RETURN TO SER AR INDEX

From: Minkin, Paul NAE
To: marsh.mike@epamail.epa.gov
Cc: Sneeringer, Paul J NAE
Subject: salt marsh sampling (UNCLASSIFIED)
Date: Wednesday, November 07, 2012 9:26:17 AM

Classification: UNCLASSIFIED
Caveats: NONE

Mike,

This article seems an appropriate source and citation for restoring salt marsh vegetation and monitoring its success:
http://www.masgc.org/gulfhydrorestoration/documents/13MonitoringSaltMarshVegetationRomanetal2001.pdf

I still recommend that for mitigation monitoring they use percent aerial cover for the overall site. However, if they wish to use something more formal, the procedures outlined in this document, i.e., using the point intercept method in a minimum of 40 1-meter squared plots along 4 transects across the site, is appropriate.

Let me know if you have any questions.

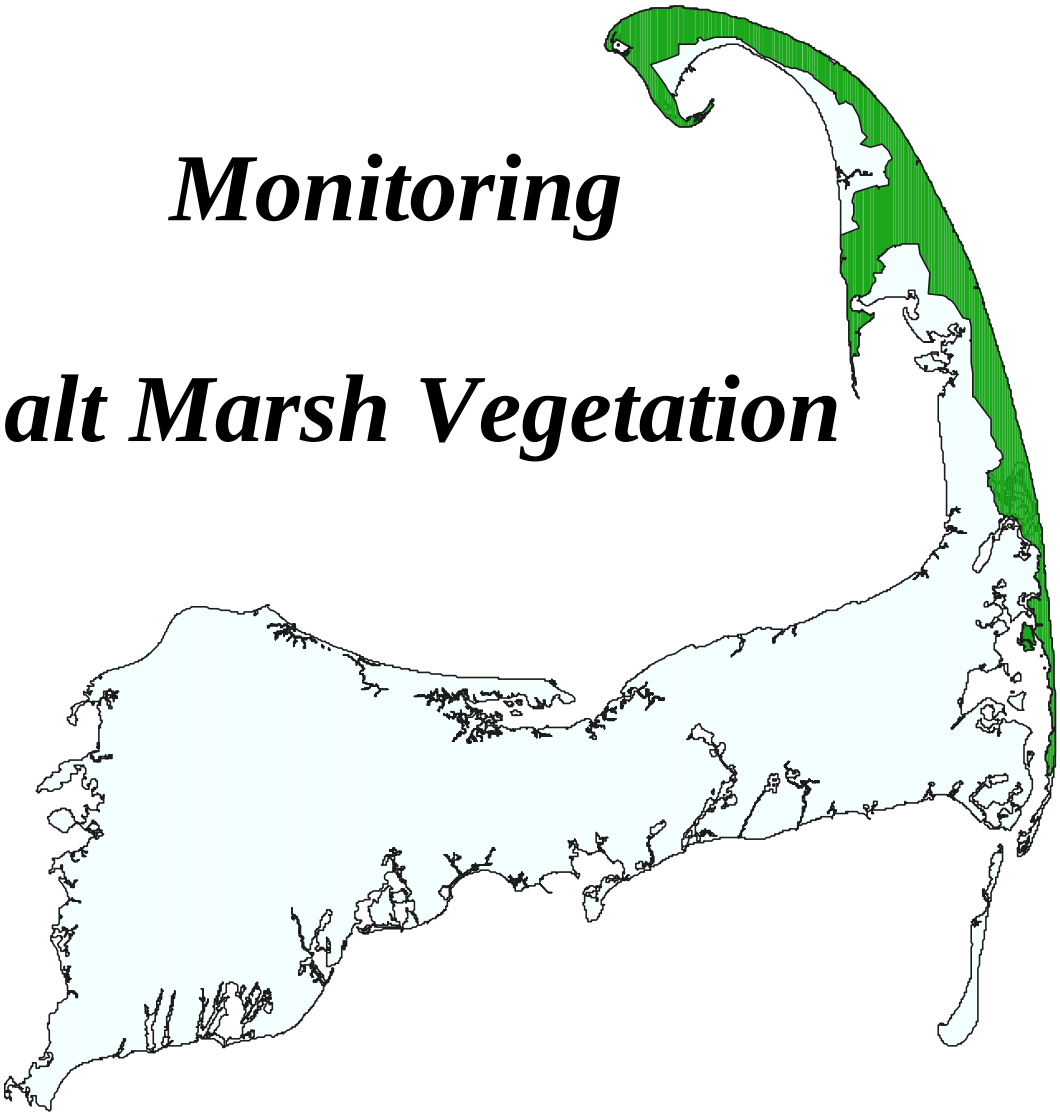
Paul

Classification: UNCLASSIFIED
Caveats: NONE

SDMS Doc ID 529008

Monitoring

Salt Marsh Vegetation



USGS Patuxent Wildlife Research Center

Cape Cod National Seashore

MONITORING SALT MARSH VEGETATION

A Protocol for the Long-term Coastal Ecosystem Monitoring Program
at Cape Cod National Seashore

Charles T. Roman
National Park Service
Graduate School of Oceanography
University of Rhode Island
Narragansett, RI 02882
croman@gso.uri.edu

Mary-Jane James-Pirri
Graduate School of Oceanography
University of Rhode Island
Narragansett, RI 02882

James F. Heltshe
Department of Computer Science and Statistics
University of Rhode Island
Kingston, RI 02881

December, 2001

Long-term Coastal Ecosystem Monitoring Program
Cape Cod National Seashore
Wellfleet, MA 02667

This report is on the National Park Service Inventory and Monitoring website:
http://www.nature.nps.gov/im/monitor/protocoldb.cfm

PREFACE

Overview of Long-term Monitoring Program

Cape Cod National Seashore serves as a National Park Service prototype monitoring park for the Atlantic and Gulf Coast biogeographic region. The USGS, in cooperation with the National Park Service, is charged with designing and testing monitoring protocols for implementation at Cape Cod National Seashore. It is expected that many of the protocols will have direct application at other Seashore parks, as well as US Fish and Wildlife Service coastal refuges, within the biogeographic region.

The Long-term Coastal Ecosystem Monitoring Program at Cape Cod National Seashore is composed of numerous protocols that are relevant to the major ecosystem types (Estuaries and Salt Marshes, Barrier Islands/Spits/Dunes, Ponds and Freshwater Wetlands, Coastal Uplands). The salt marsh vegetation protocol is associated with the Estuaries and Salt Marshes component of the monitoring program. Other protocols being developed within the Estuaries and Salt Marsh component are related to nutrient enrichment, nekton, waterbirds, marsh development processes, and sediment contaminants. The overall program is designed so that all of the protocols are interrelated. For example, information acquired from the nutrient enrichment protocol or marsh development protocol may be especially relevant to interpreting observed trends in salt marsh vegetation. Roman and Barrett (1999) present a conceptual description of the entire monitoring program.

Protocol Organization

To maintain some consistency among the various monitoring protocols, each protocol is organized as follows. PART ONE of the protocol is intended to provide detail on the objectives of the monitoring protocol and to provide justification for the recommended sampling program. Incorporation of relevant literature and presentation of data collected during the protocol development phase of the project are used to justify a particular sampling design, sampling method, or data analysis technique.

PART TWO is a step-by-step description of the field, laboratory, data analysis, and data management aspects of the protocol. For example, PART TWO may simply state that vegetation sampling is conducted with a $1m^2$ quadrat using a point-intercept method. PART ONE provided a detailed justification as to why the point-intercept method was selected. There is some redundancy between Part One and Two, because each part is intended as a stand-alone document.

EXECUTIVE SUMMARY

Salt marsh ecosystems provide habitat for a variety of species including recreational and commercial fishes, forage species, migratory shorebirds and waterbirds, as well as acting as erosion buffers and filters of nutrient inputs by intercepting and absorbing land derived runoff. A large percentage of the nations salt marshes have been altered, degraded, and lost over the past century. Restoration of salt marsh habitat has recently become a management tool to rectify past environmental change. To determine if restoration activities are effective, standardized protocols must be developed. This study develops a protocol for monitoring salt marsh vegetation for use in the Long-term Coastal Monitoring Program at Cape Cod National Seashore. We recommend sampling salt marsh vegetation by the point intercept method with at least 20 replicate $1m^2$ permanent plots per marsh area. Other aspects of vegetation sampling are discussed, including seasonal sampling considerations, transect and plot location, recommended sample size, and associated environmental data sampling. Developing and initiating long-term salt marsh monitoring programs will help track natural and human-induced changes in salt marshes over time and advance our understanding of the interactions between marsh ecosystems and the estuarine environment.

