ. Most industries use a constant source of Pb for their product for consistency and quality control. Therefore, Pb isotope ratios can be used to distinguish mined Pb from ambient Pb. For example, Pb that is used for batteries or gasoline or paint will show a different isotope ratio than ambient Pb, which is locally occurring Pb that is derived from natural weathering processes.

Identification of isotopic composition of Pb through thermal ionization mass spectrometry (TIMS) is a technique that has been used predominantly for geochemical studies, although one study used isotopic analyses to link Pb contamination in mussel tissue with a Pb slag deposit (Flegal *et al.*, 1987). The ratio between ^{206}Pb and ^{207}Pb in various substances or biota is characteristic of the original source of the Pb. Lead from the Earth's crust ranges from 1.220 - 1.250, dependent upon the age of the rocks. Rain and snow melt, which derive their Pb from atmospheric and crustal sources, are typically near 1.210. Ratios of 1.205 ± 0.004 designate Pb from the North American Westerlies (*i.e.*, tradewinds), which is derived from the burning of gasoline, oil and coal (Vernon *et al.*, 1994). Ratios below 1.200 indicate industrial Pb, or Pb that was mined and used in industry. For example, sediment adjacent to an abandoned copper mine in eastern Penobscot Bay has isotopic Pb ratios ranging from 1.150 - 1.158 (Boeckeler, 1996).

To our knowledge, using isotopic ratios of Pb in plant tissue to identify sources of Pb contamination has not been investigated previously. The uptake mechanism for Pb by seagrasses is primarily passive (Lyngby and Brix, 1984; Bond *et al.*, 1985; Everard and Denny, 1985); we assumed that Pb isotopes accumulated in plant tissue reflect that Pb to which the plants are exposed. The isotopes ^{206}Pb and ^{207}Pb differ by one atomic mass unit, a difference so small that we assumed it precludes a plant showing an affinity for the uptake of one over the other. In other words, it is not likely that isotope fractionation plays a major role in Pb uptake by eelgrass. If a plant is exposed to a source of industrial Pb, *i.e.* Pb from battery leachate, the degree to which its $^{206}Pb/^{207}Pb$ isotope ratio falls below 1.200 will depend on the amount of Pb from battery leachate compared to the amount of ambient Pb that it absorbs. By identifying the Pb isotopic composition in deployed eelgrass, we show that eelgrass can be used to identify water-borne sources of contaminant Pb.

We implemented a technique that separated the water column from the sediment as a source of Pb for eelgrass. Shoots of eelgrass were deployed, unrooted in the water column, at specific locations along the shore. In this way, we removed eelgrass from direct exposure to sediment as a source of Pb.

METHODS

Site Description

Seavey Island is located in the Portsmouth Harbor of New Hampshire and Maine at 43° 05′ latitude and 70° 44′ longitude and is the site of the Portsmouth Naval Shipyard (Fig. 5.1). Two coves on Seavey Island that border the southeastern and northeastern shores of the JIL, Clark and Jamaica Cove, respectively, were the locations for the six eelgrass deployment sites used in this study. Stations 1, 2, and 6 were in Jamaica Cove and stations 8, 12, and 16 were in Clark Cove. The JIL was used for as a depository for industrial waste for many years both before and after World War II, including the disposal of mercury, lead-acid batteries, plating sludges and dredge spoils. Clark Cove is periodically dredged and falls off sharply from the shoreline near station 8. There is a fairly expansive mudflat between station 12 and the shore. Station 16 was located at the mouth of Clark Cove near the only natural bed of eelgrass in the cove. Jamaica Cove, in contrast, is shallow and

