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# The effect of physical and biological site characteristics on the survival and expansion of transplanted eelgrass (Zostera marina L)

Ryan Clark Davis University of New Hampshire, Durham

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**UMI** 

# THE EFFECT OF PHYSICAL AND BIOLOGICAL SITE **CHARACTERISTICS ON THE SURVIVAL AND EXPANSION OF** TRANSPLANTED EELGRASS (ZOSTERA MARINA L.)

**BY** 

# **RYAN C. DAVIS** B.A., Virginia Tech, 1985 B.S., Towson State University, 1993

### **DISSERTATION**

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

> Doctor of Philosophy  $\mathbf{i}$ **Natural Resources**

> > May, 1999

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This dissertation has been examined and approved.

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Dr. Tom Lee, Associate Professor, Department of **Plant Biology** 

#### **DEDICATION**

This dissertation is dedicated to all my family and friends who provided support over the past six years, especially to Laura and Clark...you are my life.

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### **ACKNOWLEGDEMENTS**

I thank my advisor, Dr. Frederick T. Short, for sharing his knowledge and appreciation of eelgrass and estuaries with me. I'll never look at shallow water the same again. I also thank Fred for funding, patience, and guidance over the last six years. Thanks for taking a chance on an English major!

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Many UNH students and the staff at Jackson Estuarine Laboratory helped in more ways than I can recall. Special thanks go to all the New Hampshire Port Authority Eelgrass Mitigation Project eelgrass collectors and divers, Dan Marquis, Derek Sowers, Vidya Kesevan, Dawn Hallowell, Doug Britton, Rick Biche, Brad Aguis, Lea Houk, Keith Savage and many others who transplanted boatloads of eelgrass and also helped with the field and mesocosm experiments. Thanks to Doug Britton for doing illustrations. Thanks also to Cathy Short for editing the entire dissertation.

Finally, I thank my wife Laura, without whose support I never would have been able to finish.

iv

# **TABLE OF CONTENTS**



# **CHAPTER**

 $\sim$ 

# **PAGE**





vi

 $\hat{\mathcal{A}}$ 



 $\bullet$ 

 $\mathcal{L}_{\mathcal{A}}$ 



# **LIST OF FIGURES**



 $\ddot{\phantom{a}}$ 

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# **LIST OF TABLES**

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#### **ABSTRACT**

### THE EFFECT OF PHYSICAL AND BIOLOGICAL SITE **CHARACTERISTICS ON THE SURVIVAL AND EXPANSION OF** TRANSPLANTED EELGRASS (ZOSTERA MARINA L.)

by

Ryan C. Davis University of New Hampshire, May 1999

Eelgrass (Zostera marina L.) was transplanted at seven sites along the New Hampshire side of the Piscataqua River in 1993 and 1994. The eelgrass transplanting was one component of the New Hampshire Port Authority Mitigation Project, designed to mitigate for impacts to natural resources associated with the expansion of the port facility. Over 2.5 hectares of eelgrass were transplanted using a newly developed transplanting technique, the horizontal rhizome method, and ultimately created eelgrass habitat at several sites. However, transplants did not survive at any of the intertidal areas planted and were greatly reduced at several subtidal sites. The intertidal transplants were lost due to severe ice scouring during the 1993/1994 winter. This dissertation focused on determining which factor(s) caused the loss of subtidally transplanted eelgrass.

I investigated the relationship between subtidal transplant survival and various physical and biological site characteristics by analyzing field data and conducting field and mesocosm experiments. The results of my research show that light, current, and sediment characteristics were not limiting at the transplant sites and that transplant growth rates were not significantly different among the range of sediment types found at the transplant sites. My research also showed that belowground growth rates for eelgrass transplanted using the horizontal rhizome method were significantly higher than for eelgrass transplanted with the most commonly used transplanting method, the bundle

xii

technique. These results indicate that the variability in the survival of subtidally transplanted eelgrass was not the result of the transplanting technique or physical site characteristics, but was due to some other factor such as bioturbation.

I then conducted experiments to quantify the effect of bioturbation on transplant survival. The results of a mesocosm experiment showed that green crabs (Carcinus *maenas*) can significantly decrease transplant survival when they occur in densities of greater than 4.0 per square meter. Similarly, the results of a field experiment showed that the clam worm (Neanthes virens) can significantly decrease transplant survival by pulling the tips of the eelgrass blades into the sediment. These results demonstrated that bioturbation by N. virens and C. maenas significantly decreased survival rates of transplanted eelgrass, and that protecting transplants from the bioturbating activity of these organisms significantly increased transplant survival.

The final aspect of my research was to create a transplant site selection model by combining all factors that influence transplant survival (as demonstrated by the research conducted for this dissertation and as documented in the literature). The result of this effort was the development of the Preliminary Transplant Suitability Index (PTSI) and Transplant Suitability Index (TSI). The PTSI/TSI methodology provides a framework and quantitative approach for selecting potential transplanting sites. The indices were applied retroactively to the New Hampshire Port Authority eelgrass mitigation sites and correctly differentiated between the successful and unsuccessful sites. The model is now being used as a site selection tool in other estuaries on the East Coast of the United States and will provide natural resource managers with an effective tool for identifying and prioritizing potential seagrass restoration sites.

#### xiii

#### **CHAPTER I**

#### **INTRODUCTION**

#### **Background**

Eelgrass (Zostera marina L.) is a flowering plant that grows underwater, rooted in the sediment. Elegrass is widely distributed in estuarine and coastal areas throughout the Northern Hemisphere (Phillips and Menez, 1988). Each shoot of eelgrass generally contains between 2 to 5 flat leaves enclosed in a bundle sheath. The leaves elongate as the plant grows and can reach up to 2 meters in length (Phillips and Menez, 1988). The plants also grow belowground by elongation of the rhizome. This type of vegetative growth strategy allows eelgrass to expand both above and belowground to form dense meadows (Tomlinson, 1980).

Eelgrass meadows create important habitat and form a basis of primary production that supports ecologically and economically important species (Thayer et al., 1984; Orth et al., 1984; Heck et al., 1995). Eelgrass, and seagrasses in general, are an essential component of healthy estuarine and coastal ecosystems (Short et al., 1993). Eelgrass plants baffle wave energy (Gambi et al., 1990; Fonseca and Cahalan, 1992), creating a depositional environment (Ward et al., 1984), and provide sediment stabilization (Ward et al., 1984; Hine et al., 1987). The plants also filter and retain nutrients from the water column (Kenworthy et al., 1982; Short and Short, 1984).

The importance of eelgrass to estuarine and coastal productivity was highlighted in the 1930's, when a large scale die-off of eelgrass occurred on both sides of the Atlantic due to the wasting disease (Rasmussen, 1977). The wasting disease is caused by a pathogenic slime mold, Labyrinthula zosterae Porter et Muehlstein, and has been reported in several species of Zostera (Short et al., 1987; Muehlstein, 1989). The disease

attacks eelgrass leaves and destroys mesophyll cells via enzymatic degradation (Muehlstein et al., 1991), causing leaf loss and eventually plant death. The disease resulted in the loss of over 90% of the North Atlantic eelgrass population; the loss had a catastrophic effect on estuarine productivity (Milne and Milne, 1951). Several fisheries, for example the North Carolina bay scallop industry, were decimated and have never fully recovered (Thayer et al., 1984).

In recent decades, losses of eelgrass, which had slowly recovered much of its former range, have occurred again. This time the losses have been attributed to a variety of causes, including water quality degradation resulting from eutrophication (Kemp et al., 1983; Twilley et al., 1985; Cambridge et al., 1986), aquacultural practices (Everett et al., 1995), coastal development (Short and Burdick, 1996), human-induced disturbance and storm events (Short and Wyllie-Echeverria, 1996), and a recurrence of the wasting disease (Short et al., 1986; Short et al., 1988). The current consensus among scientists and resource managers is that reduced light availability caused by poor water quality resulting from the pollution associated with increased human population and development is the most important cause of seagrass loss (Duarte, 1995; Short and Wyllie-Echeverria, 1996; Short and Burdick, 1996).

On average, seagrasses require approximately 10-20% of surface light to survive (Duarte, 1991; Dennison et al. 1993). The amount of light available underwater decreases exponentially with depth due to the scattering, reflection, refraction, and absorption of incident light caused by the water itself and dissolved and particulate constituents within the water column (Dennison et al., 1993). Recently, research and management efforts have focused on identifying which water quality parameters most affect light extinction and are correlated with seagrass survival (Batuik et al., 1992; Stevenson et al., 1993; Morris and Tomasko, 1993; Fletcher and Fletcher, 1995). In the Chesapeake Bay, the critical water quality parameters have been identified as total suspended solids, dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorus (DIP), and chlorophyll a concentrations (Batuik et al. 1992; Stevenson et al., 1993; Dennison et al. 1993). In other areas, such as the Indian River Lagoon of Florida, water

 $\mathbf{2}$ 

color (Kenworthy and Fonseca, 1996; Gallegos and Kenworthy, 1996) and dissolved organic matter (Gallegos and Kenworthy, 1996) have been shown to be important. In the Long Island Sound, the importance of tidal range (Koch and Beer, 1996) and sediment organic matter content on eelgrass distribution and losses have also been documented. Based on the results provided by these studies, many estuaries now have management plans in place to improve the quantity and quality of light reaching the bottom, primarily through a reduction in the amount of nutrient pollution, such as DIN and DIP, reaching the waterbody (Batuik et al. 1992; Morris and Tomasko, 1993; Duarte, 1995).

The reduction of nutrient pollution is expected to improve water quality and increase light availability, allowing seagrasses to recover. Johansson and Lewis (1992) documented such a recovery in Hillsboro Bay, Florida following reduction of nutrient inputs. However, whether seagrasses can fully recover their former range, and the time scale of recovery following improvements in water quality, remain largely unknown (Duarte, 1995). For example, the sites that were transplanted in 1993 and 1994 as part of the New Hampshire Port Authority (NHPA) eelgrass mitigation project were vegetated as recently as 1981, until the eelgrass was destroyed by the wasting disease (Short et al. 1986). The sites had remained unvegetated since then, despite the presence of large eelgrass beds in the surrounding area (Short et al. 1993). The lack of revegetation may be due in part to the dramatic effect eelgrass plants have on physical and biological site characteristics and water quality.

Eelgrass meadows support large and diverse faunal assemblages, often with a different species composition than that found in unvegetated areas (Orth et al., 1984; Heck et al., 1995). Consequently, once eelgrass cover is lost, research has shown that physical and biological site characteristics and water quality change. Rasmussen (1977) and Christiansen et al. (1981) documented the subsidence and loss of fine particle sediments and organic matter with the disappearance of eelgrass in Denmark following the wasting disease of the 1930's. Hine et al. (1987) reported an increase in sediment transport and decrease in sediment deposition associated with the loss of seagrass along the Florida coast. Short term water quality degradation caused by sediment resuspension

 $\overline{\mathbf{3}}$ 

was reported by Olesen (1996) and Duarte (1995) in areas where seagrass cover was lost.

Following the 1930's eelgrass die-off due to the wasting disease. Rasmussen (1977) documented the change in the benthic infaunal community from a predominantly deposit feeding community to a suspension feeding community. Similarly, Connolly (1995) showed a reduction in epifaunal species abundance following the experimental removal of seagrass canopy. The presence of seagrasses have also been shown to have a significant influence on faunal recruitment processes (Grizzle et al., 1996; Eckman, 1987) and predator-prey relationships (Heck and Crowder, 1990; Orth et al., 1984), suggesting that the loss of seagrasses can result in significant changes to the biological communities inhabiting a site. These types of changes in physical and biological site characteristics may be important determinants for the potential recolonization of historically vegetated sites, even after sufficient water quality improvements have been made.

The lack of recolonization of previously vegetated habitats has been attributed to a number of causes. Olesen (1996) and Giesen et al. (1990) suggested that short term water quality degradation caused by sediment resuspension prevented eelgrass revegetation in the Limfjorden and the Dutch Wadden Sea, respectively. The effect became even more pronounced once the roots and rhizomes had completely decomposed a few years after the eelgrass coverage was lost. Moore et al. (1996) and Burke et al. (1996) both contend that reduced light availability caused by turbidity during a spring "window of opportunity" prevents eelgrass from sequestering enough carbon reserves during this crucial growing phase so that even if plants do initially colonize a site, they will not persist. A reduction in the number of propagules or seeds produced from smaller remaining populations can also limit the extent and speed with which eelgrass can expand its range (Orth et al. 1994; Olesen, 1996). Because of the potential obstacles that can slow or prevent natural recolonization, transplanting has been used as a means of reestablishing seagrass populations in historically vegetated sites (Fonseca et al., 1998).

Transplanting seagrass has also been used as a means for mitigating impacts to naturally occurring seagrass beds due to coastal development. The work conducted for

 $\overline{\mathbf{4}}$ 

this dissertation was based on an eelgrass mitigation project undertaken to offset impacts to an eelgrass bed resulting from the expansion of the New Hampshire Port Authority (NHPA) pier facilities in Portsmouth, New Hampshire (Bosworth and Short, 1993). The impetus behind this research was the differential success of the eelgrass transplanted at sites along the Piscataqua River in 1993 and 1994 (Figure 1). By identifying the factors most responsible for the differential transplant survival, I hope to provide information that can be used to improve the site selection process and the overall success of future eelgrass restoration efforts.

#### **Transplanting Seagrass**

Historically, the overall survival rate for transplanted seagrass shoots is approximately 40% (Fonseca et al., 1998). As described later in Chapter 2, survival rates for eelgrass transplanted for the NHPA mitigation project varied considerably, from 0% at most intertidal sites and two subtidal sites, to 99% at four subtidal sites. Fonseca et al. (1996) state that a 49% transplant survival rate is acceptable and provides adequate coverage for a seagrass population to eventually recover without further human intervention. However, such a high rate of transplant loss leads to unacceptable increases in the time and cost of a project, and can lead to poor public perception of restoration efforts. The mechanisms responsible for historically low survival rates need to be identified and quantified to improve the success of future transplanting efforts. The NHPA eelgrass mitigation project provided an ideal means by which this could be accomplished. Numerous types of biological and physical site characteristic data were collected in both the transplanted and naturally occurring eelgrass beds over a number of years. These data included eelgrass parameters such as above ground biomass and shoot density, benthic infaunal species composition and abundance, fish species composition and abundance, sediment parameters, depth, current, and light availability. To augment these field data, I conducted a number of field and mesocosm experiments to quantify the effects of selected physical and biological site characteristics on the survival of transplanted eelgrass.



which they were transplanted. Control sites begin with the letter "C." The donor bed from which all transplant Authority Migitation Project. Transplant sites begin with the letter "T" and are numbered in the ordered in Figure 1. Site map showing the location of all transplant and control sites for the New Hampshire Port material was collected is also shown near the mouth of the Piscataqua River.

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 $\overline{6}$ 

Of the possible factors that can directly influence the survival of transplanted eelgrass, poor site selection (Fonseca et al., 1988; Harrison 1990) and insufficient light associated with poor water quality (Zimmerman et al., 1991; Reid et al., 1993; Stevenson et al., 1993; Zimmerman et al., 1995) have been identified as the major limiting factors. Zimmerman et al., (1991) state that "the success of any seagrass revegetation effort is strictly dependent upon a physical environment that will not only ensure initial establishment, but will support long-term growth as well." While this is true to a certain extent, i.e., light is certainly the most important criteria, it belies the influence biological site characteristics can have on recolonization or restoration efforts. One such characteristic, bioturbation, has been shown to be a significant factor that can greatly reduce the survival and expansion of both naturally occurring (Orth, 1975; Suchanek, 1983; Philippart, 1994) and transplanted (Harrison, 1987; Philippart, 1994; Fonseca et al., 1994; Molenaar and Meinesz, 1995; Fonseca et. al., 1996) seagrasses. For example, Fonseca et al. (1994) transplanted two species of seagrass in Tampa Bay, but lost many transplants to bioturbation by foraging rays. In areas where the rays were not a factor, the seagrass grew extremely well (Fonseca et al., 1996). Molenaar and Meinesz (1995) lost the majority of their experimental transplants to the burrowing shrimp Callianassa tyrrhena. At sites not inhabited by the shrimp, survival rates were as high as 62%.

For the NHPA eelgrass mitigation project, the majority of transplants at intertidal locations were lost due to ice scouring in the severe winter of 1993-1994 (Davis and Short, 1997), which is consistent with previous findings that the shallow edge of eelgrass beds in this geographic area are set by physical disturbances (Dennison and Alberte, 1986) such as ice scour (Robertsen and Mann, 1984). In contrast, survival rates of subtidal transplants were generally over 80%, except for two sites. At these two sites, 99% and 80% of the subtidal transplants did not survive. Preliminary evidence from field observations suggested that bioturbation or feeding activity by the clamworm (Neanthes virens) and the green crab (Carcinus maenas) were decreasing transplant survival. The overall goal of my research was to separate physical site characteristics from biological interactions and quantify their relative effects on the survival of subtidally transplanted eelgrass. To accomplish this goal, I established a number of objectives which involved

analyzing the physical and biological site characteristic data collected for the NHPA project and conducting mesocosm and field experiments to quantify the effects of sediment characteristics and bioturbation by clamworms and green crabs on the survival of transplanted eelgrass.

#### **Objectives**

The objectives of my research were to investigate the physical and biological site characteristics of the NHPA eelgrass transplanting sites (Figure 1) to determine the extent to which they explain the differential survival rates of transplanted. Specifically, I attempted to answer the following questions:

1. Are the physical site characteristics (i.e., light availability, current, and/or sediment type) limiting transplant survival?

2. Is the new transplanting method developed for the NHPA project successful and a preferable method to those previously used?

3. Can benthic invertebrate community data from potential transplant sites be used as a tool to predict how well transplants will survive?

4. Are bioturbating organisms limiting transplant survival at any of the NHPA eelgrass transplanting sites?

5. Can physical and biological site characteristics be used together to improve the site selection process?

#### **Organization of Additional Chapters**

Chapter II provides an overview of the mitigation project through which the eelgrass transplanting was completed and a thorough description of the new transplanting method that was developed for this project. Chapter II has been published as an article in *Aquatic Botany*, a peer reviewed journal, with coauthor Frederick T. Short. The other chapters describe the field data collected and the experiments that were completed to augment the field data. The first component of my research was to investigate the physical characteristics of the transplant sites to determine if suitable growing conditions existed. Specifically, I measured light and current levels at all transplant and naturally occurring eelgrass (control) sites to determine if light (Dennison, 1987, Duarte, 1990;

Zimmerman et al. 1995) or current velocity was limiting (Fonseca et al., 1998; Fonseca and Fisher, 1986). I also conducted mesocosm experiments to determine if the sediments can support eelgrass or if significantly different eelgrass growth rates occurred in the different sediment types at the transplant sites (Kenworthy and Fonseca 1977, Short 1987), and whether the new transplanting method used for the NHPA project affected eelgrass growth. The results from these data collections and mesocosm experiments are presented in Chapter III.