TABLE OF CONTENTS

PREFACE iii

EXECUTIVE SUMMARY iv

TABLE OF CONTENTS v

LIST OF FIGURES AND TABLES vi

ACKNOWLEDGEMENTS vii

PART ONE (Background and Justification for Protocol)
INTRODUCTION..... 1
MONITORING QUESTIONS 2
Habitat Restoration 2
Long-term or Large Scale Changes 4
SAMPLING METHODS..... 5
Site Selection 5
Sampling Unit (Permanent Quadrats) 7
Spatial Sampling Frequency..... 9
Sample Design 9
Sample Size: How many quadrats? 10
Temporal Sampling Frequency..... 14
Data Collected for Each Sample Quadrat..... 15
Associated Environmental Data 17
DATA ANALYSIS TECHNIQUES..... 18

PART TWO (The Vegetation Monitoring Protocol)
SUMMARY..... 24
PROTOCOL 25
Site Selection..... 25
Sample Location 25
Segmentation of the Marsh 25
Transect Layout 25
Permanent Plots along Transects 26
Marking Permanent Stakes 26
Sampling Gear and Field Methods..... 28
Point Intercept Method 28
Associated Environmental Variables 32
Water Table Level..... 32
Materials 32
Groundwater Well Fabrication 32
Well Installation..... 33
Sampling Procedure 34
Soil Water Salinity..... 35

Materials 35
Soil Probe Fabrication 35
Sampling Procedure 36
Soil Sulfides 38
Materials 38
Sampling Procedure 38
Data Management 39
Data Analysis Techniques 40
Equipment List 41
Personnel 41

LITERATURE CITED 42

LIST OF FIGURES

1. Agents of change and ecosystem responses 3
2. Map of Hatches Harbor salt marsh 6
3. Species area curves for different sized quadrats 8
4. Hypothetical marsh illustrating the arrangement of transects and plots 11
5. Power curve of the number of samples required per marsh 13
6. Photo of point intercept method 16
7. Height of *Phragmites australis* before and after restoration 18
8a. Detrended Correspondence Analysis (DCA) plot of Hatches Harbor vegetation quadrats 22
8b. Time-trajectory lines for individual vegetation plots in the tide-restricted/ tide-restored marsh from 1997 to 2000 22
9. Hypothetical marsh illustrating the arrangement of transects and plots 27
10. Diagram of quadrat orientation relative to plot stake 28
11. Diagram of the sample quadrat and arrangement of rods 29
12. Diagram of location of groundwater wells 33
13. Diagram of water table well 34
14. Photo of soil salinity probe 36

LIST OF TABLES

1. ANOSIM results for Hatches Harbor vegetation analysis 20
2. Dissimilarity results for Hatches Harbor vegetation analysis 21
3. Summary of salt marsh vegetation protocol 24
4. Example data sheet for the point intercept method 30
5. Cover type categories for point-intercept method 31
6. Example data sheet for water table and soil salinity measurements 37
7. Example of spreadsheet for vegetation entry 39

ACKNOWLEDGEMENTS

Development of this protocol was supported by the USGS-Biological Resources Division, with funds administered by the USGS Patuxent Wildlife Research Center at the University of Rhode Island. Special thanks are extended to the Superintendent and natural resource management staff at Cape Cod National Seashore for providing logistical support. Evan Gwilliam and Kelly Chapman are thanked for their dedicated assistance with data collection. Nels Barret is gratefully acknowledged for his many contributions to early drafts of this protocol.

PART ONE
Background and Justification for the Vegetation Monitoring Protocol

INTRODUCTION

Cape Cod salt marshes, and those elsewhere throughout the northeast, provide essential nursery habitat for recreational and commercial fishery species (Nixon and Oviatt 1973a, Able *et al.* 1988, Heck *et al.* 1989 and 1995, Ayvazian *et al.* 1992) and are an especially important habitat for forage species (Roman *et al.* 2000). The role of salt marshes in supporting migratory shorebird and waterbird populations is well-documented (*e.g.*, Burger *et al.* 1982, Brush *et al.* 1986). Salt marshes may also serve as nutrient filters, intercepting and absorbing land-derived runoff, thereby reducing nutrient input to estuarine and coastal waters (*e.g.*, Howes *et al.* 1996). Physically, salt marshes can buffer upland areas from erosion and storm waves (Dean 1979).

An estimated fifty percent of the nation's coastal wetlands have been completely lost, mostly by filling and dredging activities (Dahl 1990, Tiner 1984). Salt marshes that remain often have a long history of alteration from extensive networks of ditching for mosquito control or salt hay farming purposes, from restriction of tidal exchange by roads, causeways, bridges, and dikes, and from widespread watershed development activities (Daiber 1986, Roman *et al.* 2000). The plant species composition of salt marshes dramatically changes in response to ditching activities (*e.g.*, Bourn and Cottam 1950, Niering and Warren 1980) and restriction of tidal flow (*e.g.*, Roman *et al.* 1984, 1995). With ditching, the marsh may become drier and less salt- or flood-tolerant species may dominate (*e.g.*, *Iva frutescens* and high marsh species), while restriction of tidal flow often results in conversion of *Spartina*-dominated to *Phragmites australis*-dominated marshes. Conversely, re-establishment of hydrologic conditions that were altered by ditching or tidal restriction often initiates a change or recovery back to typical marsh vegetation (Burdick *et al.* 1997).

Sea-level rise also influences salt marsh vegetation. The rise in sea level along the Atlantic coast is estimated to increase by 0.5m by 2100 (Intergovernmental Panel on Climate Change, 1995). Changes in vegetation or the conversion of marsh to mudflats or open water may result (Titus 1991). Salt marshes in New England appear to be keeping pace with sea level rise, but at some locations recent studies have documented vegetation changes indicating that the marshes are getting wetter and tending toward submergence or drowning (Warren and Niering 1993, Roman *et al.* 1997).

Sea level rise is a global climate change phenomenon. Other factors related to climate change can also affect salt marsh vegetation. For example, with increased air temperatures, evaporation will accelerate leading to an increase in marsh salinities, perhaps resulting in the expansion of extreme salt tolerant halophytes and unvegetated marsh pannes. At present, salt marshes in more southern latitudes (*e.g.*, southeast Atlantic), with warmer climates, generally have a greater occurrence of halophytes adapted to extremely high soil salinity conditions (Bertness 1999).

Increased loading of nutrients to salt marshes can also cause vegetation changes. With nutrient enrichment of the coastal zone it is expected that primary production of marsh plants will increase and vegetation patterns may be influenced. Nixon and Oviatt (1973b), sampling along a nutrient gradient in Narragansett Bay, found that *Spartina alterniflora* production was substantially greater in high nutrient areas of the Bay compared to the lesser-developed and low nutrient sites.

Figure 1 shows many of the linkages between human-induced and natural environmental stressors and associated responses of salt marsh plant communities. The salt marsh vegetation monitoring program presented in this report will focus on long-term assessment of plant species composition and abundance, including tracking of invasive, non-native, and rare species. The overall Long-term Coastal Ecosystem Monitoring Program at Cape Cod National Seashore is designed to not only monitor ecosystem responses to natural and human-induced stressors, but to also monitor key stressors in order to acquire an understanding of why the ecosystem is changing (Roman and Barrett 1999). Regarding an evaluation of stressors related to salt marsh vegetation change (as depicted in Fig. 1), other protocols are focused on estuarine nutrient enrichment monitoring, shoreline change/geomorphic monitoring, and hydrologic monitoring of estuarine tidal restriction and tidal restoration. It is also noted that information derived from monitoring changes in salt marsh vegetation patterns will be especially useful to interpreting any observed long-term changes in salt marsh nekton and bird communities.

MONITORING QUESTIONS

Habitat Restoration

Salt marsh restoration is a major resource management goal at Cape Cod National Seashore (Godfrey *et al.* 1999). Dikes have restricted normal tidal flow to several Seashore salt marshes for many decades (Roman *et al.* 1995, Portnoy and Reynolds 1997). These systems include Hatches Harbor (Provincetown), Pilgrim Lake (Provincetown and Truro), Pamet River (Truro), and Herring River (Wellfleet). After 7 decades of tidal restriction, hydrology is being restored to the Hatches Harbor salt marsh and investigations are underway to evaluate the feasibility of restoring tidal flow at the other systems. Monitoring the species composition and abundance of salt marsh vegetation provides an excellent indicator of habitat degradation under regimes of tidal restriction, and conversely, an indicator of habitat recovery under tide-restored conditions (*e.g.*, Roman *et al.* 1984, Barrett and Niering 1993, Burdick *et al.* 1997).

Also, and as previously noted, most salt marshes within the Seashore and throughout the northeastern US have been ditched for mosquito control or production of salt hay, with some ditching activities evident on New England marshes since Colonial times. Vegetation changes often resulted from the draining of these ditched marshes (Niering and Warren 1980). Now, the practice of Open Marsh Water Management (OMWM) for mosquito control (Wolfe 1996) and modifications of OMWM are designed to restore marsh hydrology.