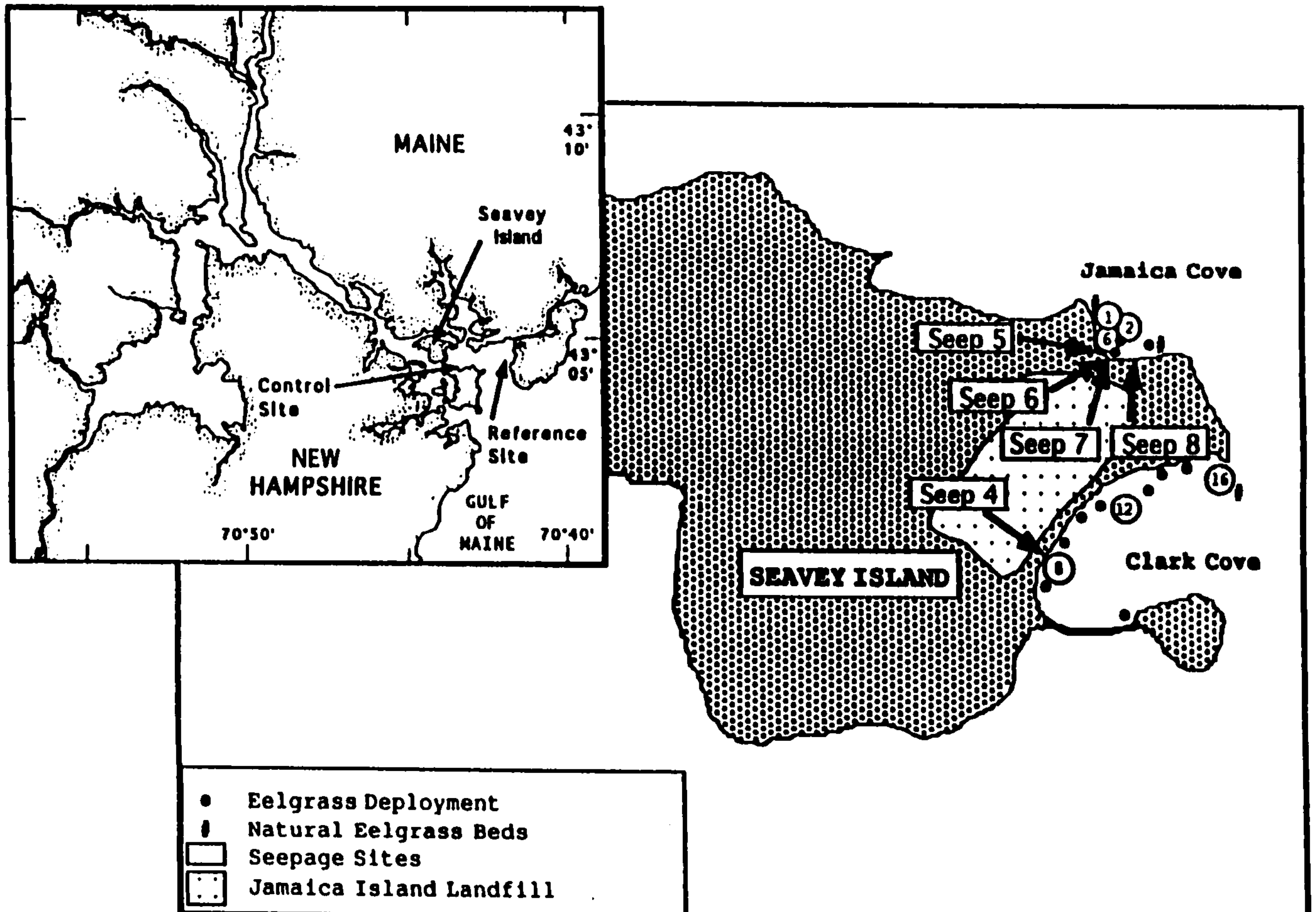

Figure 5.1. Eelgrass deployments and seepage sites around Seavey Island, Maine. Station numbers of eelgrass sampled for Pb analysis are circled.

broad. For example, station 2 is in the center of the cove and was only half a meter deeper than the two other deployment stations that were near the shore. Jamaica Cove also has a small natural eelgrass bed near its mouth.