For the second component of my research, I attempted to quantify the effects of bioturbating organisms on the survival and expansion of transplanted eelgrass. The benthic infaunal species composition and abundance from each transplant site were analyzed to determine if potentially important differences in the benthic communities existed which were correlated with transplant survival. Finally, I conducted field and mesocosm experiments to determine if N. virens and/or C. maenas can decrease transplant survival, and if so, whether protecting the transplants from the activity of these organisms can increase transplant survival. The results of the benthic infaunal community data analysis are presented in Chapter IV. Chapter V describes the results of the N. virens field experiments. Chapter V has been submitted as an article to the Journal of Experimental Marine Biology and Ecology, a peer reviewed journal, for consideration of publication, with co-author Frederick T. Short. Chapter VI describes the results of the C. maenas mesocosm experiment and has been published as an article in Restoration Ecology, a peer reviewed journal, with co-authors Frederick T. Short and David M. Burdick. Chapter VII describes the eelgrass transplant site selection model designed to combine physical and biological site characteristics to create a comprehensive site selection tool for future transplanting efforts. The rationale behind the site selection model development is described, as well as an application of the model to the NPHA eelgrass mitigation project sites. The site selection model is currently being field tested in New Bedford Harbor, Massachusetts and the lower Chesapeake Bay.

#### **CHAPTER II**

# RESTORING EELGRASS (ZOSTERA MARINA L.) HABITAT USING A NEW TRANSPLANTING TECHNIQUE: THE HORIZONTAL RHIZOME METHOD

#### **Introduction**

Eelgrass (Zostera marina L.) meadows are highly productive components of estuarine and coastal ecosystems and support large and diverse faunal assemblages (Thayer et al., 1984; Heck et al., 1995). Eelgrass plants filter and retain nutrients from the water column (Short and Short, 1984), provide sediment stabilization (Ward et al., 1984), and baffle wave energy (Fonseca and Fisher, 1986), thereby reducing erosional forces and protecting adjacent shorelines (Christiansen et al., 1982). Eelgrass biomass production serves as a major component of the detrital food chain (Thayer et al., 1984). Worldwide, eelgrass abundance has declined significantly since the turn of the century due to pollution associated with increased human populations (see review Short and Wyllie-Echeverria, 1996) and episodic occurrences of the "wasting disease" (Short et al., 1986; den Hartog, 1994). In the United States, seagrass habitats are protected under Section 404(c) of the Clean Water Act (33 U.S.C. 1341-1987). According to these regulations, any person who undertakes any activity which may potentially impact seagrasses must mitigate for those impacts by first, avoiding as many potential impacts as practicable; second, minimizing any impacts that will occur, both spatially and temporally; and finally, replacing the functional values of the habitat lost due to impacts (Federal Register 3/12/90). When proposed plans to expand the existing New Hampshire

Port Authority pier facilities in Portsmouth, NH, USA (43º 05' 00"N, 70° 45' 40"W) included impacts to an eelgrass bed, federal and state regulatory agencies required mitigation to prevent further loss of eelgrass habitat within the Great Bay Estuary.

The mitigation plan (Bosworth and Short, 1993) for the New Hampshire Port project required transplanting eelgrass at several sites in the estuary, totaling 2.5 hectares. to replace the functional values lost due to both direct and indirect impacts on eelgrass beds adjacent to the proposed pier facility. Although the project had to meet the requirements of a large public-works undertaking, it was designed in an experimental framework to determine our ability to restore eelgrass habitat and to evaluate the time course of transplant survival and habitat functional development. The transplanting method described here was developed specifically for the mitigation project.

Since the 1940s, numerous projects of varying size and complexity have attempted to restore seagrass habitats (Addy, 1947; Phillips, 1974; Churchill et al., 1978; Fonseca et al., 1996). Other studies have focused on evaluating the plants' morphological and physiological responses to transplanting (Kenworthy and Fonseca, 1977; Phillips and Lewis, 1983; Dennison and Alberte, 1986; Molenaar and Meinesz, 1995). Fonseca (1992) summarized five main goals for a seagrass mitigation or restoration project: (1) development of persistent vegetative cover; (2) equivalent acreage of vegetative cover gained for that lost; (3) increase in acreage where possible; (4) replacement of the same seagrass species as was lost (in-kind mitigation) and; (5) development of faunal population structure and abundance equivalent to that of natural, control beds. In our transplanting project, we attempted to meet these five goals in addition to satisfying the U.S. Army, Corps of Engineers' requirement of in-place (as well as in-kind) replacement of the habitat functions and values lost, while working in an experimental framework. This paper reviews other transplanting techniques and describes the technique developed for our project.

11

#### **Background**

Seagrass transplanting methods can be grouped into three broad categories: 1) shoots with sediment intact, known as cores or plugs; 2) seeds; and 3) shoots with bare roots. Extracting cores of shoots with the sediment intact has been recommended as the preferred transplanting method (see review Phillips, 1990), but costs can become prohibitive (Fonseca et al., 1996). A variety of devices has been used to extract cores of seagrass with the roots, rhizomes and sediment intact, including PVC pipe (Phillips, 1990), small metal cans (Kelly et al., 1971; Harrison, 1990), sod pluggers (Fonseca et al., 1996), and shovels (Addy, 1947; Churchill et al., 1978). The cores are moved to the transplant site and placed into excavated holes. The advantage of the core/plug method is that a large, well developed root and rhizome system remains intact, including a portion of the sediment type and nutrient pool to which the plant is adapted. The major disadvantage or the core/plug method is the creation of holes in a healthy donor site that must be filled and, even then, may be susceptible to erosion. Additionally, transportation and labor requirements are high.

Seeds have also been used in seagrass restoration efforts (Addy, 1947; Lewis and Phillips, 1980; Fukuda, 1987). Seeds are collected by taking reproductive shoots from natural beds or from along the wrack line and storing the shoots in seawater until the seeds mature and are released. The major advantage of this method is that once a suitable number of seeds have been collected, they can be sown over large areas rather quickly and easily. However, currents and bioturbation can transport seeds so there is no guarantee they will germinate where they are sown, and creating a bed in a specific location is difficult. Other disadvantages include the substantial amount of time it can take to collect a suitable number of viable seeds due to variable seed production, as well as unpredictable germination time, low seedling viability, and highly variable survival rates (Churchill et al., 1978; Lewis and Phillips, 1980; Churchill, 1983; Phillips et al.,

 $12$ 

1983; Harrison, 1991; Moore et al., 1993). Additionally, because of the relatively short dispersal distance of seeds from existing beds (Orth et al., 1994), large scale seed collection may reduce natural seedling recruitment in the immediate vicinity.

The bare-root method involves removing seagrass shoots along with a small length of rhizome (2-20 cm, depending on species) from a donor site. The shoots are then planted singly or in groups, with or without an anchor (Churchill et al., 1978) such as a nail or a piece of steel reinforcing bar (Phillips, 1990). Phillips and Lewis (1983) collected lengths of tropical seagrass rhizome growing unrooted in the water column with 5-6 shoots attached and anchored them in place with a "u"-shaped metal sod staple. Alternatively, single shoots have been grouped together into bundles of 10 shoots and anchored in place with 8-gauge metal "u"-shaped sod staples (Fonseca et al., 1982) or with biodegradable popsicle stick anchors (Merkel and Hoffman, 1990). Individual shoots also have been woven into a mesh fabric that is anchored with steel pins (Homziak et al., 1982). We considered each of these techniques in the development of our revised planting methodology.

#### **Site Location**

The Piscataqua River forms a natural border between southern Maine and New Hampshire, USA. The New Hampshire side of the Piscataqua River is heavily industrialized and the tidal range along this portion of the river is over 3 meters, both factors that limited the area and number of potential transplant sites. All our transplant sites were located on the New Hampshire side of the river (six sites located between 43<sup>0</sup> 06' 05"N, 70<sup>o</sup> 47' 20"W and 43<sup>o</sup> 06' 50"N, 70<sup>o</sup> 50' 30"W, Figure 1). A naturally occurring eelgrass bed in the immediate vicinity on the Maine side of the river was used as a control (43<sup>o</sup> 07' 10''N, 70<sup>o</sup> 48' 30"W). The donor site for the project was located near the mouth of the river (43<sup>o</sup> 04' 35''N, 70<sup>o</sup> 41' 50''W), and the impact site of the proposed pier construction was located in Portsmouth, New Hampshire, adjacent to the

existing New Hampshire Port Authority facility.

#### **Methods**

#### **Eelgrass Collection**

All eelgrass used for transplanting was collected from a large, healthy, intertidal, 6.0 hectare donor site. In 1993, all collecting was confined to three adjacent 150 m x 300 m rectangles within the donor bed. Eelgrass was collected by progressing north-south through the length of each rectangle, which allowed us to track our progress accurately and minimize potential impacts to the bed by dispersing the effects of collecting both temporally and spatially. In 1994, we randomly selected new collecting locations outside of the 1993 harvest area and used the same collecting method.

All collectors were trained to collect vegetative shoots and to minimize the taking of reproductive shoots. Collectors knelt in an unvegetated area adjacent to a patch of eelgrass and collected one or two shoots at a time from the edge of the patch. Plants at the edge of a patch are easier to remove and may be better suited for transplanting (Thom, 1990). The collector followed the blades of the shoot(s) to the substrate, uprooted approximately 3-5 cm of the rhizome by digging under the rhizome by hand, and snapped the rhizome to remove the plant. This technique allowed us to minimize disruption of the root-rhizome layer. The donor bed had an average of 445 shoots  $m^{-2}$ . Only 50 shoots were collected from a 1  $m<sup>2</sup>$  area of the donor bed before the collector changed location. At the donor site, eelgrass was temporarily stored in large coolers with a small amount of seawater to prevent exposure and desiccation and was later transferred to floating cages attached to a dock in the estuary. Collected eelgrass remained viable for transplanting for up to 72 hours when stored in this manner. Total collection from the donor site was 250,000 plants over two years, of which 19% were unused because they were damaged, reproductive, or unneeded.

#### **Eelgrass Transplantation**

The horizontal rhizome method consists of anchoring two mature eelgrass shoots with a biodegradable staple. The rhizomes are aligned parallel, pointing in opposite directions, and are pressed horizontally into the top 2 cm of the sediment, and held in place with a bamboo skewer bent in half. Each planting unit (PU) is created in the field at the time of planting which eliminates any intermediate plant handling or preparation after collection. Bamboo skewers were selected to anchor the PUs because they are biodegradable, less expensive than metal staples, and avoided potential human health risks of the metal staples traditionally used in bare root transplanting (Fonseca et al., 1982). Skewers were soaked for at least 48 hours before use to waterlog them and reduce buoyancy.

Transplanting occurred from June to September, 1993 and May to July, 1994. Planting units were installed on 0.5 m intervals. Spacing was maintained by using 10 m x 10 m planting frames constructed with nylon rope and 6.25 cm diameter PVC piping so that 400 PUs were planted uniformly per grid. The planting frame was removed after each grid was transplanted. A total of 252 grids, or 2.52 hectares (6.23 acres) of eelgrass, were planted. The number of grids transplanted at each site varied based on topography and bathymetry (Table 1). Grids were arranged linearly, parallel to the shoreline, with 2 to 6 rows of grids per site. All transplanting was done by SCUBA divers. Diving was necessary for all subtidal work because of water depth. The intertidal transplant areas were not accessible at low tide due to the soft-grained and easily resuspended muds and, at high tide, water depths in the intertidal required diving for transplanting (depth of transplanting ranged from  $+0.5$  m to -2.0 m mean low water).

#### **Transplant Protection**

Bioturbation has been shown to be a significant factor that can greatly reduce the survival and expansion of naturally occurring (Orth, 1975, Suchanek, 1983, Philippart,

15





np - indicates not planted

1994) and transplanted seagrasses (Molenaar and Meinesz, 1995, Fonseca et al., 1996). In the Great Bay Estuary of New Hampshire, horseshoe crabs (Limulus polyphemus) have foraging habits which can uproot unprotected transplants (F.T. Short, personal observation). Green crabs (Carcinus maenas), an introduced species shown to have disruptive foraging habits elsewhere (Cohen et al., 1995), also damage transplants (R.C. Davis, personal observation). In test transplanting studies, both organisms destroved eelgrass PUs. Therefore, a method of protecting transplants from these bioturbating organisms was devised using temporary cages similar to those reported in Fonseca et al. (1994). The cages were constructed by hammering 2 m oak stakes into the sediment around the perimeter of the planted plots at 1 to 2 m intervals. Monofilament gill netting (2 cm mesh, 2 m high) was then attached to the oak stakes with plastic cable ties. The extra netting at the bottom of the stakes was stretched out to form a skirt covering the sediment and secured to the bottom with 45 cm long 8-gauge metal "u"-shaped sod staples. All subtidal cages were constructed by divers; intertidal cages were constructed by wading workers at low tide. Once the cages were in place, unbaited crab pots were placed inside the cages and emptied of green crabs twice a week. Forty-two percent of the grids were caged. The majority of caging material was left in place for the 1993 and 1994 growing seasons; all caging materials including the sod staples were removed in summer 1995.

#### **Monitoring and Evaluation**

Because transplanted eelgrass habitats may take five years or more to become established, a 15-year monitoring period including sampling and comparing vegetation, benthic invertebrates, and fish from the transplanted beds and control beds was specified for this project (Bosworth and Short, 1993). Eelgrass at each of the transplant sites was initially sampled for overwintering survival, and is now being sampled yearly. Overwintering survival rates were assessed for site comparison  $(n=2)$  in April of the year following transplanting by randomly selecting two of the transplant grids at each site that
still had plants, and counting the number of PUs that remained of the 400 originally planted per grid. Grids with no plants were assigned 0% survival. Subsequent annual sampling includes production (leaf biomass), shoot density, and 2-sided leaf area index. Aerial photography is obtained annually to assess bed continuity and calculate areal extent of the beds.

For annual sampling of eelgrass characteristics, a 100 m primary transect was placed within each control bed and transplanted bed, across which four 20 m secondary transects were laid perpendicularly at 33 m intervals. Knots were located every meter along the 20 m transects to indicate sampling points, and two points along each secondary transect within the eelgrass bed were randomly selected for sampling (n=8 for each site: stratified random design provided a representative mean for the entire bed). A 1.25 m x 1.25 m sampling square (divided into 25 cm x 25 cm sub-quadrats) was centered over the knot and the total number of sub-quadrats containing eelgrass was counted to determine percent cover (Fonseca et al., 1990). The sampling square was 1.25 m on a side to avoid error in sampling PUs on 0.5 m centers. Two of the sub-quadrats containing eelgrass were randomly selected and all aboveground vegetation within was removed by cutting the plants above the primary meristem, keeping the sheath intact. Shoot density, biomass, and 2-sided leaf area index were determined using procedures modified from Phillips and McRoy (1990).

## **Results**

A total of 250,000 eelgrass shoots, less than 1% of the shoots present, was collected from the donor site in 1993 and 1994 with no observable damage to the donor bed. Aerial photography before (1992) and after (1995) collecting confirmed the lack of impact. Mean eelgrass shoot density and leaf biomass of the donor site were significantly higher (alpha = 0.05) in 1995 (782 shoots/m<sup>2</sup>  $\pm$  SE 58 and 120 g/m<sup>2</sup>  $\pm$  12; n=8) than in

1993 (445 shoots/m<sup>2</sup>  $\pm$  56 and 79 g/m<sup>2</sup>  $\pm$  7; n=8). The areal extent of the donor bed was also greater in 1995, after the collecting period, than before the project began.

Approximately one person hour was required to collect 150 shoots. An average of 4.5 person hours were required to transplant a 100  $m<sup>2</sup>$  area (i.e., one grid), depending on visibility and current velocity. An average of 5.5 person hours were required to construct a single subtidal cage by divers, depending on visibility and current velocity. Intertidal cages were constructed at low tide by field workers in an average of 4.5 person hours.

Overall, the eelgrass transplanting project was successful. Of the five sites planted in 1993, three still have eelgrass in subtidal areas which continues to grow and expand (Figure 2, 3 and 4); all the intertidal portions of these five sites, plus two of the subtidal areas, did not survive. The average overwintering survival rate (area-adjusted) for 1993 transplants was only 25%. The majority of intertidal plants at all 5 sites did not survive due to ice damage. Subtidal plants at two sites were severely bioturbated and did not survive (Table 1). At the subtidal, non-bioturbated 1993 sites, 87% of PUs survived. In contrast, the average overwintering survival rate (area-adjusted) for the five 1994 transplant sites was 56%. The 1994 subtidal transplant success rate was 97%. The 1994 intertidal success rate was 14%. Over the two years of transplanting, 71% of all subtidal transplants survived and expanded, while only 10% of intertidal transplants survived and expanded (Table 1). By the completion of the 1995 monitoring, 1.2 hectares (2.96 acres) of newly restored eelgrass habitat was growing in the estuary.

Leaf biomass at the three transplant sites increased slightly from 1993 to 1994, and showed a larger increase from 1994 to 1995 (Figure 3). At the same time, biomass at the control site showed a slow, steady increase. Eelgrass shoot density increased at all three transplant sites, and surpassed that of the control site within two years (Figure 4).



Figure 2. Aerial photographs of the T3 transplant site in July 1994 (a) and again in August 1996, (b) one year and three years after transplanting, respectively. Note the patchiness of the intertidal and shallower subtidal areas in 1994 that were disturbed by ice during the winter and that were lost by 1996. Deep subtidal transplants expanded and began to coalesce in the first year and had formed a bed by 1996.



Figure 3. Eelgrass leaf biomass  $(g/m^2)$  at the three transplant sites (T1-T3) where eelgrass survival was high and a control site (C1). Mean biomass increased each year at all transplant sites. Leaf biomass at T3 exceeded that of the control site after two full growing seasons. Data shown are means  $\pm$  SE (n=8).

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Figure 4. Eelgrass density (shoots/ $m<sup>2</sup>$ ) at the three 1993 transplant sites (T1-T3) where eelgrass survival was high and a control site (C1). The control site decreased consistently in shoot density. Transplant sites T1 and T2 showed continuing increases, while site T3 decreased in the second year after achieving the highest density measured through the period. Data shown are means  $\pm$  SE (n=8).

Shoot density at the control site showed a slow decline over the sampling period (Figure 4).

#### **Discussion**

To maximize success in our transplanting project, only those sites at which eelgrass was known to have previously existed and that exhibited physical characteristics which could support eelgrass were initially selected for transplanting. Even with these site selection criteria, transplant survival varied widely. Subtidal transplant survival ranged from a low of 1% at sites T4 and T5, to 99% at T2 and T3. Intertidal transplant survival ranged from 2% at T2 to 15% at T1. The mean percent survival (71%) we obtained for eelgrass transplanted subtidally with the horizontal rhizome method equals or exceeds that reported with previous methods (Phillips, 1974, Thom, 1990, Fonseca et al., 1996). Our survival rate of 98-99% in year 2 for four subtidal areas (Table 1) testifies to the value of the horizontal rhizome method of eelgrass transplanting. Increase in shoot density of our subtidal transplants was comparable to the only transplant spread rate reported in the literature, for bare-shoot transplants in sub-tropical areas (Fonseca et al., 1996). Shoot density at one site exceeded that of the control site one year after transplanting (Figure 4).

Eelgrass biomass increased over the first two years at the subtidal portion of the three surviving 1993 transplant sites as well as at the control site (Figure 3). In contrast, shoot density showed continuous increase at two sites (T1 and T2), increase followed by leveling off at one site (T3), and a constant decrease at the control site (Figure 4). The differential adjustment of eelgrass leaf biomass and shoot density at the control site and T3 results from the change in bed structure, representing a shift from small, dense plants to a taller and less dense bed (Olesen and Sand-Jensen, 1994).

The overall survival rate of 48% for all transplants, both intertidal and subtidal, resulted from the failure of the majority of intertidal transplants as well as a portion of the subtidal transplants. These intertidal and subtidal losses were not a result of the transplanting methodology, but largely due to physical and biological disturbances. respectively.