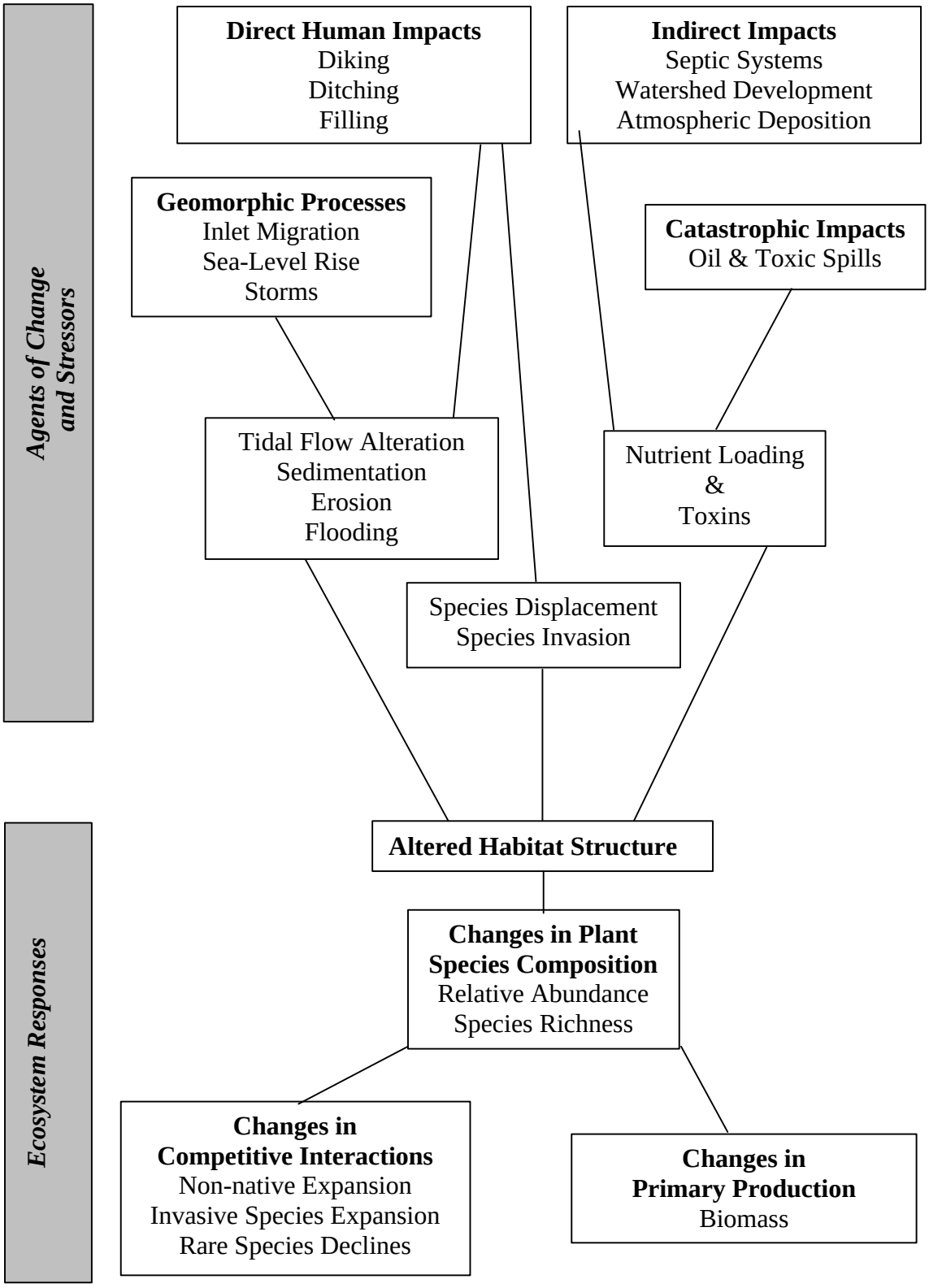


Figure 1. Salt marsh vegetation linkages and responses to agents of change and environmental stressors.

Some specific monitoring questions related to salt marsh restoration initiatives at the Seashore are as follows;

- What is the extent of vegetation difference between the hydrologically impacted (*e.g.*, tidal restriction, ditched) marsh and reference (unimpacted or control marsh) marsh?

- What is the response of salt marsh vegetation to restoration of hydrologic conditions?

  - Is there a reduction in the spatial distribution and growth of the invasive *Phragmites australis* with tidal restoration, and conversely, are typical salt marsh vegetation patterns being re-established?

  - Are the vegetation communities of tide-restored (the marsh undergoing restoration) and reference marshes converging and what is the temporal scale of this convergence?

  - What are the principal factors responsible for observed vegetation changes (*e.g.*, soil salinity, water table level, soil biogeochemistry, *etc*.)?

Long-term or Large-scale Changes

It is also important to monitor the response of salt marsh vegetation to long-term changes in sea level, climate (temperature, precipitation), nutrient loading, and barrier spit and inlet dynamics. To emphasize the potential for vegetation changes in response to these factors, consider one of the Seashore's major salt marsh ecosystems – Nauset Marsh. At this site, the barrier spit fronting the marsh is highly dynamic, with a migrating inlet that dramatically influences hydrologic characteristics of the marsh-dominated estuary (Aubrey and Speer 1985). In response to the inlet dynamics and sea level rise, historic vegetation changes have been documented (Roman *et al.* 1997). It is also noted that nutrient loading, mostly from coastal development served by on-site septic systems, is quite elevated throughout portions of the Nauset Marsh estuary (Portnoy *et al.* 1998). The response of marsh vegetation to fertilization by nutrients is known (Valiela *et al.* 1975), but responses to long-term chronic levels of nutrient enrichment are less well understood.

The following monitoring questions are relevant;

- Are salt marsh vegetation patterns changing over time (*e.g.*, decades)?

- Are the observed changes resulting in an expansion of invasive species, change in the ratio of marsh-to-open water, or shifts from low marsh to high marsh dominated species?
- What factors are contributing to observed vegetation changes (*e.g.*, altered hydrology, nutrients, salinity)?

If long-term vegetation changes are noted, if there is some understanding of the causes of change, and if those changes are determined to be a problem or unacceptable, then resource management actions can be considered.

SAMPLING METHODS

This section of the protocol provides justification and supporting documentation for various aspects of the protocol, including site selection, justification for permanent quadrat sampling, spatial and temporal sampling frequency, justification for inclusion of associated environmental monitoring variables, and recommended data analysis methods.

Site Selection

If the monitoring questions being addressed are related to assessment of a specific impact or impacts, then reference or control areas must accompany the impact area, and moreover, a BACI (before, after, control, impact) or modified BACI study design is recommended (Stewart-Oaten *et al.* 1986, 1992; Underwood 1992). For example, re-introduction of flow to tide-restricted marshes is a high priority management issue at Cape Cod National Seashore. Ongoing research and monitoring efforts at the tide-restored Hatches Harbor salt marsh are utilizing the BACI approach. As noted in Fig. 2, Hatches Harbor is bisected by a dike, constructed in 1930 for mosquito control purposes. Tidal flow from Cape Cod Bay and through a barrier spit and inlet system is unrestricted to the 90-ha portion of salt marsh that is downstream of the dike. Tidal exchange through the dike, and exchange with the 80-ha marsh upstream of the dike, was dramatically limited through a small 0.6-m diameter culvert, until 1999 when the culvert was replaced with multiple larger openings.

With the BACI study design, vegetation monitoring was conducted on the unrestricted (control marsh) and tide-restricted marshes before installation of larger culverts, and then, vegetation was again monitored on the unrestricted and tide-restricted (or now tide-restored) marshes after the new culverts. “After” monitoring was during the second growing season after tidal restoration and is planned for the long-term to track the response of the marsh to tidal restoration. The impact in the BACI design is the restoration of tidal flow. With this kind of design it is possible to compare, with a degree of statistical certainty, the following;

- Control marsh vs. tide-restricted marsh before tidal restoration to document the degree of difference in vegetation.

- Tide-restricted marsh vs. tide-restored marsh to document the response to tidal restoration. Continued monitoring in successive years will track the trajectory of vegetation response.