The JIL was not intentionally constructed as a landfill and was never lined with an in-ground, impermeable barrier. During the 1970s, a clay cap was placed on top of the JIL to prevent rainwater from percolating through the landfill but there was nothing to prevent groundwater or tidal water from moving into and out of the JIL. There are several locations along the shore of both Clark and Jamaica Coves that drain water from the ground into the estuary, visible during low tide. Both groundwater and tidal water enter the landfill, and can leach materials (such as metals) which then enter the estuary through these visible drainage areas that we refer to as seepage sites.

Experimental Methods

Eelgrass was tethered to the top side of plastic coated wire frames (Fig. 5.2) and deployed, unrooted in the water column, offshore from seepage sites near the JIL for two months. Forty-five pairs of shoots that were collected from a reference site were tethered with cloth taped, plastic cable ties to each of three frames at all deployment sites. The frames were weighted with bricks and placed on the bottom such that the plants were positioned approximately 15 cm above the sediment. The plants were deployed near the shore of Clark and Jamaica Coves and at a control site as part of a larger study (Hoven and Short, in prep., Fig. 5.1).

All eelgrass leaves were clipped to a uniform length of 46 cm before deployment. After the first month, six shoots from each frame were collected. The length from the meristem to the distal end and the width at midlength of the leaves was measured. Leaf material that exceeded 46 cm was considered

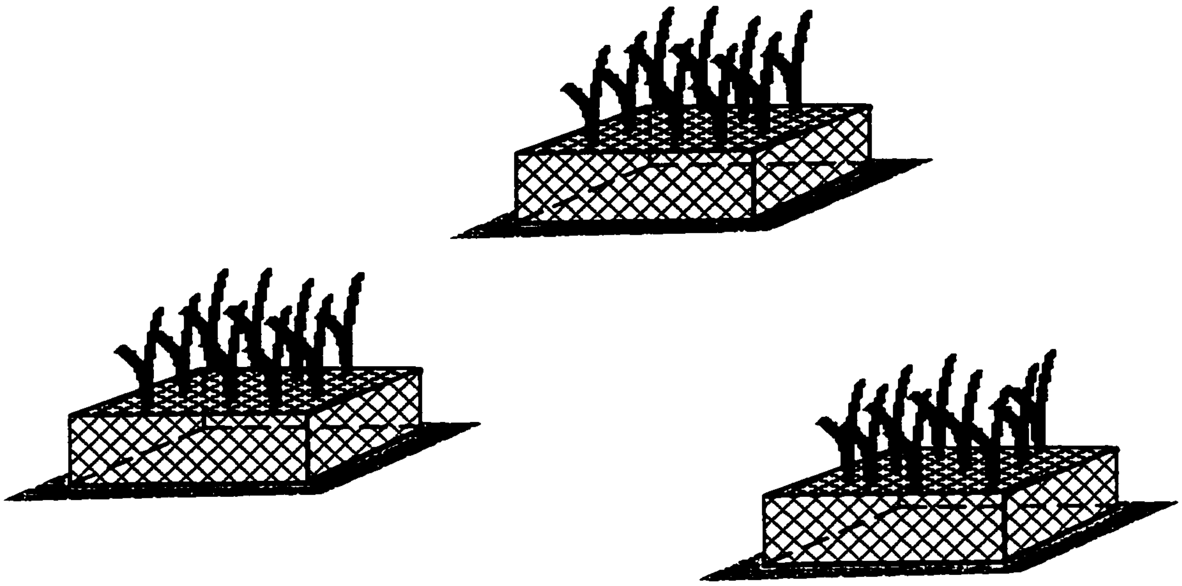

Figure 5.2. Shoots of eelgrass from the reference site were tethered to three plastic coated wire frames at each deployment site and placed on the bottom for growth while suspended, unrooted, in the water column.

new growth and divided by the number of days of deployment. After the second month, six additional shoots per frame were collected for growth and Pb analysis. By the end of the second month, most of the initially clipped leaves had been replaced, indicating that the majority of leaf growth had occurred while the shoots were deployed. Shoots from the growth collections were scraped free of epiphytic algae with a glass microscope slide, and rinsed in distilled, deionized water. Individual samples were dried at 80°C in clean glass jars for 24 hours for weight determination. Samples were stored in clean, reclosable polyethylene bags for Pb analyses.