The lack of success of the intertidal transplants was due to physical disturbance from scouring and rafting of sea ice (Table 1). This result is consistent with the findings of Robertson and Mann (1984), who observed that shallow edges of eelgrass beds are restricted by physical disturbance. In the severe winter of 1993-94, the ice rafts along the shore of the Piscataqua River were 25-45 cm thick for over three months (Figure 5). The protective caging, put in place to prevent bioturbation, may have intensified ice damage. Ice rafts often became trapped inside the cages on a falling tide, and near full low tide, scoured out portions of sediment and eelgrass. While the majority of the intertidal transplants did not survive, a small area at one site has persisted. The donor bed, nearer the coast, is completely intertidal, and eelgrass beds in the Great Bay, farther up estuary, are both intertidal and subtidal. The existence of these intertidal eelgrass populations made it reasonable to attempt intertidal transplanting. However, replanting of one intertidal area in 1994 also resulted in a very low percent survival under normal ice conditions.

Biological disturbance caused most of the losses of transplants in subtidal areas. We successfully protected the transplanted eelgrass against crab bioturbation, but were surprised to discover severe bioturbation by clam worms affecting some subtidal areas. Bioturbation from horseshoe crabs (Limulus polyphemus) and green crabs (Carcinus maenas) affected test transplanting efforts prior to the New Hampshire Port Authority Mitigation Project (Short, personal observation). Horseshoe crabs were diverted from the transplant grids by the cages and green crab disruption of new transplants was decreased



Figure 5. February 1994 photograph of intertidal transplants at T1 taken during a spring low tide. Ice rafts were trapped inside caging material, scouring transplants during the low tides.

by cages. Green crabs were frequently observed climbing on the caging material rather than dispersed among the transplants. In some cases, eelgrass did not survive well in the center of the grids; whether or not this was related to crab activity is unknown.

Clam worms (Neanthes virens) most likely caused the failure of transplants at two subtidal sites where worms were abundant (T5 and part of T1). The ability of marine worms to prevent the natural recolonization of seagrass has been observed previously (Philippart, 1994). In our transplant grids, polychaete worms appeared to be pulling the distal ends of the eelgrass blades down into their burrows. After eelgrass leaves were pulled flat against the sediment surface, they were rapidly covered by bioturbated sediments, leading to shoot death. Site T5 had the low survival rate (1%) and the highest clam worm densities (Chapter 5). These results underscore the need to assess the biological environment as well as the physical environment when selecting transplant sites.

Our horizontal rhizome method offers several advantages as a transplanting methodology (Table 2). First, only two shoots are required per PU, reducing the number of plants harvested by 80% over the most prevalent bare-root method (Fonseca et al., 1982b). Second, hand collection of individual shoots minimizes disruption of the donor site root/rhizome mat compared to coring or shoveling methods. Third, considerable time and cost savings can be realized over the coring method, because no holes need to be filled at the donor site (after removal of plants and sediment) or created at the transplant site, and there is no need to transport heavy sediment. Fourth, there is no pre-planting PU preparation, thereby reducing costs and minimizing plant handling and potential plant damage, keeping more plants viable until transplanting. Fifth, we use a biodegradable anchor to secure the PUs in the substrate, eliminating the need for leaving metal staples in the marine environment. Finally, transplanting two shoots with their rhizomes oriented in the natural horizontal growing position allows for rapid attachment and expansion in two



Table 2. Advantages and disadvantages of the horizontal rhizome method and the three most commonly used methods for

directions as evidenced by high survival rates and density increases. These advantages are particularly important when conducting large scale transplanting projects such as ours. The horizontal rhizome method is a reliable, effective transplanting technique. Survival rates, as high as 98% at several subtidal sites, and habitat development of eelgrass transplanted using the horizontal rhizome method equal or exceed those reported with other methods. The results of our project further demonstrate that transplanting is a viable method for replacing or increasing seagrass habitat area and its concomitant functions and values.

# **CHAPTER III**

# THE EFFECT OF LIGHT, CURRENT, AND SEDIMENT CHARACTERISTICS ON THE SURVIVAL OF EELGRASS (ZOSTERA MARINA L.) TRANSPLANTED IN THE PISCATAQUA RIVER, NEW HAMPSHIRE

## **Introduction**

Seagrasses exist under a wide variety of environmental conditions, but determining the specific site characteristics that are most conducive to transplant success remains a subject of much debate (Fonseca et al., 1998). Zimmerman et al. (1991) state that "the success of any seagrass revegetation effort is strictly dependent upon a physical environment that will not only ensure initial establishment, but will support long-term growth as well." The success of the New Hampshire Port Authority transplanting project, described in the preceding chapter, varied considerably among the transplant sites. Therefore, a natural question to ask was "are physical conditions significantly different among the transplant sites, and if so, do these differences explain the differential transplant survival?"

The parameters that most strongly affect seagrass survival, growth, and productivity include light, temperature, salinity, depth, nutrients, current (or wave energy), and sediment parameters (Philippart et al., 1992; Livingston et al., 1998; Fonseca et al., 1998). Determining which of these parameters is most important for initial survival, and which are more important for long-term growth, has important site

selection implications. Because of the strong flushing characteristics of the Piscataqua River portion of the Great Bay Estuary, there is little difference in temperature and/or salinity between the transplanting sites (Short et al., 1993), and these two variables were not investigated further. The influence of light, current, and sediment parameters on the survival of eelgrass transplanted at sites in the Piscataqua River (Figure 1) were investigated using a combination field measurements and mesocosm experiments.

# **Light**

Light availability is the most important determinant of seagrass production and distribution (Dennison, 1987; Duarte, 1991). Seagrasses require at least 10% of surface light in order to survive (Dennison, 1987). Dennison et al. (1993) reported that eelgrass in the northeastern United States required higher levels (18.6%), which was confirmed in mesocosm experiments by Short et al. (1995). Light availability decreases exponentially with depth due to the scattering, reflection, refraction, and absorption of incident light caused by the water itself and dissolved and particulate constituents within the water column (Dennison et al., 1993). Water column constituents [(e.g., dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorus (DIP), total suspended solids (TSS), and chlorophyll a (Chl  $a$ )] and the light extinction coefficient ( $K_d$ ; often used as a simple method for integrating the effect of all water column constituents), have been used successfully to predict seagrass production and distribution (Short, 1980; Dennison, 1987; Dennison et al., 1993; Zimmerman et al., 1995; Kenworthy and Fonseca, 1996; Olesen, 1996; Moore et al., 1997). Batiuk et al. (1992) and Stevenson et al. (1993) established minimum levels for DIN, DIP, TSS, Chl  $a$ , and  $K_d$  that were required for the survival of submerged aquatic vegetation in the Chesapeake Bay (Table 3). These parameters are generally referred to as submerged aquatic vegetation (SAV) habitat requirements. In the Great Bay Estuary, the levels of these critical light-reducing water column constituents are often below those minimum levels, except for DIP (Table 3). In

Table 3. Comparison of water quality parameters required for the survival of submerged aquatic vegetation (SAV) with water quality parameters in the Great Bay Estuary, New Hampshire in 1993 and 1994.



1. from Batuik et al. 1992.

2. Jackson Estuarine Laboratory unpublished data. Median values from data collected off dock at laboratory on Adams Point (calculated from monthly means (n=2) during the growing season (April-October).

3. No data available, but when measured other years in the Piscataqua River,  $Kd < 0.5$ .

addition to the SAV habitat requirements, epiphytic algae is another factor that can greatly reduce the amount of light reaching the seagrass leaf surface. The amount of epiphytic algae present is largely dependent on the level of nutrient enrichment (or eutrophication) of the estuary (Short et al., 1995). Quinn et al. (1988) determined that the Great Bay Estuary is not highly susceptible to eutrophication, suggesting that epiphytic algae, although present, are probably not limiting production and distribution of naturally occurring eelgrass populations. These factors, combined with the fact that eelgrass exists in nearby portions of the Piscataqua River, suggest that light is not a limiting factor within the depth range at which eelgrass was transplanted. However, because of its critical role, light was measured at all transplant sites for comparison to light levels at the nearest naturally occurring eelgrass bed. The short-term light measurements were designed to test whether light was sufficient at all transplant sites, and that trends in light availability were similar at transplant and naturally occurring eelgrass beds.

## **Current**

Current velocity (herein used to indicate any movement of water past the plant such as tidal current or wave and wake energy) directly influences a number of physiological processes in eelgrass plants including photosynthesis and nutrient uptake (Fonseca and Kenworthy, 1987; Koch, 1994). The photosynthetic performance of seagrasses generally improves with increasing current velocity due to a concomitant decrease in boundary layer thickness which improves carbon uptake and nutrient utilization (Fonseca and Kenworthy, 1987; Koch, 1994). However, at higher velocities, current can physically disrupt/damage the plant or cause sediment erosion, both of which decrease seagrass productivity. Because of the physical damage that can occur to transplants and the potential for sediment movement in areas with high current velocities, a maximum current velocity of 50 cm/sec has been recommended for proposed transplant sites (Fonseca et al., 1998).

In addition to its direct effect on seagrasses, current velocity can also affect seagrass production and distribution indirectly by interacting with sediments, causing light limiting conditions. For example, Lauridsen et al. (1993) suggested that accumulation of organic matter in the sediment may prevent subsequent colonization by macrophytes as resuspension of loose organic matter may cause a significant decline in available light. In the Great Bay, Anderson (1970) showed that sediment resuspended by current energy comprised a large percentage of total suspended solids (TSS). Therefore, if the sediment at a potential transplant site is susceptible to current-induced resuspension, either due to the sediment particle size or organic matter content, eelgrass growth may be inhibited due to light limitation (Olesen 1996). Fonseca et al. (1998) suggest that this resuspension "threshold" velocity may occur at current velocities as low as 25 cm/sec. Therefore, current velocities were measured at selected transplanting and control sites for comparison to the maximum and threshold velocities reported in the literature to determine if current velocity was limiting transplanting survival.

# **Sediment Characteristics**

Sediment organic matter positively affects plant growth because it provides nutrients following remineralization (Short, 1987; van Wijck et al., 1992). Seagrasses growing in sediments with low organic matter content have reduced productivity (Short, 1987). Conversely, aquatic macrophyte productivity can also be inhibited when organic matter content of the sediments becomes too high (Barko et al., 1991; Stevenson et al., 1993; Lauridsen et al., 1993) due to inhibition of root metabolism resulting from inadequate oxygen supply and nutrient limitation resulting from nutrient complexation with organic matter (Barko and Smart, 1986). Additionally, sediments with high organic matter are often anoxic, leading to the accumulation of hydrogen sulfide to levels that can be toxic to the plant (Goodman et al., 1995; Moore et al., 1997). Thus, there is a range of

sediment organic matter, within which seagrass growth is enhanced, and outside of which growth is inhibited (Kenworthy and Fonseca, 1977; Short, 1987). Determining the exact range of sediment organic matter content which promotes the establishment and growth of transplanted eelgrass would provide significant information for selecting future transplanting sites. In Long Island Sound, this level has been set at less than 3% organic matter content (Ron Roza, Connecticut Department of Environmental Protection, personal communication). I tested the effect of sediment characteristics on the survival and growth of eelgrass transplanted for the NHPA mitigation project by analyzing sediment samples collected from transplant and control sites, and through a series of mesocosm experiments.

# **Hypotheses**

The research described in this chapter was designed to determine whether physical site characteristics limited the initial survival of transplanted eelgrass. Additionally, the mesocosm experiments were used to test whether the Horizontal Rhizome Method (HRM; Davis and Short, 1997) limited eelgrass growth compared to one of the most commonly used transplanting techniques, the bundle technique (Fonseca et al., 1982). This was accomplished by testing the following hypotheses.

Hypothesis 1: Light availability is limiting transplant survival. This hypothesis was tested by comparing light availability at transplant sites to that of the control sites and with published minimum light requirements for eelgrass.

Hypothesis 2: Current velocity is limiting transplant survival. This hypothesis was tested by comparing maximum current velocity recorded at the transplant sites with that of the control sites and published values for the maximum current velocities recommended for eelgrass transplanting.

Hypothesis 3: Sediment characteristics are limiting transplant survival. This hypothesis was tested by analyzing sediment cores collected from the transplant and

control sites and in mesocosm experiments. Transplant survival and aboveground development were compared to the field sediment data. Mesocosm experiments were used to measure growth rates for eelgrass transplanted into the range of sediment types found at the field sites.

Hypothesis 4: The sediment at site T5, which had the lowest transplant survival. does not support eelgrass. This hypothesis was tested by transplanting eelgrass into sediment from the T5 site that had been placed in the mesocosm tanks.

Hypothesis 5: The horizontal rhizome method (Davis and Short, 1997; see Chapter 2) limits growth of transplanted eelgrass. This hypothesis was tested by transplanting eelgrass using two different techniques: the HRM and the bundle technique (Fonseca et al., 1982) in mesocosm tanks and monitoring above and belowground growth rates.

#### **Methods**

Field measurements were taken at all transplant and several control sites to determine light availability, current velocity, and sediment characteristics. Mesocosm experiments were conducted at the Jackson Estuarine Laboratory in the summer of 1995 to determine if growth of transplanted eelgrass was significantly different within the range of sediments found at the transplant sites, and whether transplanting technique affected eelgrass growth. Mean K<sub>d,</sub> bottom current, and sediment characteristics were regressed against percent survival, aboveground biomass and shoot density to determine significant trends. Significant trends/relationships are described in the results.

## Light

Light availability ( $\mu$ E/m<sup>2</sup>/sec) was measured at the deep edge of all sites in 1995 and 1996 using two Type 174 SSM Meters manufactured by Endeco, Inc., modified to record photosynthetically active radiation (PAR) with a 4 pi spherical sensor (the

Estuarine Sensor and Profiler, Short et al., 1993). The device recorded PAR every 10 seconds, but data were averaged to obtain hourly means for use in subsequent analyses. Divers placed the meters at sites for 2 to 3 days to record light availability over the complete tidal cycle. One of the meters was placed at one site for the entire deployment period, while the other meter was moved among transplant and control sites. Meters were checked daily to remove any drift material and/or epiphytic algae which had accumulated on or near the light sensor. Incident light readings were obtained at the Jackson Estuarine Laboratory using a Licor DataLogger II meter with a 2 pi flat sensor that continuously records PAR levels. These data were used to calculate the percent of surface light reaching the meters on the bottom. A Licor DataLogger II meter with a 4 pi spherical sensor was also used to take synoptic measurements of PAR at selected sites in 1993.

Light availability was recorded in 1993 (synoptic) and 1995 and 1996 (continuous). The mean light extinction coefficient  $(K<sub>d</sub>)$  for each transplant site was calculated using the Beer-Lambert equation (Kirk, 1994) by determining the daily mean K<sub>d</sub> from 8 am - 4 pm, and then averaging the daily means over the deployment period.

# **Current**

A hand-held Marsh-McBirney Model 201 Portable Water Current Meter was used to measure current velocities at all sites during the flood tide, when currents in this portion of the Piscataqua River are strongest (Bilgili, 1996). The probe was deployed over the side of a double-anchored boat (anchors were placed off the stern and bow) and held in place for one minute to stabilize the meter before readings were taken. The highest and lowest current velocities were recorded every five minutes over the flood portion of the tidal cycle. Surface current measurements were taken 0.25 m below the water surface to reduce the effect of small surface waves and wind energy on the

readings. Bottom current measurements were taken 0.25 m above the eelgrass canopy.

## **Sediment Parameters at Field Sites**

Three replicate sediment samples were obtained for all transplant and two control sites using 6.0 cm diameter clear acrylic coring tubes pressed to a depth of 15 cm into the sediment. All sediment cores were collected approximately 1.0 m below the mean low water mark at low tide. Samples were left in the coring tubes, drained of surface water, placed in a cooler, and transported to Jackson Estuarine Laboratory for processing. At the lab, sediment cores were separated into 0-2cm, 2-5cm, and 5-15cm subsamples. Only the 0-2cm subsamples were analyzed for this study, because that is the depth to which the eelgrass root/rhizomes were pressed into the sediment during transplanting. Mean particle size, sand/silt/clay ratios, and organic matter content (estimated from percent of material lost on ignition) were determined using the methods outlined in Folk (1980) and modified by Mueller et al. (1992).

#### **Sediment Mesocosm Experiments**

A series of mesocosm experiments were conducted in the summer of 1995 to test hypotheses 4 - 6. Mesocosms have been used successfully to test the effect of different environmental parameters on the growth and survival of eelgrass (Short et al., 1995; Short, 1987; Kenworthy and Fonseca, 1977). The sediments used in the experiments were collected from the donor site (at the mouth of the Piscataqua River, designated "sand") and from Adam's Point Cove ("mud") adjacent to the Jackson Estuarine Laboratory. The sediments from these two sites are representative of the end points for the range of sediments found at the transplant sites (Table 4). A third sediment type was made by combining equal parts of donor site sediment and Adam's Point Cove sediment (designated "muddy sand"). In addition, sediment was collected from transplant site T5,

the only site at which over 99% of transplants were lost in both years of transplanting at the site.

Three replicate sets of mesocosm experiments were conducted in the summer of 1995 using a randomized complete block design experiment in four mesocosm tanks (1) cubic meter each) with running seawater outside the Jackson Estuarine Laboratory. For each experiment, ten plastic buckets (26 cm diameter, 20 cm deep) were placed inside each tank and filled with approximately 12 - 15 cm of sediment. Three buckets each had sand, muddy sand, or mud sediments. One bucket contained sediment from T5. A single planting unit (two shoots) was transplanted into each bucket. After allowing one week for the plants to become rooted, water circulating pumps were turned on and one shoot of each planting unit was marked for growth (Short, 1987). After two weeks, the entire eelgrass shoot was harvested and processed to determine growth rates. These procedures were identical for the first two replicates.

For the last replicate, rhizomes of the planting units were also marked for growth in addition to the shoots. Two different eelgrass transplanting techniques were also used for the last experiment to test whether the revised transplanting technique developed specifically for this project provided growth rates comparable to those obtained using the most commonly used eelgrass transplanting method, the bundle technique (Fonseca et al. 1982). For this test, one half of the buckets were transplanted with eelgrass shoots using the horizontal rhizome method (Davis and Short, 1997), and the other half were transplanted using the bundle technique (Fonseca et al., 1982).

At the end of the experiments, sediment samples were collected from four buckets (one for each sediment type) for later analysis. Mean particle size (given in phi units), percent sand/silt/clay content, and percent lost on ignition (an estimate of organic matter

content) were determined following procedures outlined in Folk (1980) and modified by Mueller et al. (1992). Above and belowground growth rates were analyzed using ANOVA and Student-Newman-Kuels post-hoc tests to determine if significant differences existed among treatment means (Zar, 1996).

#### **Results**

## **Light**

Trends in light availability were similar at transplant and control sites, with an increase or decrease at the transplant site corresponding to an increase or decrease at the control site (Figure 6 and 7). However, light data were only available for a limited number of transplant and control sites in 1995 due to a computer problem which resulted in the loss of data. In 1995, site T5 had the highest light availability of the transplant sites for which data were available (Figure 6). Light availability at T5, the site with the lowest transplant survival, was greater than at both the shallower and deeper areas of T3 (Figure 6), the site with the highest transplant survival (Chapter 2).

In 1996, light levels were recorded at the deep edge of all transplant sites over two deployment periods, except T2 and T5. Because the transplants at these two sites were largely unsuccessful, the sites were dropped from the monitoring program. With the exception of site T4, light availability at the transplant sites exceeded that at the C1 control site at all times (Figure 6 and 7). Light availability declined during the second monitoring period (July 23 - August 6, 1996) due to reduced water clarity associated with runoff from storm events.