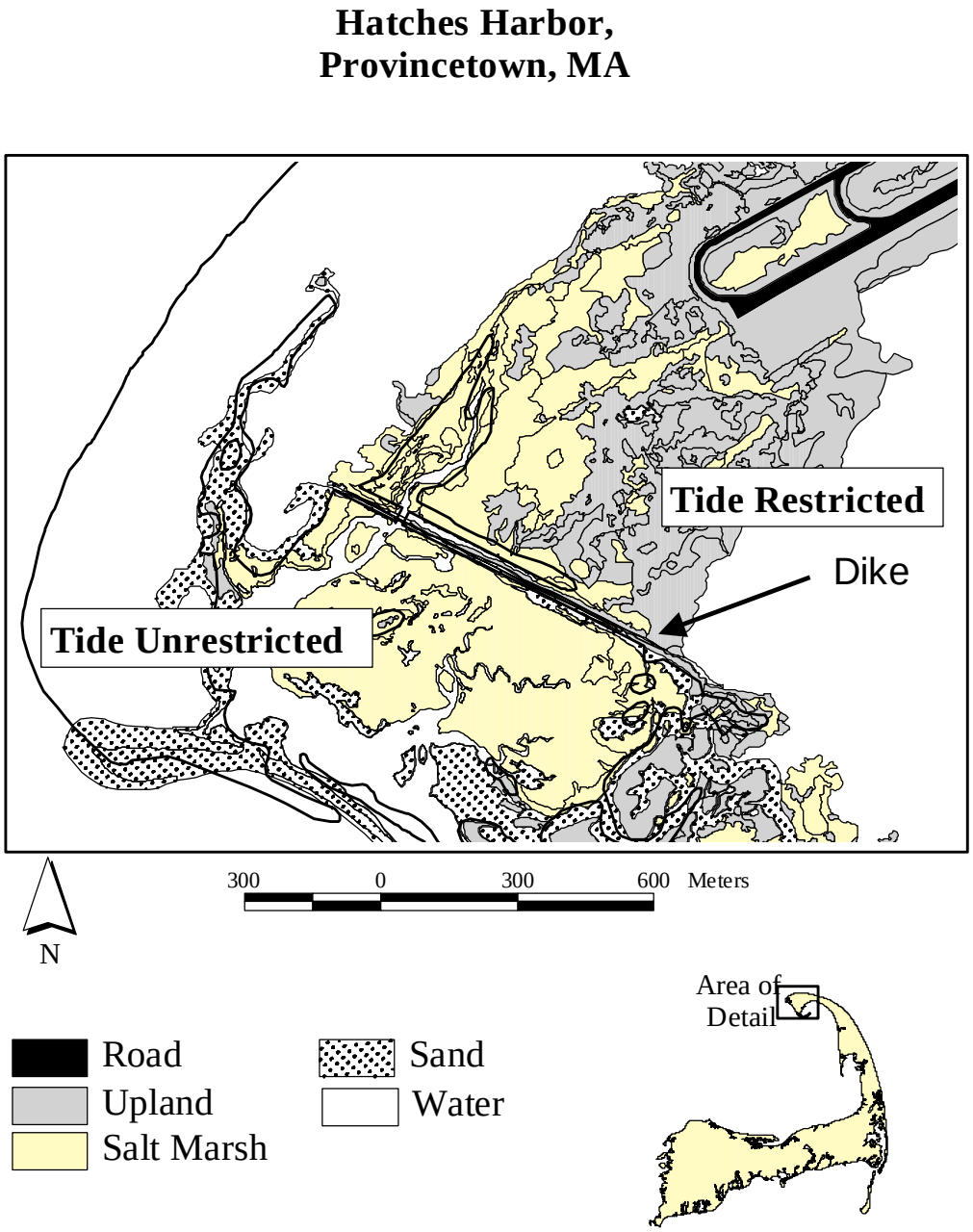


Figure 2. Map of Hatches Harbor salt marsh showing tide restricted and unrestricted portions of the marsh.

- Control marsh before vs. control marsh after tidal restoration. Conceptually, it is important to document vegetation changes of the control marsh over time. If after tidal restoration, vegetation changed within the tide-restored marsh, but the control marsh vegetation did not change, then it could be suggested, with some certainty, that the changes in the tide-restored marsh were due to increased tidal flow and not some other factors.

- It is also possible to monitor "convergence" of vegetation communities by comparing the control vs. tide-restricted, then control vs. tide-restored year 1, control vs. tide-restored year 2, *etc*. It is generally hypothesized that as restoration proceeds, the tide-restored marsh will become more similar to, or converge with, the control marsh.

The BACI study design would also be appropriate if monitoring the response of marsh vegetation to nutrient enrichment, mosquito control practices like ditching or Open Marsh Water Management, and other impacts.

It is often difficult to find appropriate control sites or reference marshes. The control site should not be influenced by the impact that is being assessed and it should be a site that has similar geomorphic/physical features to the impact site (*e.g.*, tidal range, salinity range, wetland type in terms of back-barrier vs. drowned river valley). If a control site can not be located, it would still be valuable to monitor changes in the tide-restricted marsh before and after tidal restoration.

For monitoring the response of salt marsh vegetation to large scale or long-term changes, as discussed above, it may not be appropriate to follow the BACI study design, but rather, to establish some monitoring marshes and sample repeatedly over time. The control marshes established from BACI designs, as well as other sites, such as the Seashore's Nauset Marsh estuary, would serve as appropriate sites for assessing marsh responses to sea level rise, temperature changes, and other long-term factors.

Sampling Unit (Permanent Quadrats)

Quadrats are clearly the most common type of sampling unit for grassland communities, like salt marshes (Kent and Coker 1992, Elzinga *et al.* 1998). Species area curves, from data collected at the Hatches Harbor marsh restoration site, suggest that a square $1m^2$ quadrat is appropriate (Fig. 3). As noted for the typical salt marsh habitat, like the tide-unrestricted portion of Hatches Harbor, few species occur (5 species maximum within a quadrat, and often just 2 or 3) and a quadrat size of $1m^2$ is more than adequate. In fact, a $0.5m^2$ quadrat would be appropriate (*i.e.*, increasing the quadrat size beyond $0.5m^2$ does not result in an increased number of species being recorded). However, as the vegetation community becomes more complex (up to 15 species per quadrat), like on the tide-restricted portion of Hatches Harbor, a $1m^2$ quadrat size may be required. We plotted species area curves for 20 randomly selected tide-restricted marsh plots and determined

that for 80% of the sampled quadrats the species area curves leveled-off or reached a plateau, suggesting that $1m^2$ was appropriate (Fig. 3). To further confirm that a $1m^2$

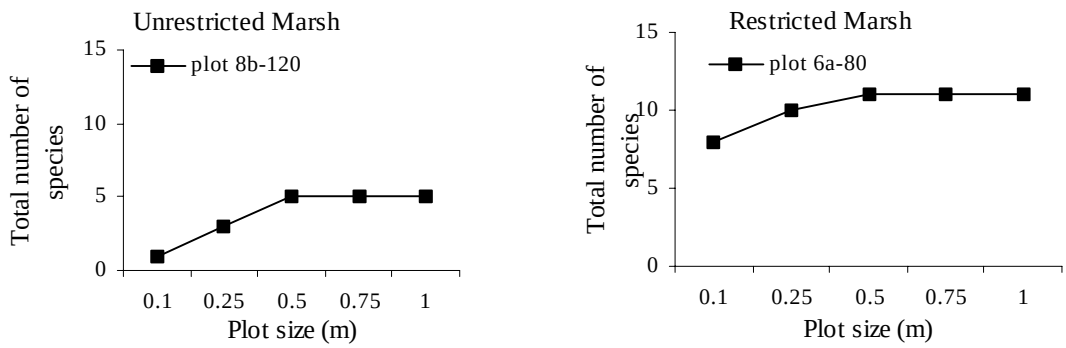


Figure 3. Species area curves of selected plots from unrestricted and tide-restricted portions of Hatches Harbor salt marsh. Species area curves were calculated for 20 randomly selected plots. Only 2 plots are presented here as examples.

quadrat is adequate, we performed a one-way Analysis of Similarity (ANOSIM; Carr 1997) on the tide-restricted marsh quadrats to compare the vegetation community (species composition and abundance) using data from $0.1m^2$, $0.25m^2$, $0.5m^2$, $0.75m^2$ and $1m^2$ quadrats. Alpha levels for the 4 pairwise comparisons ($0.1m^2$ vs. $1.0m^2$, $0.25m^2$ vs. $1.0m^2$, $0.5m^2$ vs. $1.0m^2$, $0.75m^2$ vs. $1.0m^2$) were Bonferroni adjusted and a significant difference was only noted for the $0.1m^2$ vs. $1.0m^2$ comparison. In other words, when the vegetation community as defined by the twenty $0.25m^2$, $0.5m^2$ or $0.75m^2$ quadrats was compared to the same 20 quadrats, but $1.0m^2$ in size, there was no detectable difference in the vegetation community. Therefore, we are confident that the $1.0m^2$ quadrat is adequate.

Quadrats can be established as permanent or temporary. Permanent quadrats are sampled year after year. For temporary quadrats, the location of quadrats within the study marsh is re-determined each sampling year. Permanent plots allow the application of powerful statistical tests for detecting change (Elzinga *et al.* 1998). Permanent plot studies are the most direct way to indicate the pathways of vegetation change (what happens), and can also provide insights into mechanisms and causes (how and why) of vegetation change (Pickett *et al.* 1987). Permanent plots are more efficient to resample than temporary plots and fewer numbers of plots are required to detect change or track trends. As a monitoring tool, they are uniquely suited for temporal studies of vegetation, where change may be marked by slow or variable processes and rare, episodic, or complex phenomena (Bakker *et al.* 1996).

Spatial Sampling Frequency

Sample Design

There are often distinct zones of salt marsh vegetation encountered from tidal creeks toward the upland border of New England marshes (Niering and Warren 1980). At creek banks, the marsh is flooded twice daily by tidal action, commonly called the low marsh. Here, *Spartina alterniflora* usually dominates. With a progression landward, elevation of the marsh surface is increased and the marsh is flooded less frequently. This zone is referred to as the high marsh. Typical plants of the high marsh include *S. patens*, *Distichlis spicata*, short form *S. alterniflora*, and *Juncus gerardii*. At the upland border, there is often a zone of species that is less tolerant of flooding and high soil salinities, including *Iva frutescens*, *Panicum virgatum*, and *Phragmites australis*. Because of this distinct gradient of elevation and frequency of tidal flooding, and corresponding responses of vegetation to this gradient, sampling along transects from the creek bank to the upland border is necessary. Sampling along transects, established across the gradient, will insure that all vegetation cover types along the gradient are sampled.