Sediment samples from the deployment locations in the vicinity of the JIL and a reference station, as well as from 12 additional stations in Clark and Jamaica Coves, were collected using modified, stainless steel oyster tongs. The tongs were rinsed in seawater between each grab, which collected approximately 600 cm^2 of the top 10 cm of sediment. Samples were homogenized and frozen for storage and then dried in clean glass jars at 80°C for 48 hours. Precisely 3.0000 g ± 0.0001 of sediment was disaggregated with a clean ceramic mortar and pestle and digested in ultra pure 3N HNO_3. Lead concentrations of sediment from stations 1, 2, 6, 8, 12, and 16 were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES) and sediment Pb concentration from the 12 additional locations was determined using flame atomic absorption spectrophotometry (FAAS).

Dried eelgrass leaf samples were ground by hand with a clean mortar and pestle. As much as 0.5 g of ground leaf tissue was digested in ultra pure 7N HNO_3, and Pb concentrations were determined using ICP-AES. Lead isotopic composition was measured using thermal ionization mass spectrometry (TIMS).

Seepage water was sampled at five seeps using pore water sippers (Short *et al.*, 1985). The seeps were first sampled for salinity using a hand-held refractometer to determine the tidal influence in the seepage. The seep samples were stored in clean glass jars and analyzed for Pb isotopic composition using TIMS.

Error in measurement precision of TIMS analyses was based on a 95% confidence interval and was 0.01%. Eelgrass tissue Pb concentration was compared between coves by one way ANOVA.

RESULTS

Eelgrass deployments from both Jamaica and Clark Coves often grew as well or better than control deployments (Capter IV). Specific growth ranged from 0.32 - 0.36 $mg \cdot mg^{-1} \cdot day^{-1}$ during the first month and from 0.12 - 0.14 $mg \cdot mg^{-1} \cdot day^{-1}$ during the second month. The second growth analysis occurred during October, which is late in the growing season for *Z. marina* in New England (Short *et al.*, 1993). Plants from all locations grew at similar rates within each month; in the second month, biomass was greatly reduced. The slower growth did not yield enough plant material to sample three replicates per site (one per frame). Therefore, replicate samples were combined to yield enough dried leaf tissue for Pb analyses.

Leaf Pb concentrations were approximately 10 % or less of Pb concentrations of sediment collected at the corresponding deployment locations (Table 5.1). With the exception of the deployment at station 2, all deployed leaves accumulated Pb above the reference level (P-value = 0.034). Eelgrass leaf Pb concentration was generally greater in Clark Cove but was not significantly different between coves (P-value = 0.072). Stations 6 and 8 were

Table 5.1. Lead concentrations (ppm) of deployed eelgrass, naturally occuring eelgrass (ERA samples) and sediment at the deployment locations.
* = Stations closest to the perimeter of the JIL in Jamaica and Clark Coves;
† = ERA samples, mean ± se; (Johnston *et al.*, 1995); # = USACE (1989).

	STATION	EELGRASS LEAF	SEDIMENT
Jamaica Cove	1	2.6	23
	2	1.0	60
	6 *	3.3	109
	ERA19	1.9 ± 0.82 †	44 ± 4.85 †
Clark Cove	8 *	5.3	142
	12	3.1	84
	16	3.6	43
	ERA3	1.6 ± 0.25 †	46 ± 2.65 †
Reference	ERA1	1.1 ± 0.06 †	20 ± 0.55 †
Dredge Classification for sediments #			
Low: Class I			< 83
Moderate: Class II			83- 285
High: Class III			> 285

closest to the JIL and accumulated the most Pb within their respective coves.