Figure 6. Daily irradiance (mean of 8am-4pm irradiance) measured at selected eelgrass transplant and control sites. One light measuring device was continuously deployed at the deep edge of the T3 transplant site (open bars). A second device was moved among other transplant and control sites. Deployment period was June 10-22, 1995.



Figure 7. Daily irradiance (mean of 8am-4pm irradiance) measured at selected eelgrass transplant and control sites. One light measuring device was continuously deployed at the deep est of the C1 control site (open bars). A second device was moved among other transplant and control sites. Deployment periods were: a) June 20-30, 1996; b) July 23-August 6, 1996.

Mean K<sub>d</sub> for each site was determined by averaging available light recorded from 8 am - 4 pm for the each day of the deployment, and then obtaining a site mean by averaging the daily means. Light extinction coefficients calculated from the Endeco meters ranged from a low of 0.397 at T1 to a high of 0.512 at T3 (Table 4). Because of the partial loss of computer data in 1995 (surface light readings were not available), the light extinction coefficient for site T5 was calculated using synoptic light data previously collected at that site in 1993 using a 4 pi spherical sensor as 0.336. This value corresponds with the high light levels recorded at the site with the Endeco meter.

# **Current**

Current velocities were measured 0.25 m above the eelgrass canopy on a flood tide during the neap portion of the tidal cycle at all sites, and during the spring portion of the tidal cycle at three sites. Mean current velocities ranged from a low of 6.0 cm/sec at site T2 during a neap tide, to a high of 57.5 cm/sec at T6 during a spring tide (Figure 8). At several sites, the highest current velocities were recorded on back-eddies, when the current at the sites was flowing opposite to the direction of the incoming tide in the main channel. Current velocities exceeded recommended "maximum velocities" (Fonseca et al., 1998) for transplanting at T6 on a spring tide, and "threshold velocities" (Fonseca et al., 1998) were exceeded at T7 during the neap tide, T3 during the spring tide, and T4 and T6 during both spring and neap tides (Figure 8). However, simple regression analysis of transplant survival and development with current velocity revealed no significant trends (Figure 9).

# **Sediment Parameters at Field Sites**

Mean particle size, in phi units, for the sediments collected from five transplant sites, two control sites, and the donor site varied from a high of 4.9 at T5, to a low of 1.93 at T3 (Table 4). Phi units are inversely related to sediment grain size (Folk 1980); thus,



Figure 8. Mean current velocity measured at eelgrass transplant and control sites during spring (open bars) and neap (shaded bars) tides. Threshold velocity (25 cm/sec) is defined by Fonseca et al. (1998) as the current velocity at which sediment can potentially be resuspended, creating light limiting conditions. The maximum current velocity (50 cm/sec) is the current velocity above which transplanting seagrass is not recommended due to sediment movement and resuspension; physical damage to seagrass can occur above this velocity (Fonseca et al., 1998).



Figure 9. Transplant survival (measured two months after transplanting) related to current velocity (cm/sec).

Table 4. Physical parameters for eelgrass transplant and control sites determined using procedures outlined in Folk (1980). Sediments used in mesocosms were designed to span the range of sediments found at the field sites. The light attenuation coefficient (Kd) was calculated using the Lambert-Beer equation. \* from Short et al. 1993. nd=no data available. %S/S/C indicates the percentage of the sediment that is sand/silt/clay. Mean phi is the average sediment grain size (in phi units, which are inversely related to grain size). % LOI indicates the amount of material lost on ignition and indicates the amount of organic matter in the sediment.



T5 had the smallest sediment particle size of any transplant site (Table 4). Site T5 also had the highest organic matter content of any transplant site, measured as the percent of material lost on ignition (%LOI). Control site C3 had the highest organic matter content, at 3.67%, of any site sampled. Site T3 had the lowest organic matter content of any transplant site (1.05% LOI). Mean particle size and organic matter content of the processed sediments were directly related (Figure 10). The sediment at all transplant and control sites was poorly or very poorly sorted, most likely the result of the strong and variable currents that affect the sites.

The percent of transplanted eelgrass surviving two months after transplanting was not strongly correlated with any sediment parameter (Table 5). However, the regression analysis revealed that aboveground development of the transplanted eelgrass was significantly (alpha  $=0.10$ ) related to mean phi. Four months after transplanting, aboveground biomass and shoot density were negatively related to mean phi  $(r^2=0.723)$ .  $p=0.068$  and  $r^2=0.756$ ,  $p=0.0556$ , respectively). This relationship was still significant (alpha=0.10) fourteen months after transplanting ( $r^2$ =0.810,  $p$ =0.0750 and  $r^2$ =0.841.  $p=0.0589$ , respectively) (Figure 11).

#### **Sediment Mesocosm Experiments**

Transplanted eelgrass grew in each of the four sediment types used in the mesocosm experiments. Aboveground growth was measured as length increase (cm/shoot/day), leaf mass (g/shoot) and specific growth rate (cm/cm/day). Eelgrass transplanted in sediment from T5 had the highest specific growth rate and length increase. Mean leaf mass was highest for eelgrass transplanted in the sandier sediments (sand and muddy sand) and was significantly lower (alpha  $= 0.10$ ) for eelgrass transplanted in the mud treatment (Table 6). No other significant differences in aboveground growth existed (Table 6). Belowground growth was measured as the total



Figure 10. Direct relationship between sediment grain size (mean phi) and percent organic matter content of the sediment (measured as percent of material lost on ignition (%LOI)). Data are from sediment cores collected from a) field sites, and b) tanks used in mesocosm experiments.



 $a)$ 

Figure 11. Eelgrass leaf biomass (a) and density (b) related to sediment grain size (mean phi) four months (1993) and fourteen months (1994) after transplanting.



Table 5. Correlation (R) matrix using data collected from eelgrass transplant and control sites.

49

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Table 6. Sediment parameters and growth rates for eelgrass transplanted into different sediment types. a) sediment parameters. b) values for mean aboveground growth (s.e.) from three replicate mesocosm experiments. c) mean belowground growth rates (s.e.) from third replicate only. P-values<br>were obtained using analysis of variance. The only significant difference amo

50

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number of new nodes produced, total number of new laterals, the total number of nodes on new laterals, new rhizome length (cm of rhizome/day), and new rhizome weight (mg of rhizome/day). Belowground growth rates varied among treatments, but there were no consistent trends nor significant differences based on sediment type (Table 6).

Aboveground growth rates varied between transplanting techniques tested in the third mesocosm experiment (Table 7). Eelgrass transplanted with the bundle technique (Fonseca et al. 1982) had slightly higher specific growth and length increase than eelgrass transplanted with the HRM, but these differences were not significant (Table 7). Eelgrass transplanted with the HRM had higher leaf mass than eelgrass transplanted with the bundle technique, but this difference was not significant (Table 7). All belowground growth measures were greater for transplants using the HRM than the bundle method (Table 7). The number of nodes on laterals ( $p=0.0403$ ), new rhizome length ( $p=0.0004$ ), and new rhizome weight  $(p=0.0228)$  were significantly higher for eelgrass transplanted using the HRM (Table 7).

The sediments used in the mesocosms were representative of the upper range (i.e., smaller grain size) of sediments found at transplanting and control sites (Table 4). Mean phi was smallest for the sandy sediment collected at Fishing Island (the donor site) and largest for the muddy sediment from Adam's Point Cove, adjacent to the Jackson Estuarine Laboratory (Table 4). Mean phi and percent organic matter content (%LOI) for the sediment from T5 was between that of the muddy sand and mud sediments. Percent organic matter content of the sediments was inversely related to grain size, i.e., highest for the muddy sediment and lowest for the sandy sediment (Figure 10).

#### **Discussion**

Initial survival of the transplanted eelgrass was not significantly related to any of the physical site characteristics studied (light, current, sediment characteristics), when





transplant survival was assessed two months after transplanting (1993 data, Table 5). This result is not surprising because of the similarity of these characteristics among the sites, particularly light availability. In fact, it was due to their similar characteristics that these sites were selected as transplant sites. As such, it was reasonable to expect similar eelgrass transplant survival and development at these sites. The overwintering survival of the transplanted eelgrass was likely determined by other factors such as ice scouring (Chapter 2) and bioturbation (Chapters 5 and 6). Analysis of eelgrass growth four months and 14 months after transplanting demonstrated that the development of aboveground biomass and shoot density was more strongly correlated with the percent organic matter content of the sediment (%LOI, indicating that this physical site characteristic may be an important determinant of the extent to which transplanted eelgrass will develop over a longer period.

It is important to note however, that the utility of the field data is limited due to the lack of replication and the fact that some of the data were not collected at the same time as the initial survival data. Because of this limitation, more sophisticated analyses such as analysis of variance or multiple regression, were not appropriate. This limitation did not apply to the sediment mesocosm data, as they were collected within an experimental framework (Table 6 and 7). The results of those experiments showed little difference in aboveground or belowground growth rates based on sediment type (Table 6), but significant differences in belowground growth rates due to transplanting technique (Table 7).

#### Light

There was sufficient light at all sites (above the theoretical 18% surface light level, Dennison et al. 1993) to support eelgrass growth (Figure 6 and 7). Additionally, the long-term (decadal) existence of eelgrass at the control sites indicates that sufficient
light reaches those areas to support seagrass. Mean Kd for the transplant sites were similar to those calculated for the control sites (Table 4), indicating that sufficient light reached the transplanted eelgrass. Counterintuitively, calculated light attenuation  $(K_d)$ was significantly positively correlated with transplant survival (Table 5), indicating that there was higher transplant survival at sites with greater light attenuation (less light reaching the bottom). These data indicate that factors other than light availability determined transplant survival. Light availability alone cannot adequately explain the differential survival among transplant sites over the range of light levels found in this study.

## **Current**

The survival and development of the transplanted eelgrass was poorly correlated with current velocity (Table 5). The lowest bottom current was measured at T2, a site which had fairly low transplant survival and expansion. Bottom currents at T3 and T5 were nearly identical, but the former site had the highest transplant survival and the latter site had the lowest transplant survival. Transplants survived well at sites where the current velocities exceeded recommended maximum levels (T6, Figure 8) and threshold levels (T3, T4, and T6, Figure 8) as defined by Fonseca et al. (1998). The Great Bay Estuary complex, which includes the Piscataqua River, has a wide tidal range (3-4m) resulting in strong tidal currents. Eelgrass occurs naturally throughout this area, and the plants persist and thrive in strong current velocities. The relative importance of current velocity to transplant survival may be more significant in areas with lower tidal energy, such as the North Carolina coastal bays where the current velocity criteria were established. Specific current velocity criteria have yet to be established for eelgrass transplanting in the Northeastern United States. The fact that our eelgrass transplants survived, and persisted, at sites where current velocities exceed 50 cm/sec suggests that the recommended maximum current velocity will be higher in this geographic region than previously suggested (Fonseca et al., 1998). The results of the NHPA transplanting project demonstrate that eelgrass can be successfully transplanted in current velocities ranging from 6 - 56 cm/sec, much greater than the 20 - 40 cm/sec range previously suggested for eelgrass transplanting (Fonseca et al. 1998).

As described in Chapter 2, protective cages were constructed around many of the transplanted areas and may have altered the current velocities that affected the transplants. The cages created a baffling effect, reducing current velocities, particularly for those transplants closest to the caging material. Some of the highest transplant survival was seen among the transplants closest to the caging material (Chapter 2). This may have been related to a baffling effect, but was more likely the result of decreased bioturbation in the area immediately adjacent to the cages (Chapter 2 and Chapter 4). Site T6 had very high transplant survival and the highest recorded current velocities, yet caging material was never placed at this site. Similarly, caging material was never placed at the portions of sites T3 or T4 where current velocities were recorded. Sites T1, T2, and T5 were the only sites that previously had caging material in the areas where current velocities were measured (all caging material had been removed by the time the current measurements were taken).

## **Sediment Parameters at Field Sites**

Sediment grain size and % LOI are two commonly used sediment characteristics that strongly influence macrophyte production (Grady, 1981; Harlin and Thorne-Miller, 1982; Boeger, 1992; Livingston et al., 1998). Sediment grain size and %LOI have a strong inverse relationship (Figure 10) that is the result of two processes (Folk, 1980). First, smaller sediments have larger surface to volume ratios and more surface area to which organic matter can bind; accumulation of fine sediment particles includes a high level of organic matter. Second and more importantly, smaller sized sediments can experience a higher degree of compaction and become more anoxic than larger sized

sediments. High organic matter content has been shown to limit SAV growth due to promotion of anoxic conditions which may inhibit root metabolism due to inadequate supply of oxygen (Barko and Smart, 1986) and formation of reduced compounds such as Fe<sup>2+</sup> and S<sup>2-</sup> which can inhibit plant growth (Goodman, et al. 1995; Van Wijck et al., 1992; Koch et al., 1990).

Eelgrass that was transplanted into environments with smaller sediment size and/or higher organic matter content may have been subjected to anoxic and reduced sediment conditions. Because the eelgrass had to adapt to the new sediment regime at the transplant sites, eelgrass shoots transplanted into sediment most different from the parent sediment had to adapt the most. The donor site had low %LOI and correspondingly large grain size (Table 4) and the root/rhizome structure of the eelgrass had developed to maximize nutrient use in that environment (i.e., smaller rhizomes with dense root hairs (Short, 1983)). Therefore, plants removed from the donor site and transplanted into sediments with higher %LOI and smaller grain size had to adapt the most. Eelgrass growing in these environments has larger rhizomes and fewer root hairs (Short, 1983). Eelgrass transplanted into these environments consumed more energy during the adaptive process and had less energy to put into aboveground development (Figure 11). Fourteen months after transplanting, aboveground development was still related to sediment parameters (Figure 11). This pattern is also evident in the naturally occurring populations of eelgrass in the Great Bay (Short, 1992) and reflects the level of nutrient availability in the sediments (Short, 1983; Short, 1987; Short, 1992).

It is important to note however, that eelgrass transplanted from this donor site still had higher survival and growth rates when transplanted in sediments with smaller grain size (upper bay sites) than eelgrass from upper bay donor sites (Carlson, 1997). Eelgrass that has adapted to higher nutrient environments such as those in the siltier sediments of

the upper bay may not be as suitable for transplanting as plants adapted to the sandy sediments and low nutrient conditions at the mouth of the Piscataqua River. A similar pattern in the suitability of different eelgrass populations for transplanting has also been documented in the Wadden Sea (van Katwijk et al., 1998). More extensive rhizome systems, characteristic of eelgrass growing in sandier sediments, provide the plants with storage reserves available during their initial establishment at the transplant site (Burke et al., 1996; van Katwijk et al., 1998) making them the optimal choice for transplant material. Eelgrass plants collected from siltier sediments may not have this storage reserve and may be unsuitable for transplanting into any environment. For the NHPA project, the greatest transplant success was observed with eelgrass taken from the sandier sediments.

## **Sediment Mesocosm Experiments**

The results of the mesocosm experiments demonstrated that eelgrass could survive and grow in each of the sediment types (Table 6). No significant differences existed in the growth rates of the eelgrass transplanted into different sediment types (at  $alpha < 0.05$ ), but the pattern of aboveground and belowground development was related to sediment grain size (Figure 12). As grain size decreased (mean phi increased), belowground development increased and aboveground development decreased (Figure 12). In smaller sized sediments (larger mean phi), eelgrass may allocate more resources for adapting to the new sediment environment at the expense of above ground growth. Even though eelgrass plants from sandier sediments (with more rhizomes), are a preferable source transplant material, there may be a limit to how quickly above ground development can be acheived when the plants are transplanted into increasingly dissimilar environments (Figure 11).

# Transplanting technique had a greater affect on transplant growth than did

57

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Figure 12. Growth of eelgrass transplanted into different sediment types in mesocosm experiments. Leaf mass (gm/cm) represents aboveground growth and number of new nodes per day represents belowground growth, as these two metrics had the highest level of significance based on an analysis of variance (Table 6).

sediment parameters (Table 7). The slightly higher aboveground growth rate for eelgrass transplanted using the bundle technique (Table 7) may be indicative of a light (density) effect. When eelgrass shoots are crowded in bundles (10 shoots per bundle), each shoot partially blocks light available to neighboring shoots. In response to reduced light conditions, eelgrass leaf length increases, enabling the shoots to reach more light (Short et al., 1995). However, the increase in aboveground growth using the bundle technique was offset by a significant reduction in belowground growth compared to the HRM, specifically the number of new nodes on the laterals and length of new rhizome produced each day (Table 7).

The lower belowground growth documented for the bundle technique may also be a density effect. The tightly packed rhizomes in the bundle may have been physically impeded from growing or may have been limited by nutrient competition among the 10 plants. A plant transplanted using the HRM is only in physical contact with one other plant, and the rhizomes are placed so that they grow in opposite directions, minimizing any physical impediment to growth or competition for nutrients.

Overall, the increase in aboveground growth using the bundle technique was more than offset by a reduction in belowground growth. Because eelgrass beds expand primarily through belowground vegetative (asexual) reproduction, the significantly greater belowground growth of eelgrass transplanted with the HRM demonstrates that it is a preferable technique. Additionally, a well-developed rhizome system helps to stabilize the sediment which improves the local environment, allowing for even greater expansion and development of the transplants. These factors, combined with the lower number of shoots needed from a donor site and lower costs (Davis and Short, 1997) make the HRM a preferable transplant method, particularly for large scale transplanting projects.

### **CHAPTER IV**

# **CHANGES IN BENTHIC INFAUNAL COMMUNITIES AT EELGRASS** (ZOSTERA MARINA L.) TRANSPLANT AND CONTROL SITES IN THE PISCATAQUA RIVER, NEW HAMPSHIRE.

### **Introduction**

The relative importance of seagrass habitats to estuarine productivity has been quantified in studies that compared secondary production in vegetated and unvegetated habitats (Heck et al., 1995; Szedlmayer and Able, 1996; Connolly, 1997; Bostrom and Bonsdorff, 1997). These studies have generally focused on either fish, epibenthic and epifaunal macroinvertebrate, or infaunal macroinvertebrate communities. The differences observed between the communities in the different habitat types were explained by abiotic factors such as temperature, salinity and sediment characteristics (Szedlmayer and Able, 1996) and seagrass morphological characteristics (Connolly, 1997; Bostrom and Bonsdorff, 1997).

Changes in faunal communities have also been studied in areas where seagrasses have naturally recolonized (Brown-Peterson et al., 1993), been experimentally removed (Harrison, 1987; Connolly, 1995), or been transplanted (Homziak et al., 1982; Fonseca et al., 1990; Bell et al., 1993). Only Homziak et al. (1982) and Bell et al. (1993) investigated changes in benthic infauna in transplanted seagrass beds. Homziak et al. (1982) found significant increases over time in the infaunal community in transplanted beds, but they did not compare infaunal communities in the transplanted beds to those in

natural beds. Bell et al. (1993) is the only published study that has compared infaunal communities in transplanted seagrass beds to natural beds; the study focused on the secondary production of a single polychaete in restored Syringodium filiforme beds.

Attempts to restore seagrass habitat are becoming more common (Fonseca et al., 1998). Yet the ultimate success of these efforts remains highly variable (Thom, 1990, Davis and Short, 1997; Fonseca et al., 1998), and there are no universally accepted criteria for measuring the success of transplanting efforts (Short et al., In press). Because benthic infauna are important secondary producers in seagrass habitats (Fredette et al., 1990), their status in transplanted beds must be considered when determining the overall success of a transplanting effort. Short et al. (In press) are the first to relate changes in benthic infaunal communities to transplant success. The basis for this portion of their argument is that if a transplanting effort is successful, the benthic community in the successfully restored habitat will change over time to resemble the benthic community found in naturally occurring seagrass beds. The work described in this chapter focuses on testing this assumption and a related question. First, does the benthic community at a successful transplant site change over time to resemble the benthic community at a naturally occurring bed? Second, can differences between the benthic communities at potential transplant sites be used to predict whether the transplanting effort will be successful? For the purposes of this chapter, the term "success" refers simply to the survival and expansion of eelgrass transplanted during 1993 and 1994 for the New Hampshire Port Authority (NHPA) Eelgrass Mitigation Project.