In salt marshes there is often a salinity gradient from the downstream portion of the marsh to the upstream portion of the marsh. This gradient can be especially pronounced in systems with freshwater stream input or those that have a tidal restriction, such as the Herring River. This represents another gradient (the downstream-upstream salinity gradient) that should be accounted for when designing a vegetation monitoring program. It would be appropriate to stratify the marsh system into segments, defined by the salinity gradient, and then establish transects within each segment.

In order to adequately sample the study area, it may also be necessary to systematically divide the area into sections. In this case the total number of transects should be evenly divided among the sections and then randomly located within each section. The systematic division of the area with the random placement of transects and randomization of the first plot within each transect provides better interspersion of samples within the sample area (Elzinga *et al.* 1998).

Excellent discussions are provided in the literature to justify the use of transects when sampling along environmental gradients and the use of stratified techniques (*e.g.*, the elevation gradient; Kent and Coker 1992, Sutherland 1996, Elzinga *et al.* 1998, Neckles and Dionne 2000). Taking into account the above-mentioned concepts of elevation gradient and salinity gradient, the recommended sampling design is as follows.

If there is a clearly defined gradient of vegetation from the downstream end of the marsh system to the upstream portion, then the marsh should be divided into segments. This is the case at Herring River where there is a clear gradient from *Spartina*-dominated (unrestricted) marsh at the downstream end of the marsh-estuary to freshwater-brackish water (tide-restricted) marsh toward upstream. However, in a system like Nauset Marsh that appears to have a fairly uniform distribution of vegetation throughout the system, segmenting the system would not be appropriate. It may be appropriate to systematically

divide the area into sections in order to intersperse the sampling effort throughout the study segments as mentioned above.

After segmenting of the system, if necessary, creek bank to upland transects should be located randomly within each segment. It is important to locate transects in a random manner. As stressed by Elzinga *et al.* (1998), random sampling must be incorporated into the study design to reduce bias and support the application of inferential parametric statistics. Transects should be spaced at least 10m to 20m apart. Quadrats are then systematically located along each transect from the creek bank to upland. Again, to include random sampling, the location of the first quadrat is selected randomly within the low marsh zone (even if this zone is only a few meters wide) to avoid under-sampling within this habitat. Once the first plot is located, subsequent quadrats are located at consistent intervals along each transect. Quadrats should be spaced far enough apart so that adjacent quadrats are not correlated and are considered independent. In the salt marsh environment, a distance of 10-20m, or greater, should be sufficient. There is no set number of transects to establish per marsh segment, however, it is suggested that transects should cover an area that adequately represents the marsh being studied. For example, we typically use three or more transects per marsh segment. The total number of quadrats per segment (discussed in the next section below) should then be dispersed as evenly as possible among transects.

Figure 4 provides an example of how vegetation transects should be oriented perpendicular to the tidal creek. This hypothetical marsh system was divided into 2 segments to differentiate the high salinity downstream marsh and the lower salinity upstream marsh. Then, to establish the creek bank to upland transects, each segment was divided into three equal-sized sections. One transect is located within each section in a random manner. Dividing the each segment into sections insures interspersion of plots throughout, but still maintains a random, unbiased method.

By following this design, with random location of transects and a random starting point for the quadrats along each transect, and assuming that each quadrat is considered independent, each quadrat serves as a single sample unit. Thus, it is assumed that each quadrat was selected as a simple random sample and the data set can be analyzed as such (Elzinga *et al.* 1998).

Sample Size: How many quadrats?

The total number of $1m^2$ quadrats sampled within each marsh study area should be resolved before transects and quadrats are established. We conducted a statistical power analysis to determine the minimum number of sample replicates that are necessary to detect changes between salt marsh vegetation communities. Power is a function of the differences between two populations, sample size, alpha level of the test (probability of a type I error), and variability of the measured response. In the estimate of power we used Braun-Blanquet percent cover data obtained from $1m^2$ vegetation quadrat sampling of

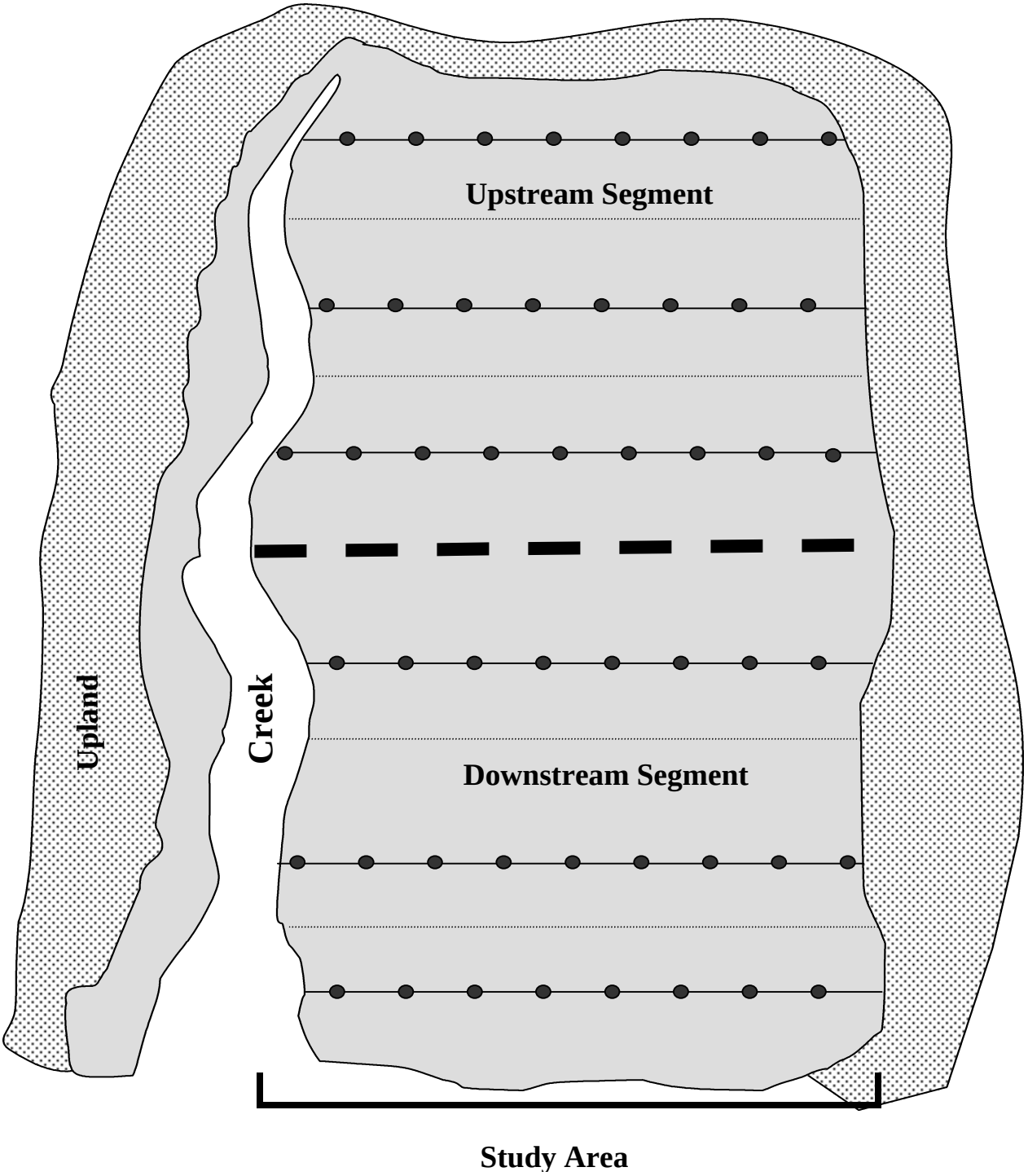


Figure 4. Sample marsh divided into upstream and downstream segments based on salinity distribution. Each segment was then divided into equal-sized sections (indicated by dashed lines) and a transect was randomly located, extending from the creek bank to upland, within each section. The first plot (near the creek bank) of each transect is randomly located, and all other plots are then systematically located along each transect. Note that each area contains at least 20 plots.

eleven salt marshes from Rhode Island (RI) to Maine. These marshes varied from relatively unimpacted to severely impacted (due to tidal restriction), and included marshes that had recently undergone tidal restoration. Seventeen pairs of data sets that exhibited a range from similar (*e.g.*, a RI marsh sampled in 1998 vs. the same marsh sampled in 1999) to very different were identified (*e.g.*, a tidal restricted marsh in RI vs. an unimpacted marsh in Maine). The power of the permutation testing procedure outlined in Clarke and Green (1988) and Smith *et al.* (1990) was evaluated. This procedure allows statistical testing of equality between two vegetation communities. The procedure uses a measure of similarity between two populations as a test statistic, and in this case a Euclidean distance similarity index (Krebs 1999) is used. Vegetation communities similar in composition will have small distances and less similar communities larger distances between them. To look at power as a function of the similarity (as measured by Euclidean distance) between two populations, pairs of vegetation data sets were selected that exhibited a range from similar to quite different vegetation composition. Using a pair of vegetation communities we randomly selected samples of size 5, 10, 15, and 20 from each vegetation community and applied the permutation testing procedure to determine a reject or fail to reject decision for each trial. Two hundred (200) trials for each sample size for each pair of marshes were performed to determine the power to detect a difference between two marshes. Empirical power was estimated as the number of rejections by the permutation procedure out of the 200 trials.