Marine sediment sampled from the perimeter of the JIL decreased in Pb concentration with increasing distance from the JIL as fit by a logarithmic curve (r^2 = 0.64, Fig. 5.3). Sediment with the highest Pb concentrations were near the perimeter of the JIL in both Clark and Jamaica Coves. The same pattern was reflected by tissue Pb concentrations of deployed eelgrass (Table 5.1).

Lead isotopic composition of the deployed eelgrass, sediment and seep water implicated the presence of industrial Pb within both coves. Eelgrass tissue from five of the six deployment sites had $^{206}Pb/^{207}Pb$ isotopic ratios below the established North American Westerlies value of 1.205 ± 0.004 (Vernon *et al.*, 1994), indicating exposure to an industrial source of Pb (Fig. 5.4). Three of the six sediment samples from the deployment locations had Pb isotopic ratios indicative of industrial rather than ambient sources of Pb as well. Sediment from station 6 of Jamaica Cove had a ratio of 1.189, and sediment from station 8 of Clark Cove had a ratio of 1.191. Station 6 and 8 had the highest Pb concentration within their respective coves (Table 5.1). Only two of the seepages that were sampled revealed isotope ratios below atmospheric inputs of Pb, and both were located on the shore of Jamaica Cove, inshore of the deployments at station 3, with a ratio of 1.166, and inshore of the deployments at station 6, with a ratio of 1.178. The seepage samples were all under 15 ppt salinity, indicating less than 50% input of tidal water into the landfill.

Deployed eelgrass isotopic ratios in Jamaica Cove were more heavily influenced by industrial Pb than eelgrass deployed in Clark Cove, *i.e.*, the Pb isotopic ratios of eelgrass were lower in Jamaica Cove than Clark Cove.

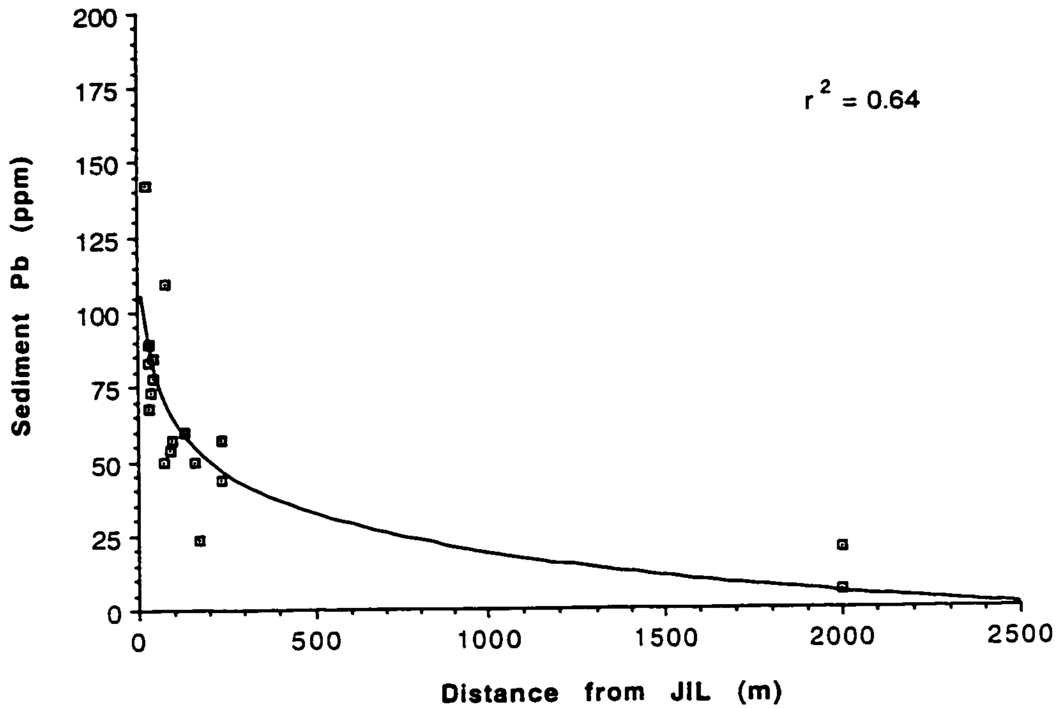

Figure 5.3. Distance from the JIL versus sediment Pb concentration.