# **Methods**

Six replicate cores were collected at each transplant and control site in October 1993 and August 1994 (Figure 1). Benthic infauna data from the five initial transplanting sites (T1-T5) and the three nearest control sites (C1-C3) were used in the analyses. Cores

were 9.0 cm in diameter (0.00785 m<sup>2</sup>), pressed into the sediment to a depth of 15 cm, and were taken by SCUBA divers to ensure that eelgrass was growing within the 9.0 cm diameter area sampled. Extracted cores were washed through a 0.5 mm mesh sieve, the resultant organisms preserved in a 5% formalin/rose bengal solution, and stored in ethanol for further processing. All organisms were identified to the lowest taxon possible.

Analysis of variance (ANOVA) was performed on untransformed species level data (number of species and abundance). Data were inspected for homogeneity of variance by plotting residuals versus estimated values. Since assumptions of analysis appeared to be met, ANOVA was run on untransformed data. The 1993 data were analyzed first to determine whether significant differences existed between sites immediately after the initial transplanting occurred. Then, an ANOVA was performed on pooled data from 1993 and 1994 to determine whether there were any significant differences between the site types (i.e., transplant or control). Year was used as a blocking factor in the latter analysis.

Data were also analyzed using multi-dimensional scaling (MDS), a nonparametric multivariate method commonly used in marine ecology for analyzing changes in community structure (Clarke, 1993). This technique is useful for investigating the relationship between environmental variables and site groups by superimposing measured environmental variables separately onto MDS ordination plots (Clarke and Ainsworth, 1993). Biological patterns are first discerned, followed by an interpretation of these patterns by relating them to the measured habitat characteristics (Schlacher and Wooldridge, 1996). Because MDS requires a similarity matrix as input, Bray-Curtis similarity matrices (Bray and Curtis, 1956; Connolly, 1997) were created on untransformed 1993 and 1994 genus level data (Vanderklift, 1996). Prior to calculating

the similarity matrix, rare (less than 1 per core) and juvenile organisms were excluded from the data set (Warwick and Clarke 1993; Schlacher and Wooldridge, 1996; James et al., 1995). Rare organisms were excluded because the density of these organisms was assumed to be too low to affect the larger scale transplanting effort. Juveniles were excluded because these organisms were not consistently identified below the family level.

The similarity coefficients from the final matrix were entered into the MDS software to create ordination plots (Clarke, 1993). Detrended correspondence analysis (DCA), a similar ordination technique, was also performed on the data using DECORANA software (Hill, 1979). The results of the DCA ordinations were nearly identical to the MDS ordinations. However, it was recently discovered that DCA has an instability associated with the input data order (Oksanen and Minchin, 1997), so the DCA results were excluded from further consideration.

The axes of the MDS plot are dimensionless (Clarke, 1993), but the distances between the points in ordination space optimally represents their dissimilarities (Kenkel and Orloci, 1986). The MDS coordinates were plotted in two dimensions and the resultant coordinate axes were then regressed against selected environmental variables to determine significant relationships (Clarke, 1993; Clarke and Ainsworth, 1993). Stress values were also determined using MDS software. The stress value indicates how closely the two dimensional plot retains the similarity rankings from the matrices, with a stress value of zero indicating a perfect match (Connolly, 1997). Similarity matrices were also analyzed using hierarchical clustering to clearly delineate site groups within the ordination plots (Clarke, 1993).

### **Results**

# In 1993, control site C2 had significantly greater number of benthic infaunal

species ( $p=0.0001$ ) and abundance ( $p=0.0001$ ) than any of the other sites (Figure 13). The mean number of species and abundance varied among the other sites and were generally higher at the control sites than at the transplant sites (Figure 13). Transplant site T2 had the highest number of species and greatest abundance of any transplant sites, and these numbers were significantly greater than those of T3 and T5 (Figure 13).

Analysis of the pooled data from 1993 and 1994 revealed a significant year by type interaction. There was a significantly greater number of species ( $p=0.0001$ ) and abundance ( $p=0.0003$ ) at the control sites in 1993 (Figure 14). The number of species and abundance decreased significantly at the control sites in 1994 to a level equal to that at the transplant sites (Figure 14).

The MDS ordination plot of the 1993 data distinguished the successful and unsuccessful transplanting sites from the control sites (Figure 15, stress value  $= 0.00595$ ). Hierarchical clustering identified three groups: T5, all control unsuccessful transplanting sites from control sites (Figure sites, and the remaining transplant sites. The first axis explained 28.4% of the variability in the benthic community composition and was significantly related to sediment size (Figure 16a,  $r^2 = 0.947$ ,  $p=0.0002$ ). The second axis explained 15.6% of the variability and was significantly related to above ground eelgrass biomass (log transformed; Figure 16b,  $r^2 = 0.548$ ,  $p=0.0357$ ). In 1994, one year after transplanting, the ordination plot showed the successful transplant sites closer to the control sites; however, the unsuccessful transplant site was still readily distinguishable (Figure 17, stress value  $= 0.00160$ ). Hierarchical clustering identified two groups: T5 and all other sites. The first axis explained 31.9% of the variability and was still significantly related to % silt ( $r^2$  = 0.833, p=0.0041, Figure 18a), though eelgrass shoot density (shoots/m<sup>2</sup>) was also important ( $r^2$  = 0.470, p=0.0605, Figure 18b). The second axis



Figure 13. Benthic infauna at transplant and control sites in October 1993: a) mean number of species (SE); b) mean abundance (SF). Different lower case letters on the right margin indicate significant differences (alpha=0.05).



a)



Figure 14. Benthic infauna at transplant and control sites in 1993 and 1994. a) mean number of species (SE); b) mean abundance (SE). Different lower case letters indicate significant type by year interaction  $(p=0.003)$ .



Figure 15. MDS plot of 1993 benthic infauna data. Delineation of site groups from hierarchical clustering on Bray-Curtis matrix.

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Log Aboveground Biomass  $(g/m^2)$ 

Figure 16. Relationship between the MDS axis coordinates and ecological parameters for 1993 data. a) Mean phi and axis 1 coordinates; b) above ground eelgrass biomass (log transformed) and axis 2 coordinates.



Figure 17. MDS plot of 1994 benthic infauna data. Delineation of site groups from hierarchical clustering based on Bray-Curtis matrix.



Figure 18. Relationship between MDS axis 1 coordinates and ecological parameters for 1994 data. a) Mean phi and Axis 1 coordinates; b)eelgrass shoot density and Axis 1 coordinates.

explained 21.4% of the variability but did not correlate well with any of the measured parameters.

Benthic infaunal data were further assessed at the genus level, revealing that certain genera were present only at transplant or control sites (Table 8 and 9). The number of genera in which such differences occurred was greater in 1993 than in 1994 (Table 8 and 9). Further analysis of the data at the species level revealed that several species were present only at site T5, these included Heteromastus filiformis, Hypereteone heteropoda (1993 only), Leitoscoloplos robustus (1994 only), Gemma gemma, and Leucon americanus. Additionally, though present at other sites, the abundance of Neanthes virens was much higher at T5, particularly in 1994 (Table 8 and 9).

### **Discussion**

Infaunal species number and abundance are generally higher in vegetated than in unvegetated habitats (Bostrom and Bonsdorff, 1997) and this fact was reflected at the newly transplanted versus the control sites in October 1993 (Figure 13). Infaunal species numbers and abundance decreased significantly at the control sites from 1993 to 1994 (Figure 14). Species numbers and abundance remained the same at the transplant sites over the same time period. These patterns are the result of two processes. First, shallowwater benthic communities show strong seasonal (temporal) variability (Whitlach, 1982). Hale and Grizzle (1992) showed that benthic community densities in the Great Bay Estuary had spring and fall maxima, with winter and summer minima. The higher species number and abundance observed at the control sites in October 1993 were likely related to the late summer-early fall recruitment (Trueblood et al., 1994). In August 1994, benthic infaunal species number and abundance may have been reduced by predation pressures which are often highest during the mid-summer. Additionally, fauna that recruit in spring attain their maximum size by mid-summer, increasing grazing

Table 8. Primary differences in genera at transplant and control sites in 1993. Shading indicates genera that are present only at transplant (light shading) or control (dark shading) sites. Underlined numbers highlight differences between T5 and all other sites.





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pressure. The significant decline in August 1994 in benthic species numbers and abundance at the control sites may be related to a self-induced decline in food availability (Trueblood et al., 1994) and predation. Second, transplanting seagrass can be equated to a disturbance of a previously unvegetated site. The aboveground portion of the seagrass changes current flow (Fonseca and Cahalan, 1992), creating processes that lead to a depositional environment (Ward et al., 1984). The belowground structure of seagrass creates localized oxygenated conditions in the sediment. These factors, caused by the presence of vegetation, effectively disturb (change) the prior conditions of the unvegetated site. Disturbances to estuarine bottom result in increased recruitment of opportunistic species (Reise, 1991; Trueblood et al., 1994) and may explain why no summer decline in abundance and species numbers was evident at the transplant sites in August 1994 (Figure 14).

Statistically, the benthic communities at the transplant and control sites were indistinguishable by 1994 (Figure 14). The fact that the benthic communities at the transplant sites were indistinguishable from those at the control sites by 1994 is largely due to a decrease in benthic species number and abundance at the control. Analysis of benthic data using simple univariate techniques makes it difficult to determine whether the benthic communities at the transplant sites are actually changing due to the presence of the vegetation. Because of such confounding factors, univariate statistical analyses may not provide the clearest picture of temporal changes in the benthic communities of transplanted seagrass beds. The MDS analysis better demonstrates how the presence of eelgrass alters the benthic community at eelgrass transplant sites.

In 1993, the MDS ordination plots showed three distinct groupings of benthic infaunal communities: those at the control sites, those at the (ultimately) successful transplant sites, and that of T5 (Figure 15). Sediment grain size was significantly related

to the first axis coordinates of the 1993 ordination (Figure 16a), consistent with previous studies that have shown sediment parameters to strongly affect benthic community composition and distribution (Kalejta and Hockey, 1991; Schlacher and Wooldridge, 1996). The second axis coordinates of the 1993 ordination were significantly related to eelgrass aboveground biomass (Figure 16b) demonstrating that the presence of vegetative cover influenced benthic community composition (Kalejta and Kockey, 1991; Bostrom and Bonsdorff, 1997).

In 1994, the MDS ordination plots showed two distinct groupings: those sites that had eelgrass present (the control and successful transplant sites) and T5, where transplanted eelgrass did not survive. The first axis coordinates were still significantly related to sediment grain size (Figure 18a). However, eelgrass shoot density was also significantly related to the first axis coordinates (alpha =  $0.10$ , Figure 18b). The shift in the significance of eelgrass characteristics from the second axis in 1993 (biomass) to the first axis in 1994 (density) indicates that the presence of eelgrass may have become a more important factor influencing the benthic community composition. Alternatively, because the presence of eelgrass can alter sediment characteristics, eelgrass density may be significantly related to the first axes coordinates simply because it is correlated with the sediment grain size. Regardless, the MDS approach provides a clearer indication of how similar the control and successful transplant site have become in 1994 because the ordination is based on a similarity matrix which takes into consideration the presence and abundance of individual genera or species.

The MDS analysis also provides additional information beyond that available from the univariate analysis. In both 1993 and 1994, the T5 transplant site was distinct from all other sites. This was the only transplant site at which all transplants were lost within a month of transplanting in both 1993 and 1994. The regression analyses of the

MDS ordinations revealed that the first axis coordinates were significantly related to sediment grain size in both 1993 and 1994 (Figure 16a and 18a). However, the mesocosm experiments described in the previous chapter showed that sediment grain size did not have any significant effect on transplant survival or growth. Therefore, the fact that the T5 site was ultimately unsuccessful is more likely related to the distinct benthic infaunal community present at the site.

Analysis of the species composition and abundance data revealed that the high abundance of N. virens was one of the main differences in the benthic infaunal communities between the transplant sites (Table 8 and 9). The high abundance of this omnivorous polychaete may account for the overall low infaunal species number and abundance observed at this site in 1993 and 1994 (Rhoads and Young, 1970; Ambrose, 1984; Figure 14). Large marine polychaetes have been shown to prevent natural recolonization of seagrass and destroy transplants (Philippart, 1994; Davis and Short, In review). Testing whether N. virens directly affects transplant survival is the basis of the field experiment described in the next chapter.

### **CHAPTER V**

# THE IMPACT OF THE CLAM WORM (NEANTHES VIRENS SARS) ON TRANSPLANTED EELGRASS (ZOSTERA MARINA L.)

### **Introduction**

Plant-animal interactions are important determinants of macrophyte species distribution and productivity in the marine environment (Johns et al., 1992). Herbivory, bioturbation, and competition for space, have been reported in a diversity of marine and estuarine habitats from salt marshes (Gerdol and Hughes, 1993; Bertness, 1985), to the rocky intertidal (Dayton, 1973), to deep water marine canyons (Williams et al., 1985). Animal interactions with seagrasses have been documented as well (see review Orth, 1992). Such interactions contribute to primary as well as secondary production of seagrass habitats, and include grazing of seagrasses and their associated epiphytes (Woods and Schiel, 1997; Heck and Valentine, 1995; Preen, 1995; Williams, 1988; Orth and van Montfrans, 1984; Thayer et al., 1984; Tubbs and Tubbs, 1983), the effect of seagrasses on faunal recruitment processes (Grizzle et al., 1996; Eckman, 1987), and the influence of seagrasses on predator-prey relationships (Heck and Crowder, 1990; Orth et al., 1984).

Other interactions do not control production directly, but influence seagrass distribution. Bioturbation by sediment-disrupting organisms can affect the colonization and distribution of seagrasses (Valentine et al., 1994, Fishman and Orth, 1996). For

example, Suschanek (1983) reported that two species of burrowing shrimp from the genus Callianassa prevented the expansion of an existing turtlegrass (Thalassia testudinum) bed in the Carribean by covering the seagrass with reworked sediment. Philippart (1994) reported that the lugworm (Arenciola marina) prevented the spread of eelgrass (Zostera noltii) on an intertidal mudflat in the Netherlands by reworking the sediment and covering the seagrass shoots with burrowing material and faecal castings. Experimental transplants that were not protected from lugworm activities were quickly lost. Harrison (1987) found that an established population of the shrimp, Callianassa californiensis, destroyed experimental Zostera marina (eelgrass) transplants after only a few weeks in Washington state.

Conversely, Harrison (1987) described how expansion of an eelgrass bed reduced the densities of Callianassa californiensis, due to restriction of the shrimp's burrowing by the root/rhizome mat. This is consistent with the findings of Brenchley (1982), who reported that movement of hard-bodied and larger taxa was particularly restricted by roots/rhizomes of Z. marina. These latter studies suggest that if the density of a potentially bioturbating organism is low, seagrass can effectively coexist with the organism, often improving its own habitat through the creation of a dense impenetrable root/rhizome mat (Philippart, 1994; Valentine et al., 1994; Harrison, 1987; Brenchley, 1982). However, if the density of bioturbating organisms is sufficiently high, the establishment or expansion of seagrass populations may be prevented (Harrison, 1987; Philippart, 1994). The density-dependent nature of this interaction is particularly significant to transplanting operations in which seagrasses are usually transplanted at low densities (e.g., on 0.5 meter centers), making them highly vulnerable to bioturbation.

As part of a mitigation project for the New Hampshire Port Authority (NHPA), we transplanted 2.52 hectares of eelgrass at several subtidal and intertidal sites (Davis

and Short, 1997). At two of the subtidal sites, more than a total of 0.5 hectares of transplanted eelgrass were lost due to bioturbation, at a cost of over \$100,000 (1994 U.S. dollars) in labor and supplies to the project. At one site, transplant loss was attributed to bioturbation by green crabs (Carcinus maenas) (Davis et al., 1998). At the other site, the tips of numerous transplanted shoots were observed to have been pulled into the sediment. Blades with their tips in the sediments were eventually pulled flat against the sediment surface, covered with sediments, and subsequently died. Personal observations of the polychaete Neanthes virens (Sars) (clam worm) extending out of their burrows at this site, led to the hypothesis that  $N$ . virens activity was affecting the survival of transplanted eelgrass.

Neanthes virens is reported to occupy and defend its temporary burrows and can impact a large area with its foraging, especially where it occurs in high densities (Miron et al., 1991). Worm densities are greatest between the surface and 4.0 cm depth (Ambrose, 1984). In New England, foraging activity of N. virens has been shown to play a significant role in regulating benthic faunal community structure (Ambrose, 1984; Commito and Shrader, 1985). Neanthes virens has been reported to be an herbivore in Massachusetts, feeding on algae and diatoms (Copeland and Wieman, 1924), and an omnivore elsewhere (Fauchald and Jumars, 1979). However, whether N. virens consumes eelgrass or can affect its distribution and productivity has not been previously investigated.

The purpose of our field experiments and flume observation described in this study was to determine if  $N$ . virens directly affected the survival of transplanted eelgrass. The flume observation was conducted to provide a photographic record of the eelgrass clam worm interaction. Field experiments were designed specifically to test the following hypotheses.

Hypothesis 1. Eelgrass transplant survival decreases as N. virens density increases. This hypothesis was tested by transplanting eelgrass shoots directly into the sediment at three sites with differential N. virens densities and monitoring growth rates, transplant loss rate and number of blades pulled into the sediment.

Hypothesis 2. Protecting transplanted eelgrass shoots from N. virens bioturbation increases transplant survival (by decreasing the rate at which shoots are lost). This hypothesis was tested by transplanting eelgrass shoots onto mesh screens which had been placed on the sediment surface to exclude large polychaetes (Philippart, 1994). Growth rates, transplant loss rate and the number of blades pulled into the sediment were recorded.

Hypothesis 3. We also tested whether water quality was sufficient to support eelgrass growth in areas where hypotheses 1 and 2 by tethering plants to plastic-coated wire frames so that the eelgrass grew above the sediment, hydroponically in the water column, approximately 10 cm above the sediment surface. This test was necessary because although sufficient light levels existed and other physical site characteristics were not limiting (Table 10), water quality parameters that may directly affect eelgrass survival and growth, such as dissolved inorganic nitrogen concentrations (Burkholder et al., 1992; Katwiyk et al., 1997) were not measured in our study (see Table 3). We assumed that if plants grew successfully in the water column, the failure of eelgrass transplanted directly into the sediment could then be ascribed to some factor(s) other than water quality.

Problems encountered during the field experiment included the loss of many transplanted shoots from one experimental site due to green crab activity (Davis et al., 1998), and the occurrence of a small oil spill during the first experimental period. Despite these problems associated with the use of field sites, the experiment provided useful data to further quantify the effect of clam worm bioturbation on transplanted

eelgrass and the important role protective measures can have on transplant survival.

# **Methods**

# **Site Selection**

Prior to the large-scale NHPA transplanting (Davis and Short, 1997), earlier test transplanting and field measurements were completed to determine whether physical site characteristics were sufficient to support transplanted eelgrass at these sites (Figure 1), and the degree to which they influenced transplant survival. The three sites used for this experiment had suitable light, current, and sediment conditions for eelgrass growth (Table 10). The minor differences in physical site characteristics could not adequately explain the variability in transplant survival. Because worm damage had been observed in varying amounts at these three sites, they were selected to test whether worm activity contributed to transplant loss and whether it could help explain the variability in transplant survival.