From Fig. 5 we can estimate the statistical power of detecting a difference between two vegetation data sets. As noted, with n=5 there is a low power to detect most differences, even for many cases where the differences between the two data sets are great. Increasing the sample size to n=10, 15, or 20 samples per marsh substantially increases the power to detect a difference between marshes even if the marshes are relatively similar. With a power above 0.9, there is a >90% chance of detecting a difference between vegetation data sets when a difference actually exists. With a low power there is an increased probability of not detecting a difference when the data sets are actually different (*i.e.*, Type II error). From the power curve (Fig. 5) it becomes clear that with n=15 or 20 there will be a high probability of detecting a change between data sets that are quite similar. If an investigator were interested in detecting subtle changes between vegetation data sets (*e.g.*, comparing vegetation from Marsh A over two consecutive years), then it would be appropriate to have a large number of replicates. If dramatic changes were of interest and expected, such as comparing a tide-restricted marsh to a natural marsh, then perhaps a smaller number of replicates would be justified.

Determining a Type II error can be quite important in ecological studies, especially when evaluating environmental impacts on sites or when management actions are being considered. For example, consider a hypothesis that states that the vegetation community of a particular marsh is the same in year 1 as in year 2, and based on a statistical test the null hypothesis (*i.e.*, there is no difference in vegetation community between the marshes) is accepted. However, in actuality the vegetation community in year 2 is different from year 1 (perhaps there was an increase in some invasive species), but by accepting the null hypothesis a Type II error was committed (accepting the null hypothesis when a difference truly exists). If the test were more powerful, the difference

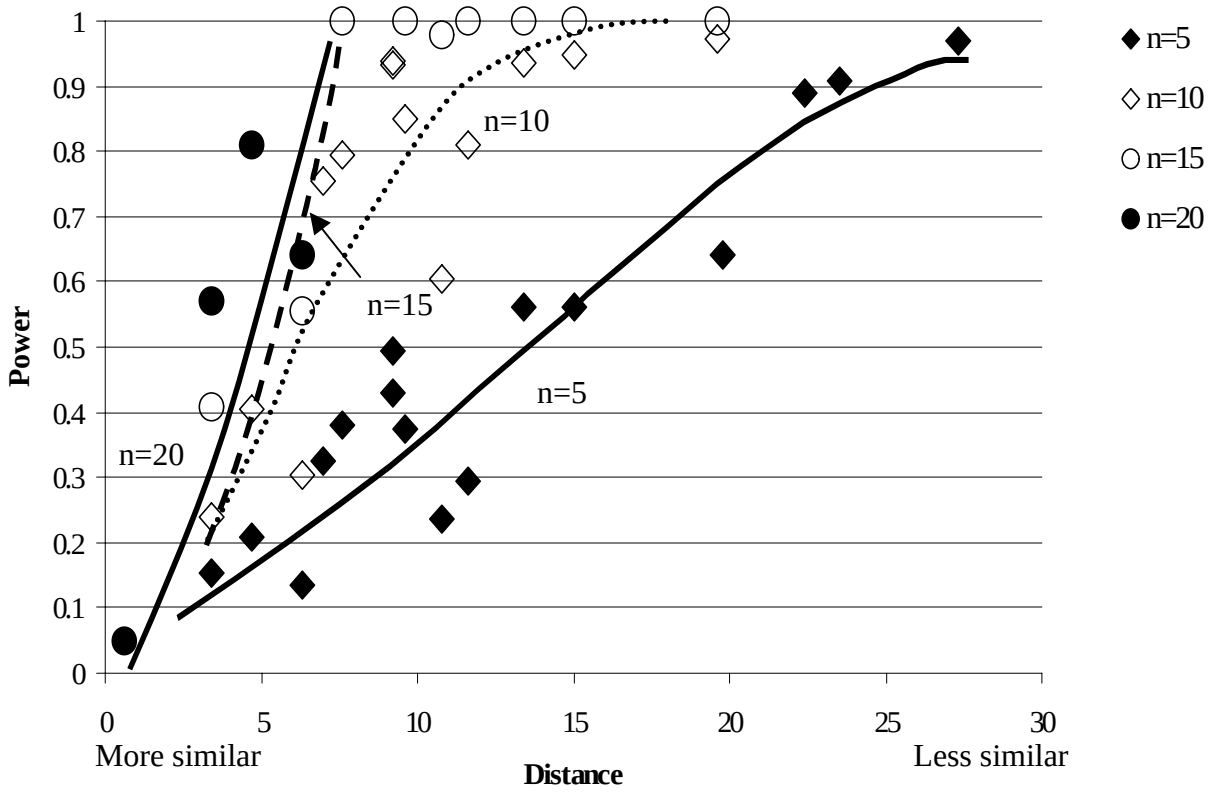


Figure 5. Estimation of power at alpha = 0.05 for 5, 10, 15, and 20 samples per marsh segment. Distance was calculated by the Euclidean distance similarity index between marsh pairs. Lines were hand drawn to assist in the identification of the appropriate sample size to achieve adequate power for a given similarity distance.

between the vegetation communities would have been detected and some management action possibly initiated. Thus, in some instances it may be advisable to set a fairly high power, possibly 0.9 or above. This would result in a greater than 90% chance of detecting a difference between two data sets.

To summarize, for salt marsh vegetation monitoring at Cape Cod National Seashore, it is recommended that a minimum of 20 permanent quadrats be established within each marsh study area. If a tide-restricted marsh is being compared to an adjacent reference "unrestricted" marsh, there should be at least n=20 in the tide-restricted marsh and n=20 in the reference marsh. It is noted that n=15 would probably be an adequate number of replicates (based on the power curves, Fig. 5) to detect the kinds of long-term salt marsh vegetation changes that are of interest in the Cape Cod monitoring program; however, given the relative ease of collecting vegetation quadrat data, we are recommending a sample size of n=20 to effectively detect even subtle vegetation changes.

Temporal Sampling Frequency

The optimal sampling time for salt marsh vegetation is during the period of peak biomass from July through early September. Plants are either flowering or fruiting during this period, thus enhancing opportunities for taxonomic identification. Sampling during multiple seasons is not required.

Sampling frequency over the long-term depends upon the projected rate of salt marsh vegetation change. For salt marshes undergoing tidal restoration, initial vegetation changes (from years to decades) can be quite dramatic and the monitoring program should be designed to reflect this change. For example, at a Connecticut salt marsh, vegetation data collected four and ten years after tidal restoration showed a progressive conversion of an impounded *Typha* marsh toward *Spartina*-dominated, with vegetation recovery still occurring after ten years (Sinicrope *et al.* 1990). In coastal Maine, vegetation monitoring for eight years following tidal restoration also found conversion of a *Typha* marsh to *Spartina* (Burdick *et al.* 1997). Regarding shorter-term vegetation changes, Roman et. al (in press) report that after just one year of tidal restoration to a Rhode Island marsh, significant vegetation changes were documented.

As noted from these studies, vegetation changes can be quite rapid when restoring tidal flow, but these changes can proceed for a decade or more. To enhance our understanding of marsh restoration processes it is important to document both the short-term and long-term vegetation changes. As a general rule, it is recommended that when addressing marsh restoration issues, vegetation monitoring should be conducted before restoration, one year following restoration, and then at five-year intervals thereafter. If dramatic vegetation changes are noted after one year of tidal restoration, then it may be appropriate to continue monitoring at year two, post restoration. Based on the literature cited above, it is certain that significant vegetation change will continue to occur for a decade, and most likely, for several decades.

In addition to monitoring that addresses salt marsh restoration, programs may also be designed to monitor vegetation change in response to long-term factors, like sea level, climate (temperature, precipitation), nutrient loading, and barrier spit and inlet dynamics (see monitoring questions in a previous section of this protocol). Because vegetation changes will most likely occur at subtle rates, the frequency of sampling does not need to be as intense as that recommended following major hydrologic alteration (*i.e.*, tidal restoration). Warren and Niering (1993), studying a Connecticut salt marsh, found that over a 40-yr period the vegetation of some portions of the marsh remained remarkably stable, while other areas displayed significant changes. The areas where vegetation did change had lower rates of marsh surface accretion, and thus, rising sea level may be a factor contributing to the changes (Warren and Niering 1993). At Cape Cod's Nauset Marsh, Roman *et al.* (1997) studied rhizomes in salt marsh peat cores and found relatively stable vegetation patterns for a century, or so; however, there was one portion of the marsh where vegetation changes were noted over the past four decades – also suggested as a response to an accelerated rate of sea level rise. To summarize, vegetation changes that are responding to longer-term factors, like sea level, as opposed to dramatic

hydrologic alterations, may occur over decades or centennial time scales, but nonetheless, significant changes do occur. Miller and Egler (1950) eloquently describe salt mash vegetation change as follows; "The present mosaic may be thought of as a momentary expression, different in the past and destined to be different in the future yet as typical as would be a photograph of moving clouds."