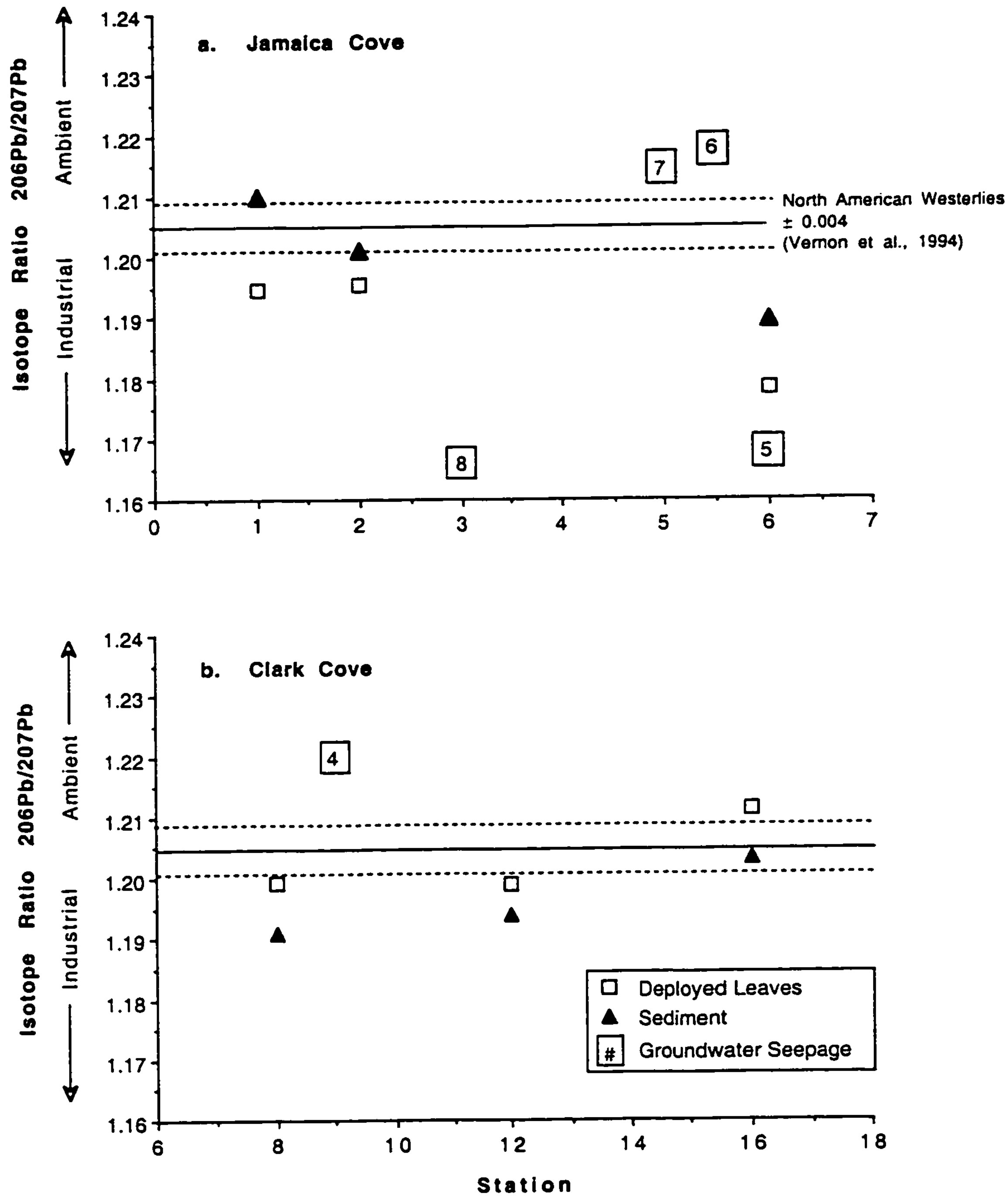

Figure 5.4. Lead isotope ratios (206Pb/207Pb) of deployed eelgrass, sediment and ground water seepage at stations in Jamaica Cove (a) and Clark Cove (b). Seep station numbers are enclosed in boxes.