To summarize the history of each site prior to the experiment: site T1 had relatively good subtidal transplant survival, but at some parts of the site, all transplants were lost. The unsuccessful areas, scattered amongst successful transplants, were selected for the experiment. Site T4 had relatively poor survival of 1993 transplants, but excellent survival of 1994 transplants. An area immediately adjacent to the 1994 transplant area was selected for the experiment. Site T5 had very poor transplant survival; within three months of transplanting, 99% of all transplants at Site T5 were lost. Areas of T5 that had been previously transplanted were selected for the experiment. Previous benthic infaunal data (mean abundances from six replicate 10 cm diameter cores) showed a density gradient of  $N$ . virens at the three experimental sites, with  $T4$ having the lowest density, T1 the middle density, and T5 the highest density (Table 10).

Table 10. Physical and biological parameters measured at the three experimental sites. Transplant survival rates are from Chapter 2 (Davis and Short, 1997). Light values were obtained using a 4 pi underwater sensor. Values shown are mean underwater irradiance from 8am-4pm, shown as a percent of surface light. Sediment parameters are for the top 2.0 cm of the sediment from each site, processed after Folk (1980). Salinity values are daily mean. Current measurements were obtained on a flood tide using a Marsh/McBirney current meter deployed from an anchored boat. Neanthes virens densities are the mean number of organisms collected in six replicate cores.



#### **Field Experiment Setup**

Three different treatments were used at each site (two replicates of each treatment), with each treatment providing increasingly more protection from N. virens. In the first treatment, 32 eelgrass shoots were transplanted directly into the sediment in a 1.0 m<sup>2</sup> area using the horizontal rhizome method (Davis and Short, 1997). This treatment provided no protection from clam worms for the transplanted shoots (referred to hereafter as "unprotected").

In the second treatment, a 0.5 mm mesh screen was sunk 2-3 centimeters into the sediment and stapled in place with 15 cm inch sod staples. Thirty-two eelgrass shoots were then transplanted into a  $1.0 \text{ m}^2$  area of sediment on the screen surface using the horizontal rhizome method (Davis and Short, 1997). The screens were designed to prevent large resident polychaetes from reaching the sediment surface to forage, and to prevent the immigration of large polychaetes into the treatment area (referred to hereafter as "screened"). Mesh screens have been used effectively to exclude specific sizes and types of benthic infauna, including N. virens, in previous field experiments (Philippart, 1994; Dittman, 1996, Caron et al., 1996). The screens were designed to protect the transplants only from bioturbation from infaunal organisms, and not to affect any other environmental parameters that might influence transplant survival.

In the third treatment, 32 eelgrass shoots were tethered to a  $1.0 \text{ m}^2$  plastic-coated wire frame using plastic cable ties. The frame suspended the transplants approximately 10 cm above the sediment surface so that they grew entirely in the water column. This treatment (referred to hereafter as "elevated") has been shown to protect plants from disturbance by epibenthic organisms (e.g., crabs) (Davis et al., 1998), and we assumed that it would protect plants from infaunal bioturbation as well.

All shoots were marked prior to transplanting for subsequent growth

measurements (Short, 1987). The entire experimental area was surrounded by protective caging to protect the transplants from green crab (Carcinus maenas) and horseshoe crab (Limulus polyphemus) disturbance (Davis et al., 1998; Chapter 6). The experiment was performed twice during the summer. The first experimental period was July 1-10, 1996; the second experimental period was August 8-29, 1996.

# **Field Experiment Sample Collection and Analysis**

Sites were assessed every two days during the experimental periods and the total number of blades pulled into the sediment and the total number of shoots surviving were recorded. Observations of disturbances or unusual activity that could potentially affect the transplants were also recorded. At the end of each experimental period, all plants including roots and rhizomes were harvested and processed at the Jackson Estuarine Laboratory for growth and biomass assessments. Survival was counted as the number of shoots remaining in each treatment at the end of each experimental period. Even if the tips of the blades were pulled into the sediment, the shoot was still counted as surviving, as long as enough of the plant remained so that it could be processed for growth measurement. The loss rate, which gives the number of shoots lost per day, was calculated by the formula:

Loss rate =  $(32 - #surviving) / # days$  of experimental period

Benthic invertebrates were collected using three replicate sediment cores taken from within each treatment area following removal of the eelgrass shoots. Cores were 9.0 cm in diameter (0.00785  $m<sup>2</sup>$ ), and taken to a depth of 15 cm. Extracted cores were washed through a 1.0 mm mesh sieve, resultant organisms preserved in a 5% formalin/rose bengal solution, and stored in ethanol for further processing. Neanthes virens were identified and counted from all treatments at the end of both experimental periods. Additionally, all organisms from the unprotected treatments were identified to

the lowest taxon possible from the cores collected between the experimental periods to characterize the benthic community at each site. Neanthes virens abundance were determined by counting the number of identifiable posterior ends in each core. Dry weight biomass of N. virens was determined by weighing worms dried at  $25^{\circ}$  C for 24 hours.

The transplant loss rate, growth rates, and number of blades pulled into the sediment from both field experiments were analyzed using ANCOVA. The independent variables used in the analysis were date (as a block), N. virens density, and treatment. The data were further analyzed using simple linear regression to assess the relationship between the number of blades pulled into the sediment and N. virens biomass and density.

# **Flume Observations**

A small flow-through flume, 3.5 m long, 0.2 m wide and 0.2 m deep, was used observe and photograph the eelgrass - clam worm interaction. The flume was constructed of smooth gel-coated fiberglass and contained a sediment box, set in the bottom of the flume, approximately 0.5 m from the outflow end (Grizzle et al., 1992). The sediment box was filled with a mixture of estuarine sediment and coarse sand that had been sifted through a 0.5 mm screen to remove organisms. Ten clam worms (mean length of 15 cm) were added to the sediment and observed until they had completely burrowed into the sediment. The following day, two eelgrass shoots (nine blades of eelgrass total) were secured within the flume up-current of the sediment box. Estuarine water flowed freely across the eelgrass plants at approximately 2.0 cm/sec. The flume was visited at hourly intervals each day from 7:00 am to 5:00 pm to observe and photograph the status of the plants. At the conclusion of the 4 day study, the sediment was removed from the flume and sifted through a 0.5 mm screen to collect and count organisms. Collected organisms

were identified to species and preserved in 5% formalin/rose bengal solution.

# **Results**

### **Eelgrass Transplant Loss, Growth, and Morphology**

The eelgrass loss rate was significantly higher  $(p=0.0277)$  for the first experimental period, due to the unexpected loss of most bottom transplants at one site (T4) from green crab bioturbation, despite the caging intended to control green crab damage (Davis et al., 1998). The loss rate was also significantly different among treatments (Figure 19). The transplant loss rate was highest for the unprotected transplants and lowest for the elevated transplants. The oil spill did not have any noticeable effect on the transplanted eelgrass.

Growth and morphology data on eelgrass transplants are presented as cm/shoot/day, total length per shoot, and mg/shoot. Transplants at site T4 had significantly lower cm/shoot/day growth than either site T1 or T5 ( $p=0.0402$ ). No other significant differences in plant parameters existed between the sites or treatments (Table 11). However, all plant parameters were significantly higher for the first experimental period than for the second. Water quality declined during the second experimental period due to runoff associated with storm events, reducing the amount of light reaching the transplants (Figure 7) and presumably resulting in the decreased growth rates we observed.

### **Eelgrass Blades Pulled Into the Sediment**

The total number of blades pulled into the sediment was significantly higher for eelgrass transplanted directly into the sediment than for either the screened or elevated treatments  $(p=0.0001$ ; Figure 20). Shoots were observed with the tips of their blades pulled into the sediment within one day after transplanting. The number of blades pulled



Increasing protection from benthic bioturbation

Figure 19. Average rate of shoot loss from two experimental runs  $(n=2)$ . Different lower case letters indicate significant difference  $(p<0.01)$ among treatment means. Neanthes virens density (#/m<sup>2</sup>) is shown in parenthesis next to the site labels.



Increasing protection from benthic bioturbation

Figure 20. Average number of eelgrass blades pulled into the sediment from two experimental runs ( $n=2$ ). Significantly more blades were pulled into the sediment in the unprotected treatment (p<0.01). Neanthes virens density  $(\#/m^2)$  are shown in parenthesis next to the site labels.




into the sediment was significantly higher  $(p=0.0047)$  at the site with the greatest N. *virens* density (T5) than at the other two sites (Figure 20).

## **Benthic Infauna**

Benthic species composition and abundances were determined from cores collected in the unprotected treatments between experimental periods (Table 12). Species composition and abundances varied among sites; T1 had the highest number of species  $(17)$ , and T5 the lowest  $(13)$ . Mean abundance varied from a high of  $1273$ individuals/m<sup>2</sup> at T1, to a low of 849 individuals/m<sup>2</sup> at T5 (Table 12).

*Neanthes virens* abundances were determined from cores collected from each treatment area after each experimental period (Table 13). Neanthes virens was not found at site T4 during either experimental period, and was collected at site T1 after the first experimental period only. Analysis of variance on  $N$ . virens abundances in the unprotected treatments revealed significantly more individuals/ $m<sup>2</sup>$  at T5 than either T1 or T4  $(p=0.0001)$ . Worm biomass did not prove to be a useful indicator of the transplanted eelgrass - clam worm interaction because many organisms were damaged (cut) by the coring tube and the actual N.virens biomass could not be determined. Therefore, worm density was used as the best measure of worm activity.

Because significantly more blades were pulled into the sediment where transplants were unprotected, the results from this treatment were analyzed separately to test the hypothesis that the number of blades pulled into the sediment was directly related to N. virens density. Simple linear regression analysis revealed a significant relationship between the number of blades pulled into the sediment and N. virens density ( $p=0.0129$ ;  $r^2 = 0.477$ ) (Figure 21).



Figure 21. The number of eelgrass blades pulled into the sediment as a function of Neanthes virens density. Data are from the unprotected treatments only.

Table 12. Benthic infauna present at sites collected between experimental periods. Abundances shown are the mean number per square meter from six replicate cores taken from the unprotected treatments at each site. Polychaete feeding guilds are from Fauchald and Jumars (1979); all others are from Whitlach (1982). np indicates not present in the cores collected during the experimental period; however, N. virens were present in previous samples at this site (see Table 10).

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#### **Flume Observations**

On the third morning of the flume observational period, the tips of four of the nine eelgrass blades (44%) had been pulled into the sediment (Figure 22). One of the blades was pulled completely flat against the flume surface. The only organisms present in the sieved sediment at the end of the observational period were N. virens. Nine of the ten clam worms originally placed in the sediment box were collected at the end of the study.

#### **Field Observations**

Despite the occurrence of two problems in the experiments (green crab damage and a small industrial oil spill), the experimental design proved robust enough to provide useful data for characterizing the eelgrass - clam worm interaction. Bioturbation by green crabs was noted during the first experimental period at T4 which resulted in the loss of eelgrass transplanted into the sediment and onto the screens. The protective caging at this site was damaged by flotsam allowing green crabs access to the site. Green crab damage to previous eelgrass transplants has been reported at this site (Davis and Short, 1997). Upon subsequent visits to the site, numerous shoots were missing entirely, resulting in higher loss rates for eelgrass transplanted directly into the sediment at this site (Figure 19). The protective caging remained intact during the second experimental period and loss rates at T4 were similar to those found at the other sites.

A 1,000 gallon oil spill occurred on the evening of July 2, 1996 during the first experimental period. The oil spill did not appear to have any significant effect on the transplanted eelgrass. Subtidal seagrass is usually not adversely affected by oil spills (Durako et al., 1993; Kenworthy et al., 1993), due to its growth strategy and occurrence below mean low water. However, the oil may have affected benthic infauna abundances. Overall abundances were lower than expected compared to the results of benthic samples collected in previous years, even when accounting for the loss of organisms due to the



Figure 22. Photograph taken in the flume of eelgrass blades pulled into the sediment. Sediment box contains N. virens. Forty-four percent of the blades were pulled into the sediment by the third day of the observation period.

larger sieve mesh size used in our experiments (Schlacher and Wooldridge, 1996). We do not know if the lower numbers of organisms were related to the oil spill, but  $N$ . virens were present in sufficient numbers at the experimental sites for statistical analyses to be performed.

## **Discussion**

*Neanthes virens* is opportunistic, feeding by extending several anterior body segments out of its burrow, grasping food in its powerful jaws and jerking it down into the burrow (Fauchald and Jumars, 1979; Pettibone, 1963). Where N. virens occurs in high densities, competition for food resources increases, resulting in increased prospecting time and an expanded foraging area (Miron et al., 1991). We observed that the worms pull the distal ends of transplanted eelgrass shoots into their burrows. Neanthes virens may be feeding on the assemblage of plants and animals growing on the eelgrass blades (personal observation). If the blades break at the point where they enter the worm burrow, the remainder of the blade can rise into the water column and continue to grow. However, in many instances, particularly at site T5, the blades were pulled flat against the substrate and quickly covered with sediment. The significant relationship between the number of eelgrass blades pulled into the sediment and N. virens density (Figure 21), combined with the flume observations (Figure 22), demonstrates the ability of this polychaete to trap transplanted eelgrass shoots and thus decrease transplant survival, providing strong support for our first hypothesis: transplant survival decreases as N. virens density increases.

The benthic communities at all three sites were dominated by deposit feeding organisms (Table 12). Deposit feeders rework the upper few sediments creating a faecalrich surface layer that is easily resuspended by low-velocity currents (Rhoads and Young, 1970). Sites T1 and T4, located along the main channel of the Piscataqua River, are

subject to strong bi-directional tidal currents (Bilgili, 1996) and much of the resuspended material is likely carried away. Site T5 is hydrodynamically distinct from the two other sites, situated in a large, shallow cove, over 300 meters from the main channel of Little Bay (which flows into the Piscataqua River). At this site, resuspended material is more likely to be trapped and resettle within the cove. However, the exact mechanism by which the blades were covered with sediment once the tips were pulled into the substrate was not examined in these experiments.

The rate at which transplanted eelgrass was lost decreased significantly with increasing levels of protection from bioturbation (Figure 19). Unprotected eelgrass shoots, transplanted directly into the sediment, were susceptible to bioturbation from both infaunal and epifaunal organisms and had the highest loss rate (mean of 1.34 shoots/day). Eelgrass shoots transplanted onto screens had the next highest loss rate (mean of 0.75 shoots/day), though it was significantly lower than the loss rate of unprotected shoots. These latter transplants were protected from bioturbation from benthic infauna such as N. *virens*, but not epifauna (e.g., crabs, Davis et al., 1998). Plants tethered to frames were protected from all bioturbation and had the lowest loss rate (mean of 0.24 shoots/day), a rate significantly lower than either the unprotected transplants or those transplanted onto the screens. Although there was some loss of shoots due to green crab bioturbation in the unprotected treatment at T4 (Figure 19), the majority of shoots were lost due to the tips of the blades being pulled into the sediment (Figure 22) in a way typical of  $N$ . virens bioturbation. These results support our second hypothesis that protecting transplants can significantly increase survival by decreasing the number of blades pulled into the sediment (Figures 19 and 20).

Growth was measured in shoots from all treatments, demonstrating that light and water quality were sufficient to support eelgrass. The growth rates for the shoots tethered

to the frames (elevated) were similar to those from the other two treatments (Table 11), while the loss rate was significantly lower. It should be noted that the frames used in the elevated treatment for this study were designed for experimental purposes and are not a realistic method of transplanting or protecting eelgrass, because the plants are not rooted in the sediment. Adequate measures for protecting transplants from infaunal bioturbation include planting at densities such that the number of transplants is sufficient to withstand the impact of bioturbating organisms. However, this approach may prove to be prohibitively expensive or time-consuming, especially when transplanting over large areas. An alternative approach would be to create a root/rhizome mat to prevent or slow the movement of some infaunal organisms (sensu Brenchley, 1982), by either transplanting portions of a mat from a donor site, or creating a reasonable substitute (e.g., an erosion control blanket constructed of natural fibers). Both of these approaches have proved successful in subsequent small-scale transplanting experiments at T5 (unpublished data).

Factors other than bioturbation may have contributed to the loss of some shoots (e.g., transplant shock, poor donor material, improper transplanting). However, the rate at which transplants were lost decreased significantly with increasing levels of protection from clam worms (Figure 19). When transplants were left unprotected, over 63% of the transplanted shoots were lost. At sites where benthic infauna know to affect eelgrass exist such as N. virens (this study) or lugworms (Philippart, 1994), all transplanted shoots may eventually be lost. Protecting transplants from infaunal bioturbation by anchoring screens to the sediment decreased transplant loss to less than 36%. These results underscore the need to adequately consider bioturbation issues in selecting potential seagrass transplanting sites.

#### **CHAPTER VI**

# **QUANTIFYING THE EFFECTS OF GREEN CRAB DAMAGE TO EELGRASS TRANSPLANTS**

## Introduction

Zostera marina L. (eelgrass) forms beds that provide a wide array of functions important for maintaining healthy estuarine and coastal ecosystems (Short and Wyllie-Echeverria, 1996; Heck et al., 1995). In the United States, seagrasses are protected under Section 404(c) of the Clean Water Act (33 U.S.C. 1341-1987). According to these regulations, any person who undertakes an activity that may impact seagrasses must mitigate for those impacts. In 1993 and 1994, we transplanted 2.52 hectares of eelgrass at several sites within the Great Bay Estuary. The planting was part of a large mitigation project to replace the functional values lost due to both direct and indirect impacts on eelgrass beds adjacent to a proposed pier facility (Bosworth and Short, 1993). The project was successful in creating over 1.5 hectares of new eelgrass habitat at unvegetated sites, using a revised transplanting method (Davis and Short, 1997). Four months after transplanting, survival rates varied widely from a high of 98% at four subtidal sites to as low as 1% at three intertidal and two other subtidal sites (Davis and Short, 1997). The lower survival rates for intertidal and subtidal transplants were the result of physical and biological disturbances, respectively. Intertidal transplants were lost due to physical disturbance from scouring and rafting of sea ice during the winter, while biological disturbance, or bioturbation, by green crabs and clamworms caused most of the subtidal

transplant losses (Davis and Short, 1997).

Plant-animal interactions have been shown to be important determinants of seagrass distribution and productivity (Orth, 1992). These interactions include animal grazing on seagrasses, seeds, and epiphytes (Heck and Valentine, 1995; Williams, 1988; Orth and van Montfrans, 1984; Tubbs and Tubbs, 1983), the effect of seagrasses on faunal recruitment processes (Grizzle et al., 1996; Eckman, 1987), and the influence of seagrasses on predator-prey relationships (Heck and Crowder, 1990; Orth et al., 1984). Bioturbation, which we use here to mean a plant-animal interaction in which an animal disturbs a plant, has been shown to affect the distribution and survival of both naturally occurring and transplanted seagrasses. Burrowing shrimp from the genus Callianassa have been shown to prevent the expansion of Thalassia testudinum

(turtlegrass)(Suchanek, 1983), Zostera marina (Harrison, 1987), and Posidonia oceanica (Molenaar and Meinesz, 1995) by burying the seagrasses with reworked sediment. The shrimp also destroyed experimental Zostera and Posidonia transplants (Harrison, 1987; Molenaar and Meinesz, 1995, respectively). Foraging activity by rays has also destroved naturally occurring (Orth, 1975) and transplanted seagrasses (Fonseca et al., 1994, 1996). Arenciola marina (lugworms) prevented the spread of Zostera noltii (eelgrass) on an intertidal mudflat in the Netherlands by reworking the sediment and covering the seagrass shoots with burrowing material and fecal castings (Philippart, 1994). Experimental transplants that were not protected from lugworm activity were quickly lost. Similarly, the effects of certain crab species on natural seagrasses has been investigated. Fishman and Orth (1996) and Wigand and Churchill (1988) demonstrated that Callinectes sapidus (blue crab) and *Pagarus* spp. (hermit crab) consume seagrass seeds and seedlings, respectively. Valentine et al. (1994) documented the role of stone crabs in controlling the spatial expansion of *Thallasia* beds. Our transplant method paper (Davis and Short, 1997) reported observations of damage to eelgrass transplants by green crabs. However,

no study has quantified the effects of crabs on transplanted seagrass. In this paper, we examine the impact of green crab bioturbation on the survival of transplanted eelgrass.