When addressing questions of vegetation change in response to long-term and large-scale issues, it is recommended that sampling initially be established at 3-5yr intervals. If significant changes are occurring during this interval, then more frequent sampling should be considered. Alternatively, a longer interval, perhaps 7-10yrs could be adopted if initial monitoring reveals a stable community. It is also recommended that monitoring should be conducted following any major events, such as hurricanes, formation of new inlets, or oil spills.

Data Collected for Each Sample Quadrat

Species composition and abundance of each species within each sampled quadrat must be determined. Cover is a common measure of species abundance in vegetation studies. Two methods of estimating percent cover (the point intercept estimate and the visual cover estimate) are widely used in grassland habitats and the merits and shortcomings of each have been reviewed by many (*e.g.*, Poissonet *et al.* 1973, Floyd and Anderson 1987, Kent and Coker 1992, Elzinga *et al.* 1998). In brief, for the point-intercept method the observer records each species that is intercepted by each point in a grid of 50 or 100 points within each quadrat. This method has a sound theoretical basis; the proportion of points intercepted equals the cover of that species. For the visual cover estimate, the observer stands over the quadrat and visually estimates the cover of each species present within the quadrat. Cover is typically estimated within standard cover classes, such as the Braun-Blanquet cover scale (<1-5%, 6-25%, 26-50%, 51-75%, 76-100%).

The point intercept method is considered by many to be the least biased and most objective method (*e.g.*, Floyd and Anderson 1987, Elzinga *et al.* 1998). The observer merely needs to record the species that each point hits or intercepts. With the visual cover method, the observer must decide the cover class that each species should be assigned. Observer bias can be quite high with the visual estimate method (*e.g.*, Greig-Smith 1983, Kennedy and Addison 1987); however, others strongly argue that the visual method yields similar results when compared to intercept methods (*e.g.*, Poissonet *et al.* 1973; Smartt *et al.* 1974, 1976; Kent and Coker 1992).

Based on the literature, use of either method could clearly be justified. However, to reduce observer bias (*i.e.*, decrease subjective decision-making by the observer) in a monitoring program that will be ongoing for several decades and will include many different teams of field personnel, we recommend the point-intercept method. It should be noted that we have used the visual cover method in salt marsh vegetation studies and find it to be a reliable method (Roman *et al.*, in press). In that study we compared

vegetation among several sampling years, but the same team of field observers was used reducing any bias associated with the subjective assessment of vegetation cover.

The point-intercept method is described as follows. As shown in Fig. 6, the $1m^2$ quadrat is divided into a grid of 50 evenly spaced points. A thin rod (3mm diameter), or bayonet after Poissonet *et al.* (1972), is held vertical at each point and dropped straight through the canopy to the sampling point on the ground. At each point of the grid, all species that touch/hit the bayonet are recorded. To calculate cover, for example, species A had 10 hits, yielding a 20% cover (10 hits/50 total points). Prior to sampling the quadrat it is useful to record all species within the plot on the data sheet.

Figure 6. Point intercept method for sampling 50 points within a $1m^2$ quadrat.

We determined that 50 points per $1m^2$ were appropriate for sampling by the point intercept method. We compared the species composition and abundance for 45 randomly selected plots within Hatches Harbor as sampled by 50 and 100 points. Analysis of Similarity showed no difference in the vegetation community when comparing the same quadrats with a 100 vs. 50 point grid. We also evaluated the ability to detect rare species when using the 50-point grid. Some investigators have noted that the point-intercept method may tend to miss rare species that occur within quadrats (see Elzinga *et al.* 1998). We have no data to quantify the species missed by sampling with a 100-point grid per m^2, however, we can state with some certainty that missing rare species was not a problem.

A rare species was defined as one that occurred in just one of the 45 quadrats sampled <u>and</u> with a cover of $\leq$ 3%. Assuming that the 100-point data set sampled all rare species, we missed only 4 species from a total of 68 species when analyzing the data based on a 50-point grid. These missed species were extremely rare. Using the 50-point grid, we

detected 85% (23 of 27) of the rare species (as defined above) that were present in the 100-point grid. Thus, we were successful in detecting extremely rare species most of the time.

Associated Environmental Data

It is important to quantify vegetation changes and it is also valuable to understand why the species composition or abundance of salt marsh plants is changing. Several interacting factors influence salt marsh vegetation patterns, such as frequency and duration of tidal flooding, salinity, substrate, soil oxygen, nutrient availability, disturbance by wrack, and competition among plant species (*e.g.*, Niering and Warren 1980, Nixon 1982, Bertness 1999, Roman 2001).

Frequency and duration of tidal flooding are mostly responsible for one of the more noticeable patterns of the New England salt marsh, the delineation between low marsh and high marsh. The low marsh is flooded twice daily by tidal action and is often dominated by a single species, *S. alterniflora*. With increasing elevation and less frequent flooding, the high marsh is typically occupied by *S. patens*, *Distichlis spicata*, and *Juncus gerardii*. Soil salinity can be relatively constant within the low marsh, but extremes in soil salinity on the high marsh contribute to the pattern or mosaic of vegetation. Hypersaline areas, in excess of 60 parts per thousand can occur in response to evaporation. In fact, it has been suggested that marshes of southern temperate latitudes have higher soil salinities because of more intense solar radiation and the vegetation is composed of more salt tolerant species (Bertness 1999). With global climate change, perhaps shifts toward more salt tolerant plants on New England marshes will be observed.

Control of *Phragmites australis* is an important issue at Cape Cod National Seashore and elsewhere throughout the northeastern US coastal zone. Increased soil salinity is an important factor contributing to the reduction in *Phragmites* height (Hellings and Gallagher 1992). Also, anoxic waterlogged soils can stress *Phragmites* growth, principally through sulfide toxicity (Chambers *et al.* 1998). It would be especially interesting to know if restoration of tidal flow to degraded salt marshes is resulting in increased soil salinity or altered sulfide levels.

In association with the $1m^2$ vegetation quadrats, it is recommended that the following variables be monitored in an effort to enhance our understanding of causal mechanisms for observed vegetation changes.

Water table level – Indicator of soil drainage or soil waterlogging.
Soil salinity – Indicator of salt stress.
Soil sulfide – Indicator of soil toxicity levels.

In addition to monitoring the above physical/chemical variables in association with vegetation plots, some investigators may be interested in targeting the response of species

of concern. For example, at a Rhode Island marsh restoration site, we have monitored *Phragmites* height prior to tidal restoration and annually after tidal restoration (Fig. 7, Roman *et al.* in press). This represents a fairly simple measure to document restoration trends.

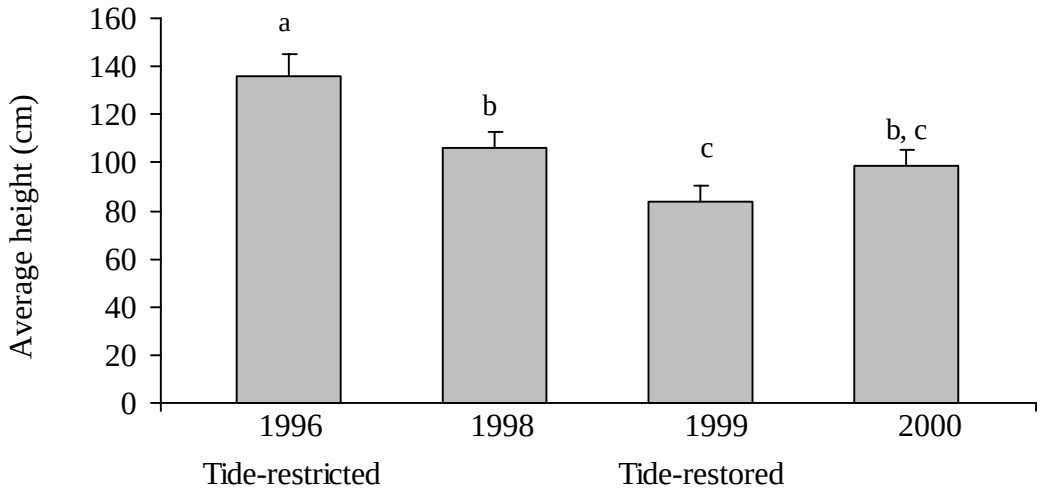


Figure 7. Height (cm ± SD) of *Phragmites australis* under tide-restricted (1996) and tide-restored (1998 – 2000) conditions at Sachuest Point salt marsh, Middletown, RI. Analyses by one-way ANOVA with different letters indicating significantly different means by Least-squared Mean Test (Source: Roman *et al*. in press).

DATA ANALYSIS TECHNIQUES

There are numerous statistical analysis techniques available for evaluating trends in salt marsh vegetation. As an example, we present a non-parametric permutation procedure for detecting significant changes in vegetation communities at different time intervals or detecting differences between various treatments (*e.g.*, pre-restoration vs. post-restoration). Also, we discuss some ordination techniques that are commonly used to elucidate patterns in vegetation data. Kent and Coker (1992) provide excellent reviews of these and other vegetation analysis techniques.