Eelgrass leaves had ratios lower than sediment ratios in Jamaica Cove, and higher ratios than sediment in Clark Cove. The eelgrass Pb isotopic ratio most heavily influenced by industrial Pb was at station 6 in Jamaica Cove, and the two lowest isotope ratios of all samples were from water seepages in Jamaica Cove near stations 3 and 6.

DISCUSSION

Lead was prevalent in most of the sediment collected around the perimeter of the eastern end of Seavey Island, and concentrations fell within the "moderate" classification in the Maine Classification of Dredged Material (USACE, 1989; Table 5.1). Both Jamaica and Clark Coves have sediment Pb concentrations elevated above the Class I level of Pb according to the Maine Classification of Dredged Material. Lead concentrations were conspicuously higher in sediment collected less than 100 m from the JIL than sediment collected farther away (Fig. 5.3).

Eelgrass deployed within Jamaica and Clark Coves of Seavey Island accumulated levels of Pb that were slightly elevated over reference eelgrass Pb levels (Table 5.1). Although eelgrass Pb levels were not significantly different between coves, eelgrass deployed at stations 6 and 8 had the highest Pb concentrations and were closest to the JIL within their respective coves.

By comparing Pb isotopic composition of eelgrass to that of the nearby seepage water and estuarine sediment, a pathway of industrial Pb from landfill groundwater to marine plant tissue was revealed. The isotope ratio $^{206}Pb/^{207}Pb$ in eelgrass samples from stations 1 and 2 was less influenced by industrial Pb (Fig. 5.4). Both stations 1 and 2 were further from seepage locations and the JIL than eelgrass deployed at station 6 (Fig. 5.5). Because the