Carcinus maenas, the European green crab, also known simply as the green crab, is a non-native species, which was found on the central east coast of the United States by the late 1800's (Glude, 1955). The crabs have foraging habits which can significantly alter endemic benthic community structure and ecological interactions, such as support for higher trophic levels and fisheries production (Cohen et al., 1995; Grosholz and Ruiz, 1995). Green crabs show a preference for Mya spp. (soft-shell clam) and Mytilus spp. (blue mussel), both commercially important bivalves (MacPhail et al., 1955; Glude, 1955; Grosholz and Ruiz, 1996). In foraging, the crabs rework the top few centimeters of the sediment (Cohen et al., 1995) and can impact a large area. Green crabs occupy primarily shallow subtidal habitats and often move into intertidal mudflats at high tide (Aagaard et al., 1995), such as the areas in which eelgrass was transplanted for our project. In the Great Bay Estuary of New Hampshire, we have observed green crabs damaging both naturally occurring and transplanted eelgrass shoots by tearing or cutting the sheath bundle (Figure 23) through their foraging, burrowing or other behavior (hereafter, foraging). Green crab foraging is especially disruptive to transplants because they are more vulnerable to disturbance.

To quantify the effects of bioturbation by green crabs on eelgrass transplants, we conducted a series of mesocosm experiments in the summer of 1996. One experiment elevated plants above the bottom to determine whether the crabs were damaging eelgrass as part of their foraging activity or were directly attracted to the plants themselves. By quantifying the damage caused by green crabs, and characterizing the crab-eelgrass relationship, we can improve the transplant site selection process and upgrade methods for protecting transplants from crab foraging activities in future transplanting projects.

#### **Methods**

Mesocosm tanks were used to experimentally test the effects of green crabs on the survival of transplanted eelgrass. Eight replicate  $1.4 \text{ m}^2$  outdoor mesocosm tanks were filled with approximately 15 cm of estuarine sediments and connected to a flowing seawater system (Short et al., 1995) at the Jackson Estuarine Laboratory. Before each experiment, eighteen planting units were transplanted into each tank using the horizontal rhizome method (Davis and Short, 1997) and allowed to stabilize for one week. Each planting unit consists of two eelgrass plants for a total of 36 shoots per tank. Field densities of green crabs, used as a basis for establishing crab densities in the mesocosm experiments, were obtained by placing two  $1.25$ m<sup>2</sup> quadrats at a transplant site and recording the number of crabs within the quadrats after one hour. Green crabs were then placed in the tanks for one week periods, and shoots that were clipped at the bundle sheath or dislodged from the sediment were collected daily and counted as crab-damaged shoots (Figure 23).

Three separate experiments were initiated in June, July, and August, 1996 using a range of crab densities encompassing those measured in the field. For Experiment One, four tanks were assigned a low crab density of one crab per tank  $(1.0 \text{ crabs/m}^2)$ , and four tanks a high crab density of 10 crabs per tank  $(7.0 \text{ crabs/m}^2)$ . Frames, constructed of plastic-coated wire mesh, were added to two tanks of each crab density. In these tanks, all plants were tethered to the frames (approximately 10 cm above the sediment surface) instead of being transplanted into the sediment to test whether crabs were attracted to eelgrass when it was removed from the sediment surface. For Experiment Two, four tanks were assigned a high crab density (7.0 crabs/ $m<sup>2</sup>$ ), and four tanks a very high crab density of 20 crabs per tank  $(15.0 \text{ crabs/m}^2)$ . For Experiment Three, three tanks had no crabs (used as a control), one tank was assigned a low crab density (a single crab was inadvertently left in this tank), and the other four tanks a moderate crab density of five crabs per tank  $(4.0 \text{ crabs/m}^2)$ . Frames were not used in Experiments Two or Three.

The assumptions of normality required for an ANOVA could not be met by transforming the damaged shoot count data. Therefore, the mean number of total shoots damaged per tank for each experiment was analyzed using ranked data, which allows parametric tests such as ANOVA to be performed on nonparametric data (Conover and Iman, 1981; Zar, 1996). To test for the effect of elevating the plants above the sediment. crab density was used as a blocking factor for analysis of Experiment One. To test for the effect of crab density, the combined results from all experiments without frames were analyzed with experiment number as a blocking factor to remove any seasonal (light, temperature) effects.

#### **Results**

After the introduction of the crabs, damaged shoots were recovered from all tanks with moderate (4.0 crabs/m<sup>2</sup>), high (7.0 crabs/m<sup>2</sup>), and very high (15.0 crabs/m<sup>2</sup>) crab densities, and three tanks with low  $(0.7 \text{ Crab/m}^2)$  crab densities. No damaged shoots were observed in the controls. The dislodged or cut shoots exhibited damage to the sheath bundle (Figure 23). Three shoots became dislodged during the experiment due to broken rhizomes, but no crab damage was evident. These shoots were excluded from the analysis.

The highest level of crab damage was recorded in the tanks with moderate crab densities, in which 39% of all transplants were lost within one week (Table 14). The number of shoots damaged in tanks with moderate, high, and very high crab densities was not significantly different (Figure 24). Significantly more shoots were damaged in the tanks with moderate, high, or very high crab densities  $(p=0.0016)$  than in the tanks with low density or the controls (Figure 24). A total of five shoots was damaged in the tanks with low crab densities, not significantly different from the controls (no damage). In



Figure 23. Damage to sheath bundle (indicated by arrow) caused by green crab in mesocosm experiments (a). Most damaged sheaths were partially cut above the meristem. In addition to sheath bundle damage, the transplanted shoots were often dislodged from the sediment or broken along the rhizome. These shoots floated to the surface following crab bioturbation. Shoots collected from a field transplant site showing crab damage (indicated by arrow) similar to that observed in the mesocosm experiments.



Figure 24. Mean number of shoots damaged at different crab densities. The number of shoots damaged with 4, 7, or 15 crabs/m<sup>2</sup> was significantly higher ( $p=0.0016$ ) than the number damaged in the tanks with lower crab densities. Different lower case letters indicate significant differences among treatment means at the 0.05 level. Error bars are 1 SE.



Table 14. Percentage of the initial 36 shoots that were damaged by green crab bioturbation at the end of the week-long experimental period.

Experiment One, in which eelgrass was elevated on frames, no shoots were damaged in the elevated treatment (Table 14, Figure 25), even though crabs were observed both underneath and on the frames throughout the experiment.

#### **Discussion**

Our mesocosm experiments showed that as much as 39% of transplanted shoots were lost within one week when exposed to crab densities of 4.0 crabs/ $m<sup>2</sup>$ . There was no damage to eelgrass tethered on frames above the sediment. Additionally, we have seen no evidence of green crabs consuming eelgrass shoots, although this activity has been reported for other crab species (Wigand and Churchill, 1988). Green crabs forage in the top few centimeters of the sediment (Cohen et al., 1995), and we have observed them damaging naturally occurring eelgrass and transplants in situ, and transplants in mesocosm experiments during this foraging process (Figure 26).

Weekly loss of up to 39% of transplants would greatly increase the time and costs required for transplanting efforts. Additionally, this high rate of loss would potentially reduce the chance for a root-rhizome system to develop if the 39% loss rate were compounded over the few weeks it takes for transplants to become established. The establishment of a well developed root-rhizome system can prevent sediment penetration and disruption by bioturbating organisms (Harrison, 1987; Valentine et al., 1994; Philippart, 1994). The initial establishment and expansion period is particularly important for transplanting operations in which seagrasses are usually installed at low densities (e.g., 0.5 m on center). For our transplanting project in the Great Bay Estuary. cages (Figure 27) were constructed around the majority of transplants to exclude green crabs and Limulus polyphemus (horseshoe crab). The cages were successful in excluding horseshoe crabs but were not consistently effective in excluding green crabs from transplanted areas (Davis and Short, 1997). In the future, additional measures will need to be taken, e.g., placing baited crab traps inside the cages, to better protect eelgrass



Figure 25. Mean number of shoots damaged in the treatment where was elevated above the sediment was significantly less  $(p=0.0146)$ than damage to the shoots transplanted in sediment. Shoots were tethered to frames (10 cm above the sediment surface) in the elevated treatments. Different lower case letters indicate significant difference among treatment means at the 0.05 level. Error bars are 1 SE.



Figure 26. Series of photographs taken in mesocosm tanks illustrating foraging activity of the green crab. Crab moves behind shoots in tanks (a); crab holding eelgrass shoot in chela (arrow indicates shoot) bends shoot to the right (b); crab turns while holding the shoot, bending the shoot farther to the right and toward the sediment surface (arrow indicates shoot). This activity by the green crab resulted in damage to the sheath bundle of the eelgrass shoots (see Figure 23) and dislodged the shoots from the sediment.



Figure 27. Illustration of protective caging erected around 10 x 10 m plots of transplanted eelgrass. The 1 m tall cages were constructed of gill netting and oak stakes, with a buoy line attached to one corner to mark the plot (for details on cage construction see Chapter 2). Green crabs were observed on and inside intact cages. Unbaited crab traps were added to several cages near the end of the transplanting period (emptied twice a week), but did not appear to affect crab densities inside the planted area.

transplants where naturally occurring densities of green crabs are sufficient to significantly reduce the survival of transplanted eelgrass. In the Great Bay Estuary, crab densities of 5.4 crabs/m<sup>2</sup> were found at one site where crab activity likely caused the loss of transplants in 1993. At this site, 0.5 hectares of transplanted eelgrass were lost within four months of transplanting.

The results of our mesocosm experiments show that green crabs can significantly decrease eelgrass transplant survival by directly damaging eelgrass shoots and highlight the detrimental impact that biological organisms can have on attempts to restore eelgrass beds. These results have important implications for seagrass restoration efforts. The significant influence of biological organisms on transplants has only recently been acknowledged and has yet to be fully incorporated into the site selection process.

#### **CHAPTER VII**

# THE PRELIMINARY TRANSPLANT SUITABILITY INDEX AND THE TRANSPLANT SUITABILITY INDEX: A METHOD FOR PRIORITIZING POTENTIAL EELGRASS TRANSPLANT SITES

#### **Introduction**

Eelgrass has been transplanted in estuaries of the United States beginning as early as the 1940s (Addy, 1947; Thom, 1990; Short et al., 1993; Davis and Short, 1997; Fonseca et al., 1998). The overall success of these transplanting efforts has been highly variable, even when transplanting sites are in close proximity to naturally occurring eelgrass populations (Davis and Short, 1997; R. J. Orth, personal communication). If percent survival is used as the measure of success, then the success of recent plantings ranges from 0 to 100%, with a mean of 42%, (Fonseca et al., 1998). Transplanting failures can sometimes be attributed to planting methodology, but are largely due to unfavorable conditions (poor light availability and bioturbation) (Fonseca et al., 1998). Therefore, properly selecting the transplant sites is the single most important step of any transplanting project (Fonseca et al., 1998).

The eelgrass transplant site selection methodology described here includes two parameters that combine and quantify the importance of physical and biological site characteristics. The Preliminary Transplant Suitability Index (PTSI) and the Transplant Suitability Index (TSI) are evaluated with reference to existing eelgrass in the area (Figure 28). Existing eelgrass populations indicate that water quality is sufficient to support. When eelgrass is present and there is a potential for it to expand, an obvious question arises: why transplant? Several important answers come to mind. First, eelgrass

expands primarily through vegetative asexual reproduction (Tomlinson, 1974; Phillips et al., 1983). Even if the existing population expands in a local area, there is the possibility that it may not recolonize more distant sites (Orth et al., 1994). Additionally, when seagrass cover is lost, the character of the site may change so that it can no longer support recolonization (Chapter V). Second, transplanting can increase eelgrass habitat more quickly than may occur naturally. This provides for the realization of the full functionality of eelgrass habitat in a shorter time period. Third, larger eelgrass populations may be able to better withstand environmental and anthropogenic perturbations. Transplanting provides a means of increasing the overall population in a relatively short time period. Fourth, transplanting is sometimes required as part of a mitigation project to offset impacts to naturally occurring populations. Lastly, almost every coastal and estuarine area around the world has lost a portion of its seagrass populations from variety of factors (Short and Wyllie-Escheveria, 1996). Therefore, if site conditions are sufficient to sustain seagrasses, transplanting can be an effective tool for reestablishing historical populations.

This last statement brings up a crucial point, i.e., attempts to restore eelgrass (or any seagrass) can only be considered if conditions which caused the loss of eelgrass in the area where you are going to work have been ameliorated (Burkholder et al., 1992; Fonseca et al., 1998). Fredette et al. (1985) pose the question, "if seagrass does not currently exist at the (chosen) site, what makes you believe it can be successfully established?" As Fonseca et al. (1998) state, "in the absence of site history information, one must then assume absence of seagrass indicates some inherent difficulty in colonization or persistence of seagrass." However, there are several factors that can prohibit seagrasses from naturally recolonizing a site that can be overcome by careful site selection and transplanting. First, because eelgrass populations have declined or are declining in many areas, there may no longer be a sufficient seed source for establishing new populations. Transplanting offers a means of getting plant material to a site that may only be lacking a seed or propagule supply. Second, bioturbation is a significant factor contributing to loss of both transplants (Davis et al., 1998, Fonseca et al., 1998) and natural seagrass populations as well (Valentine et al., 1994; Townsend and Fonseca,

1998). Properly excluding bioturbating organisms can greatly increase the survival and expansion of both transplanted (Davis et al., 1998) and naturally occurring seagrasses (Harrison, 1987; Philippart, 1994).

Other transplant site selection methodologies are largely based on physical site characteristics (Phillips 1980, Zimmerman et al. 1991, Fonseca et al. 1998) or water quality (Batiuk et al. 1992, Goshorn et al. 1998). The PTSI/TSI method improves upon past methodologies because it incorporates a wide range of biological and physical site characteristics into the overall site selection process, and provides a framework for progressing through the site selection process in a quantitative manner (Figure 28). The process begins with the calculation of the PTSI, followed by collection of site specific data and test transplanting. The results of the field data and test transplanting are then used for calcuation of the TSI. Additionally, the PTSI/TSI method considers larger scale (e.g., proximity to existing eelgrass beds) and smaller scale (e.g., density of bioturbating organisms) issues to quantify the restoration potential of a site. No other methodology exists that combines such a large number of characteristics critical to the survival of transplanted eelgrass.

The PTSI and TSI were developed based on work conducted as part of the New Hamsphire Port Authority (NHPA) eelgrass mitigation project (Davis and Short, 1997), described in the previous chapters. The indices are designed to rank areas of potential eelgrass habitat for selection as transplanting sites. Potential eelgrass habitat is defined as those areas that are currently unvegetated, but that may have been historically vegetated, with the appropriate depth (to ensure sufficient light) and substrate to support eelgrass. Potential eelgrass habitat should be the focus of any transplanting operation. The PTSI/TSI method was developed from a large scale transplanting project (> 2.52 hectare) When smaller areas are being considered for restoration  $( $0.4$  hectares), it is more cost$ efficient to go directly to test transplanting (Fonseca et al., 1998), rather than collect and analyze the data required for the PTSI/TSI.

### **Preliminary Transplant Suitability Index**

The PTSI considers depth, sediment characteristics, water quality, potential for bioturbation, and other characteristics of vegetated and unvegetated areas to identify possible restoration sites (Table 15). The use of a geographic information system (GIS) is an integral part of the development of the PTSI and facilitates obtaining certain values needed to calculate the final index for each site. Prior to calculating the PTSI, areas that support active shellfish beds and areas of high boat traffic should be identified and excluded as possible transplant sites and incorporated into the GIS database (Figure 28). Then, the first step in calculating the PTSI is to obtain historical and existing eelgrass distribution, bathymetry, sediment grain size, and water quality information to delineate existing and potential eelgrass habitat. The data are used to quantify the environmental conditions that sustain the existing eelgrass populations (e.g., depth range, sediment type, and water quality), and to identify areas of potential eelgrass habitat with similar environmental conditions to existing eelgrass beds.

Once existing eelgrass distribution information is obtained, the GIS can be used to calculate two additional parameters for potential transplant sites needed for the PTSI: exposure and proximity to naturally occurring eelgrass beds. Exposure is a metric which combines the predominant wind direction, maximum wind speed, and fetch to create a single value (modified after Murphey and Fonseca, 1995) for existing eelgrass populations. Proximity to naturally occurring eelgrass populations is used because sites distant from natural beds are less likely to be colonized by seeds (Orth et al., 1994) and therefore are given higher priority for transplanting in the PTSI. Bathymetry data from navigational charts or direct field measurements should be digitized and incorporated into the spatial (GIS) database. Areas above -0.5 meters MLW may be subject to ice scouring, dessication or temperature extremes (Davis and Short, 1997) and areas below 2 meters generally do not receive adequate light (Batiuk et al., 1992; Dennison et al., 1993; van Katwijk et al., 1998).



Figure 28. Schematic representation of the site selection methodology based on the PTSI and TSI. Unboxed items represent data required to reach the next level. Diamond shaped boxes represent decisions and rectangular boxes processes that need to be made/completed to move to the next level.



Table 15. Data layers to be used for identification of potential transplanting sites based on the Preliminary Transplant

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Water quality characteristics such as total suspended solids, dissolved inorganic nutrients, chlorophyll a, and light attenuation affect the survival of seagrasses (Dennison et al., 1993). Batiuk et al. (1992) have set specific levels for five water column constituents that they believe determine whether or not seagrasses will survive (see Table 3). The water column parameters are used as indicators to ensure sufficient light reaches the plants. The criteria were developed in the Chesapeake Bay and may not be entirely applicable to other geographic areas. However, because no water quality criteria have been established for the Great Bay, the criteria established by Batiuk et al. (1992) have been incorporated into the PTSI. Sites that do not meet at least three of the water quality parameters will be dropped from further consideration in the PTSI.