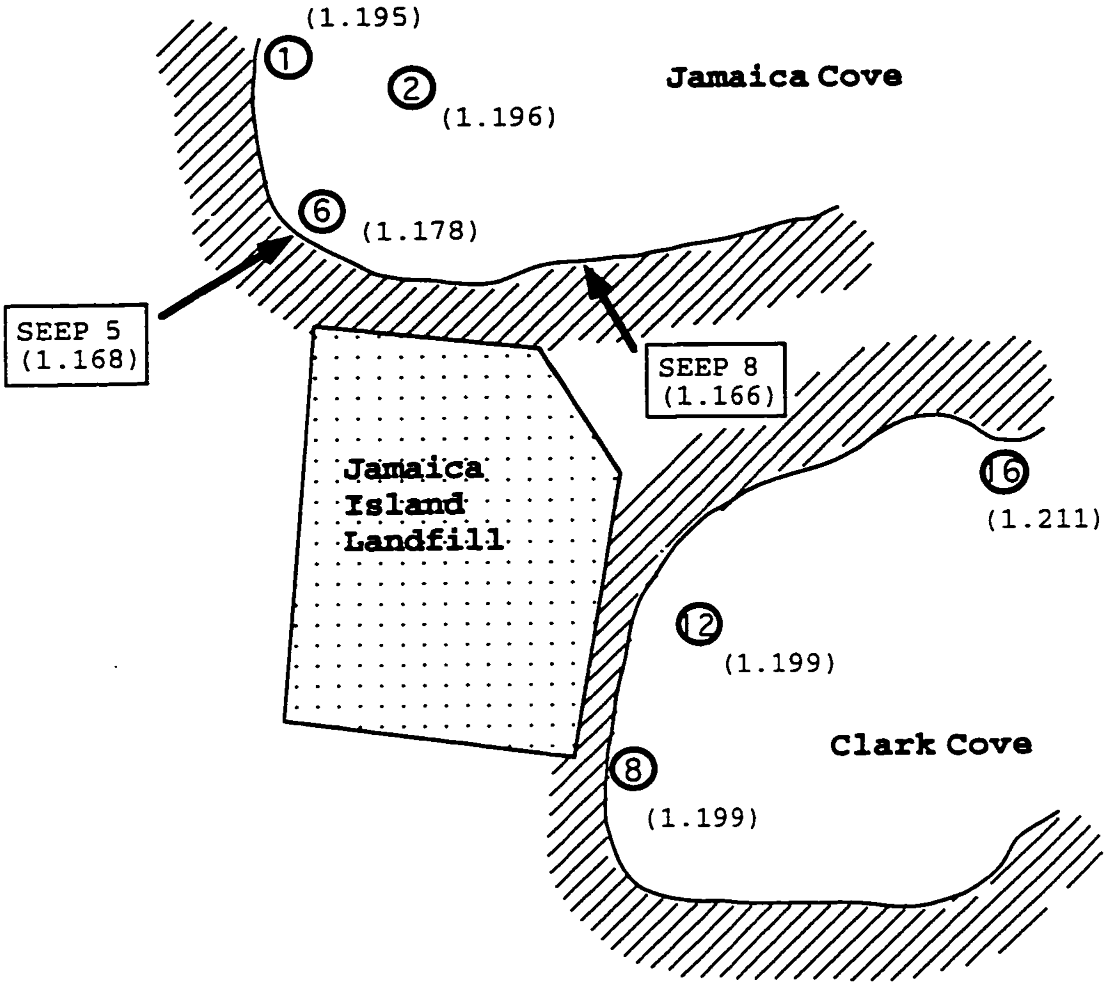

Figure 5.5. Representation of Clark and Jamaica Coves showing positions of the eelgrass deployments (circled numbers) relative to the JIL. 206Pb/207Pb ratios of eelgrass are in parentheses. Coves and JIL are not drawn to scale.

isotope ratio of the eelgrass deployed near station 6 was much lower than the isotope ratio of sediment collected at that same location and approached that of the nearby seepage (Fig. 5.4), I conclude that the deployed eelgrass absorbed Pb from the seepage water that was entering the estuary. Lead isotopic composition in the eelgrass was not identical to that in the seepage water because plant isotopic composition is an accumulation of Pb isotopes absorbed from seawater which includes ambient Pb, as indicated by the data. While eelgrass deployments in Clark Cove had $^{206}Pb/^{207}Pb$ ratios below atmospheric inputs from the Westerlies, the isotope ratios were above those of the corresponding sediment, suggesting the Pb absorbed by the eelgrass deployments in Clark Cove was from a combination of industrial Pb from resuspended sediment, ambient sources, and perhaps Pb from unmeasured seepage. Although there was no evidence of a source of Pb associated with seepage in Clark Cove, only one seepage site was sampled. Since the isotope ratio of the sediment at station 8 was similar to that of station 6 in Jamaica Cove, there may be other seeps in Clark Cove that contain industrial Pb.

Ward (1987) first proposed that the seagrass, *Posidonia australis*, was a "sentinel accumulator" for indicating the extent and spatial distribution of contamination of metals near a lead smelter in Australia. Carter and Eriksen (1992) showed that *Z. muelleri* could be used as a sentinel accumulator for copper in the estuarine environment. In the present study, eelgrass worked as a sentinel accumulator of industrial Pb in the Portsmouth Harbor of the Great Bay Estuary. The data strongly suggest that industrial Pb is currently leaching from the JIL into Jamaica Cove inshore of deployment stations 6 and 3. In past ecological risk assessments, it has been difficult to pinpoint sources of contaminants that are found in water or its biota. Once a contaminant is dispersed in water, its association with its source is lost. However, the isotope ratio $^{206}Pb/^{207}Pb$ in eelgrass confirmed a vector of transport from groundwater to the estuary.

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