Visual surveys should be used to determine to what extent bioturbating organisms inhabit a site. Bioturbation has been shown to be one of the primary factors causing transplant failure (Davis et al. 1998, Fonseca et al. 1998). The burrowing/foraging behavior of certain species, such as green crabs (Carcinus maenas) and horseshoe crabs (Limulus polyphemus) can destroy transplanted eelgrass, and crab densities greater than 4.0 crabs/ $m^2$ , have been shown to significantly reduce survival of transplanted eelgrass (Davis et al. 1998). In addition to the visual surveys, six 9.0 cm diameter benthic cores should be collected from each site and sieved using a 1.0 mm mesh to quantify the number of clam worms (Neanthes virens) that inhabit the site. Research has shown that these organisms are a significant source of bioturbation when they occur in densities greater than one per core (see Chapter 5). Sites are assigned values for bioturbation of  $0$ (present), 1 (potential), or 2 (not present) based on the results of our field assessments. More precise criteria can be specified for each of the values if data are available. For example, based on the research conducting in Great Bay, New Hampshire, site(s) are assigned a value of zero when green crab densities are  $\geq 4.0$  per m<sup>2</sup> and/or N. virens densities are  $\geq 1$  per core; a value of 1 when crab densities are from 1.0 - 3.8 per m<sup>2</sup>, and/or  $N$ . virens densities are from 0.5-0.8 per core; and a value of 2 when crab densities are <1.0 per  $m^2$ , and/or *N. virens* densities are < 0.5 per core. The density at which

bioturbating organisms can reduce transplant survival should be developed for the bioturbating organism in other areas (e.g., spider crab densities in Rhode Island) to make full use of this parameter. However, even without knowing specific densities, the bioturbation parameter can still lower the overall PTSI score and more importantly, the surveys provide critical information as to whether bioturbating organisms are present and protective measures will need to be taken if the site(s) is planted.

At the same time as the cores for assessing N, virens densities are collected. additional cores should be taken for determination of sand/silt/clay ratios at the potential transplanting sites and existing eelgrass beds. Transplanted eelgrass tends to grow faster and have better survival in sediments that are cobble free and are predominantly sandy (Fonseca and Kenworthy 1977, Short 1987, and Chapter 3).

The results of the field survey and the GIS information are used to calculate the PTSI based on the physical and biological site characteristics listed in Table 15. Values for each parameter are added to calculate the PTSI for each site, with a maximum possible value of fourteen  $(14)$ . Areas with a PTSI greater than seven  $(7)$  are selected for test transplanting and additional field measurements. Sites with a PTSI of less than seven (7) are discouraged, but can be selected for test transplanting if there is not sufficient area at the higher rated sites to meet project acreage goals.

## **Field Measurements and Test-Transplanting**

To ensure that only those sites with the highest probability of success are selected for full-scale restoration, test transplanting eelgrass should occur at the sites with high PTSI values. Essentially these sites are potential eelgrass habitat that showed no signs of eutrophication or bioturbation during the field surveys and with suitable sediment texture and water quality. Determination of the TSI to select the final transplanting sites for fullscale restoration is based on obtaining site-specific light data and quantifying the survival, growth and C:N ratio of the test transplants from those sites identified by the PTSI.

Test transplanting should occur at a number of the possible restoration sites. Preferably, there should be two to three times as many test sites as there will be full-scale restoration sites to allow for excluding sites that do not meet criteria. At each test site, a sufficiently large number of shoots needs to be transplanted to maintain a sufficient sample size. Based on previous experience, the mean percent survival of transplanted seagrass is approximately 42% (Thom, 1990; Davis and Short, 1997; Fonseca et al., 1998). A minimum of 400 shoots is recommended for transplanting at each test site for determination of percent survival.

Numerous methods exist for transplanting eelgrass (see review in Chapter 2 and Fonseca et al. 1998), but the preferred methods are the horizontal rhizome method (HRM) and the "Transplanting Eelgrass Remotely with Frame Systems" (TERFS). The HRM is described fully in Chapter 2 and in Davis and Short (1997). The TERFS method is a new transplanting technique that allows for eelgrass to be transplanted at a known density and location without the use of divers. The technique was developed and field tested at the University of New Hampshire and provides an excellent method of eelgrass restoration that is relatively low-cost because it avoids intensive hand planting using SCUBA. TERFS are 0.6 m by 0.6 m wire frames with twenty-five planting units (two shoots per planting unit) each. The planting units are held in place on the wire frame by ties made of paper. The ties disintegrate within two weeks so that the frames can be removed, leaving the transplants in place. TERFS are weighted to prevent movement due to wave energy or currents. The frames are lowered over the side of the boat and released approximately 2-3 feet above the bottom. In deep areas, ropes are passed through the wire frame to enable lowering of the frame closer to the bottom before releasing. In areas with lower water clarity, snorkeling may be required to ensure the TERFS are properly set. TERFS have been successfully deployed in areas of the Great Bay, New Hampshire and in New Bedford Harbor, Massachusetts with transplant survival averaging 76% (unpublished data, F.T. Short, personnel communication).

Four TERFS should be deployed at each test site and in the nearby naturally occurring eelgrass beds early in the growing season (April-May). Eelgrass shoots are tethered to the center of the frame so that their roots and rhizomes are embedded in the sediment when the TERFS are installed. Additionally, ten shoots are tethered to the edge of the frame so that the roots and rhizomes are elevated approximately 10 cm above the sediment surface. These latter plants grow entirely in the water column in a hydroponic fashion and are used as a biological integrator to assess water quality. In addition to the eelgrass transplanted with TERFS, an additional 200 eelgrass shoots (100 planting units) should be transplanted directly into the sediment on 0.25 meter intervals using the HRM. The geographic location of each test transplant site should be recorded with a hand-held Global Positioning System (GPS) and added to the GIS database.

The source of the donor plants can affect the survival and growth of the transplants (Carlson, 1997; van Katwijk et al., 1998). Although not directly part of calculating the indices, the selection of the donor site is a critical step (Figure 28). Eelgrass plants that have a wasting disease index greater than 40% (Burdick et al., 1993) or that are experiencing nutrient stress are not suitable for transplanting. Nutrient stress of possible donor plants is determined by calculating the carbon-to-nitrogen ratio  $(C:\mathbb{N})$  in the leaf tissue. Low C:N ratios indicate nutrient stress (eutrophication) or low light levels (Grice et al. 1996). If at a given site the C:N ratio is lower than the ratio at 99% of the C:N ratios at all potential donor sites (mean - 3\*S.D.), then the site will be excluded as a source of donor plants. Finally, plants from sandier sediments tend to have higher survival and better growth than plants from siltier sediments (Carlson, 1997; Van Katwijk et al., 1998). This pattern is probably related to the larger rhizome structure and correspondingly greater carbohydrate reserves characteristic of eelgrass plants from sandy sediment (Short, 1987).

Once the donor site has been chosen, site specific data must be collected from the potential transplanting sites. An Estuarine Sensor and Profiler (ESP, Short et al., 1993), a device especially configured to record underwater light levels, should be deployed for 3-4

days at the sites to collect light, salinity, temperature, and depth data. Deployments should occur during the spring of the year in which the test transplanting is to occur. Light levels during spring, when eelgrass emerges from winter dormancy, have been shown to be a critical determinant of the long-term survival of eelgrass (Moore et al. 1997). One ESP meter should be placed at a naturally occurring eelgrass bed in close proximity to the potential transplanting sites (in an opening such that it will not be shaded by the plants), and another moved among potential transplanting sites on 3-4 day intervals. This approach is a relatively quick, reliable method of determining the amount of light reaching the bottom at the potential transplant sites relative to the natural eelgrass site (Koch and Beer, 1996 and Chapter 3). The existence of eelgrass (at the naturally occurring site) demonstrates that sufficient light reaches the bottom to support the plants. Therefore, if light levels at potential transplant sites equal or exceed those at the naturally occurring site, when measured under the same environmental conditions, light levels will likely be sufficient to support transplants. Of course, at other times of year the light may vary, but the above method has been proven via longer-term light levels measured in the Piscataqua River, NH, to be a good general indicator of light regime suitability (Short et al., 1993).

After three to four weeks, the hydroponic shoots and 10 shoots rooted in the sediment at each site must be collected and processed to determine shoot growth (Short, 1987) and C:N ratios. Survival rates must also be determined by quantifying the number of shoots remaining of the 400 originally transplanted at each site. Short-term survival rates are important but are not as indicative of the long-term potential of the site to support eelgrass as over-wintering survival rates (Davis and Short, 1997). Carbon and nitrogen ratios are obtained from laboratory analysis of only the hydroponic eelgrass shoots to determine the level of water-borne nutrient pollution affecting the site (Short, In prep). Rhizome growth, production of new lateral shoots, and presence/absence of root-rot in the transplants should be qualitatively assessed to determine if the eelgrass is adversely affected by the sediment characteristics of any site.

## **Transplant Suitability Index**

The TSI is calculated for each test transplant site based on data obtained from the test transplanting and the PTSI (Table 16). Specifically, the additional data used to calculate the TSI are light availability, percent survival of test transplants, growth rates of the transplants compared to naturally occurring eelgrass, and the results of the C:N analysis (Table 16). For example: The TSI value for growth is assigned a value of zero (0) if the leaf growth for transplanted shoot (cm/cm/day) is less than the mean leaf growth of shoots in the natural bed minus two standard deviations. This indicates that the transplanted shoots are growing slower than 95% of the naturally occurring eelgrass shoots. The TSI value for growth is one (1) if the leaf growth for the transplanted shoots is equal to the mean leaf growth of the shoots in the natural bed plus or minus two standard deviations. The mean and standard deviations are based on measurements of shoots naturally growing in the nearest existing eelgrass bed. Eelgrass shoots are also placed at the existing beds using TERFS (i.e., hydroponically) and collected after three to four weeks for determination of C:N ratios for direct comparison with the values obtained from the test sites.

The TSI is calculated as follows:

 $TSI = PTSI * percent survival * growth * C:N * light$ 

Because each parameter in the formula is a multiplier, the TSI would go to zero and cause a site to be rejected if a test site did not have sufficient light, the transplants grew poorly or had low survival, or the C:N ratio indicated severe nutrient stress. The maximum possible TSI value is eight (8). Sites with TSI values of zero will be rejected from further consideration. Sites with TSI values of four (4) or greater can be selected as priority sites for full-scale restoration.

## Applying the PTSI/TSI to Eelgrass Transplant Sites in the Great Bay Estuary, NH

As a first test to validate the PTSI/TSI methodology, the indices were calculated post priori for the New Hampshire Port Authority eelgrass mitigation sites. Data not collected directly at the eelgrass transplant and nearby control sites included exposure (for the PTSI), C:N ratios (for the TSI) and growth rates (for the TSI). Mean exposure was



estimated from the fetch calculated for each site using USGS topographic maps. Three fetch measurements were used to obtain a mean and standard deviation: the greatest fetch, northeast fetch, and northwest fetch (Table 17). The latter two directions were chosen because those are the headings from which the strongest storms approach in this region. Growth rates for transplant sites were estimated from the results of the mesocosm experiments described in Chapter 3 and assigned to sites with the most similar sediment characteristics as those used in the mesocosm experiments (Table 17). Growth rates in the natural eelgrass beds were obtained after Short et al. (1993). The values for exposure. bathymetry, proximity to natural eelgrass beds, and growth rates used to calculate the PTSI are shown in Table 17. The source for all other data used to calculate the PTSI/TSI are indicated in the first column of Table 18 and 19.

All of the NHPA transplant sites received relatively high PTSI scores, with values ranging from 10 to 13 (Table 18). This result is not surprising since all sites underwent a screening process in 1992 prior to being selected as transplant sites in 1993. Most importantly all sites had historically supported eelgrass and had sufficient light to support eelgrass. Site T5 did receive the lowest PTSI due to its siltier sediment and the presence of bioturbating organisms. At this point in the site selection process, site T5 could be excluded from further consideration if there are no funds for constructing protective devices for the transplants. For the NHPA project, construction of protective caging material had been incorporated into the time and cost estimates at the initiation of the project. Therefore transplanting did occur at T5.

The final TSI was calculated based on the results of the actual transplanting project (Table 19). Overwintering survival at sites T4 and T5 were low (Table 1) and both sites received a score of zero (0) for the percent survival parameter (Table 19). Therefore, because parameters in the TSI are multiplied, both sites received a final TSI of zero (0). According to the methodology, sites T4 and T5 should not have been selected for full scale transplanting. In reality, a different area at T4 in close proximity to the original site that scored a TSI of zero was planted in 1994. The transplants at this portion of T4 had


Table 17. Values for specific data layers used to calculate the PTSI/TSI. The source of the data are given beneath the Growth rates for the control site are the mean of growth rates for naturally occurring eelgrass populations reported in data layer name. Exposure values for all sites are the mean of the largest fetch, northeast fetch, and northwest fetch. Short et al., 1993. Depth is the mean of the depths at the shallow and deep edges of the site referenced to mean low water.

126



Table 18. Calculation of the Preliminary Transplant Suitability Index (PTSI) for New Hampshire Port Authority<br>colorase mitiastion sites



 $\ddot{\phantom{0}}$ 



much higher survival rates and have persisted for four years (unpublished data). Similarly, small patches of eelgrass were eventually established in 1994 and 1995 at T5 by using different protective measures than those that were originally used for the 1993 transplants. The original (1993) transplants at T5 were lost due to bioturbation by clam worms (Davis and Short, In review; Chapter 5), but the protective cages were designed to prevent crab bioturbation.

Sites T1, T2, and T3 each received a final TSI of six (6). The eelgrass transplanted in 1993 at T1, T2 and T3 continued to grow and expand for at least two years after transplanting (Figures 3 and 4). By the end of the third year (1996), T1 and T3 supported healthy eelgrass beds, but the eelgrass at T2 had disappeared (unpublished data). The intertidal transplants at T2 were lost due to ice scouring in winter 1993/1994 (Davis and Short, 1997; Chapter 2). The subtidal transplants may have been lost due to bioturbation. The subtidal transplants were present at T2 through the August 1995 monitoring period (Figures 3 and 4). The protective caging (Davis et al., 1998; Chapter 6) that had been installed at this site was removed in the summer of 1995. By the time of the sampling in 1996, no eelgrass remained at T2.

#### **Field Testing to Validate the PTSI/TSI Methodology**

The next step for the development of the PTSI/TSI will to field test the method prior to initiation of a large-scale transplanting project. Currently the indices are being applied and refined in New Bedford Harbor, Massachusetts where 1.6 hectares of eelgrass transplanting is proposed (Short and Burdick, 1999). The test transplanting phase of this project was completed in the summer of 1998. An additional test of the indices is being conducted in the lower Chesapeake Bay. Test transplants were installed on the Back River, adjacent to Langley Air Force Base in the fall of 1998. The results of the test transplanting will be used to calculate the final TSI for three potential full scale transplanting sites. The PTSI/TSI will also be applied at the Little Creek Naval Amphibious Base in Norfolk, Virginia beginning in the fall of 1999. These latter two field tests are important because they are located near the southern limit of eelgrass

distribution on the east coast of the United States (Short et al., 1993). The results of these three tests will provide field validation of the PTSI/TSI methodology.

The PTSI/TSI methodology improves upon previous transplant site selection methodologies by incorporating physical and biological site characteristics into the site selection process. From the initial field tests it appears that the methodology is very effective for separating potential transplanting sites from those with poor potential (unpublished data). Based on the application of the model to the NHPA data, the indices may need to be further refined to better delineate between sites with relatively high potential for transplanting. For example, T1, T2, and T3 received the same final TSI score, yet only sites  $T1$  and  $T3$  were successful over a longer ( $>4$  year) period. Further quantification of specific parameters (e.g., assigning values from 0-4 for light, instead of only 0 or 1) may improve the ability of the TSI to better delineate transplant sites. It should be noted though, that the eventual loss of the transplants at T2 may not have been related to transplanting. The transplants did survive and expand at T2 for over two years (Figures 3 and 4). Currently, there is no defined time period during which transplanted seagrasses should be assessed using transplanting criteria, and when they should be assessed as natural occurring populations (Fonseca et al., 1998; Short et al., In press). It will be necessary to develop additional metrics for predicting how transplanted eelgrass will respond (e.g., functional trajectories after Simenstad and Thom, 1996) three to four years after it has been transplanted.

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**APPENDIX A** 

 $\Delta \sim 10^{11}$  mass  $^{-1}$ 

# **ORIGINAL DATA**

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#### **CURRENT MEASUREMENTS (cm/sec)**



# Current Measurements (cm/sec) taken at selected sites during Spring Tides<br>7/23/97 at Simplex - 1m off bottom 8/20/97 at DFN - 1m off bottom<br>Time Min velocity Max velocity Ave veloticy Time Min Velocity Max



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# NHPA EELGRASS SHOOT MEASUREMENTS IN 1993



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# NHPA EELGRASS SHOOT MEASUREMENTS IN 1994



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# NHPA EELGRASS SHOOT MEASUREMENTS IN 1995



149

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# SEDIMENT GRAIN SIZE ANALYSIS - MESOCOSM EXPERIMENT (1/2 MUD, 1/2 SAND)





150

# SEDIMENT GRAIN SIZE ANALYSIS - MESOCOSM EXPERIMENT (MUD)





DATE FMPTY AL DISH WEIGHED-

# SEDIMENT GRAIN SIZE ANALYSIS - MESOCOSM EXPERIMENT (SAND)





152

# SEDIMENT GRAIN SIZE ANALYSIS - NHPA FIELD SITES



## WATER QUALITY DATA (SURFACE WATER) FOR DETERMINATION OF TSS NUTRIENTS AND CHLOROPHYLL A

Adams Point, Great Bay. Values for High (H) and Low (L) tides. average of 2 reps.



# WATER QUALITY DATA (SURFACE WATER) FOR DETERMINATION OF TSS NUTRIENTS AND CHLOROPHYLL A

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#### ABOVEGROUND GROWTH RATES FOR EELGRASS TRANSPLANTED INTO DIFFERENT SEDIMENT TYPES IN MESOCOSM EXPERIMENTS



# **SEDIMENT TYPES IN MESOCOSM EXPERIMENTS**<br>Tank Method Mean phi Sediment Rep. mg/sht/da

#### ABOVEGROUND GROWTH RATES FOR EELGRASS TRANSPLANTED INTO DIFFERENT SEDIMENT TYPES IN MESOCOSM EXPERIMENTS

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#### ABOVEGROUND GROWTH RATES FOR EELGRASS TRANSPLANTED INTO DIFFERENT SEDIMENT TYPES IN MESOCOSM EXPERIMENTS



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#### BELOWGROUND GROWTH RATES OF EELGRASS TRANSPLANTED INTO DIFFERENT SEDIMENTS USING TWO DIFFERENT TRANSPLANTING TECHNIQUES



# LIGHT AVAILABILITY MEASURED AT SELECT TRANSPLANT AND CONTROL SITES



# LIGHT AVAILABILITY MEASURED AT SELECT TRANSPLANT AND CONTROL SITES

 $\Box$ 


## LIGHT AVAILABILITY MEASURED AT SELECT TRANSPLANT AND CONTROL SITES



#### LIGHT AVAILABILITY MEASURED AT SELECT TRANSPLANT AND CONTROL SITES



## LIGHT AVAILABILITY MEASURED AT SELECT TRANSPLANT AND CONTROL SITES



# MEAN Kd (8am-4pm) FOR TRANSPLANT AND CONTROL SITES IN 1996



Endeco 108 was always at Adlington Creek (C1)



# **BENTHIC INFAUNA DATA - 1993** VALUES ARE TOTAL ABUNDANCE FROM 6 CORES



#### **BENTHIC INFAUNA DATA - 1994** VALUES ARE TOTAL ABUNDANCE FROM 6 CORES



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 $172$ 

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174

 $\mathbb{Z}^2$ 



175



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#### CRAB MESOCOSM EXPERIMENT DATA - NUMBER OF SHOOTS/BLADES CUT



#### CRAB MESOCOSM EXPERIMENT DATA - NUMBER OF SHOOTS/BLADES CUT



178

## CRAB MESOCOSM EXPERIMENT DATA - NUMBER OF SHOOTS/BLADES CUT



# FETCH CALCULATIONS FOR PTSI VALUES OBTAINED FROM USGS TOPO QUAD







**AS** 

€

G. W



IMAGE EVALUATION<br>TEST TARGET (QA-3)







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