An example of a non-parametric permutation procedure is one-way ANOSIM (Analysis of Similarities; Carr 1997, Clarke and Warwick 1994) which tests for differences between groups of community samples (species composition and abundance). First, using a similarity measure (such as the Euclidean Distance measure), a similarity matrix is created that allows for the objective identification of sample plots that have similar (or dissimilar) vegetation communities in terms of species composition and abundance or cover. An analysis of similarities randomization test (ANOSIM) is then applied to the

matrix to test for significant differences between groups of sample plots. All pairwise comparisons are summarized into a test statistic using Clark's R that compares between-group to within-group dissimilarities. Monte Carlo permutation tests are then used to derive a significance level. ANOSIM is considered a non-parametric analog to MANOVA (Clarke and Green 1988). Assumptions of normality can generally not be satisfied with vegetation data sets, and thus, MANOVA is not an appropriate analysis method. Pairwise comparisons between groups of sample plots are defined *a priori* to detect differences in vegetation (*e.g.*, unrestricted vs tide-restricted, tide-restricted vs tide-restored, *etc*.). A Bonferroni correction for the experiment-wise error is made based on the number of comparisons being tested (Zar 1999). For example, if there are 4 pairwise comparisons and the desired probability level is 0.05, the adjusted alpha level would be 0.05/4 or 0.0125. Any comparisons having p-values below 0.0125 would be significantly different.

For pairwise comparisons that were significant, or had dissimilar vegetation communities, it is often desirable to know what contribution the individual cover types made to the dissimilarity. The proportion of the overall dissimilarity that was contributed by each individual cover type can be calculated as follows;

$$1 - \frac{D}{D_{max}} = 1 - \frac{(C_{1i} - C_{2i})^2}{\Sigma\ (C_{1i} - C_{2i})^2}$$

Where;
D = Distance
C_{1i} = cover of species i in marsh 1
C_{2i} = cover of species i in marsh 2

The outcome is a list of cover types or species ranked in order of their percent contribution to the dissimilarity between significant pairwise comparisons. D_{max} provides an overall measure of dissimilarity for each pairwise comparison.

ANOSIM was performed on the vegetation data from Hatches Harbor – a restoration monitoring program that employs a BACI design (before, after, control, impact). As shown in Fig. 2, the marsh located downstream of the dike serves as the control area or unrestricted marsh. The upstream marsh is the treatment area (*i.e.*, portion of marsh to undergo tidal restoration). Vegetation in both the control and treatment areas was monitored in summer 1997, before tidal restoration, and then after tidal restoration in summer 2000. Results from the ANOSIM are presented in Table 1 and as noted, the vegetation of the unrestricted and tide-restricted marshes was significantly different in 1997. Tidal restoration commenced in winter 1998 and following two growing seasons of restored tidal flow the vegetation remained quite different in 2000. It is also noted that vegetation of the unrestricted marsh, serving as a control in the BACI study design, as was expected did not change. At Hatches Harbor, tidal flow is being increased to the tide-restored marsh in small increments. As these increments increase it is expected that significant changes in vegetation will be noted.

Pairwise ANOSIM Comparisons	*p* value	D_{max}
1997 Unrestricted vs. 1997 Tide-restricted	0.001	28.4
2000 Unrestricted vs. 2000 Tide-restored	0.001	31.0
1997 Tide-restricted vs. 2000 Tide-restored	0.045	-
1997 Unrestricted vs. 2000 Unrestricted	0.200	-

Table 1. Results of one-way ANOSIM tests on pairwise comparisons of vegetation community data from Hatches Harbor salt marsh. Unrestricted marsh serves as the control. In 1997 the treatment marsh was under a regime of restricted tidal flow, while in 2000 the same treatment marsh was under restored tidal flow conditions. Bonferroni adjusted alpha = 0.05/4 = 0.0125.

Table 1 also presents D_{max}, a measure of overall dissimilarity (based on Euclidean Distance) for pairs that were significantly different. It is noted that D_{max} changed little when comparing the vegetation of the unrestricted and the tide-restored marshes in 1997 and again in 2000, two growing seasons of restored tidal flow. As D_{max} diminishes towards zero the marshes are becoming more similar. As restoration proceeds, we would expect this dissimilarity to decrease, as expressed by a decreasing D_{max}, suggesting that the tide-restored marsh is converging toward or becoming more similar to the unrestricted control marsh. This convergence can be used as a measure of restoration success. For example, at the Sachuest Point salt marsh (Rhode Island), average D_{max} between vegetation communities of an unrestricted marsh and tide-restricted or tide-restored marsh progressively decreased from 16.5 (during pre-restoration conditions), to 14.5, two years post restoration (Roman *et al*., in press).

The dissimilarity in vegetation noted between the unrestricted and tide-restored marshes in 2000 was mostly due to *Spartina alterniflora* having an average Braun-Blanquet cover rank in the unrestricted marsh of 3.14 (equates to an actual percent cover of 6-25%) and a much reduced average cover rank of 0.45 (equivalent to <5% cover) in the tide-restored marsh, as would be expected (Table 2). This difference accounted for 23% of the overall dissimilarity between the unrestricted and tide-restored marsh vegetation. High percentages of terrestrial species (*e.g. Rubus*) and the invasive wetland species, *Phragmites*, were found in the tide-restored marsh compared to the unrestricted control. As the Hatches Harbor monitoring program proceeds, we expect to find shifts in percentages, perhaps with *S. alterniflora* cover increasing in the tide-restored marsh and *Phragmites* decreasing.

In addition to ANOSIM, there are several ordination techniques that will prove useful in defining trends in salt marsh vegetation over-time. Ordination represents an objective means of ordering or arranging a multidimensional vegetation data set into fewer dimensions (*e.g.*, a 2-axis plot) such that any pattern that the data possess becomes more apparent. Ordination techniques arrange data such as vegetation samples or quadrats in relation to each other in terms of their similarity of species composition and/or associated environmental controls (Kent and Coker 1992). The interpretation of ordination analyses is carried out in relation to quadrat ordination diagrams and species ordination diagrams.

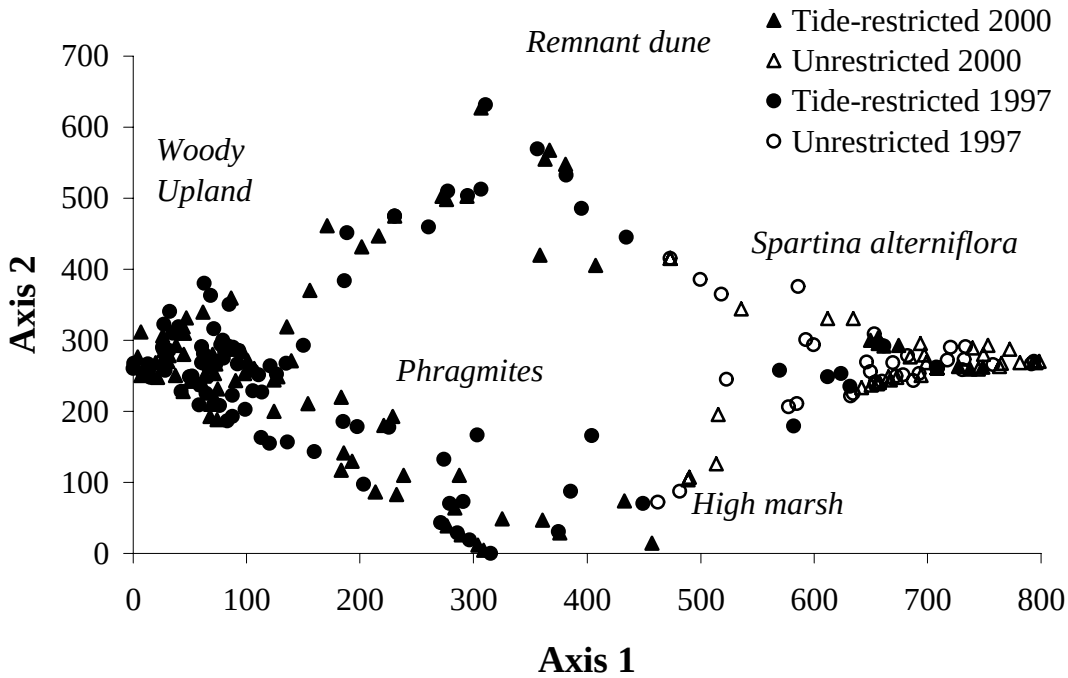
Table 2. Euclidean Distance measures and contribution of each species to the vegetation community dissimilarity noted by ANOSIM. Data presented are for the 2000 unrestricted vs. 2000 tide-restored Hatches Harbor marshes. Average cover ranks are according to the Braun-Blanquet scale (0=0%, 1=<1-5%, 2=6-25%, 3=26-50%, 4=51-75%, 5=76-100%). Only the top ranked species are shown here.

Species	Avg Cover Rank 2000 Unrestricted	Avg Cover Rank 2000 Tide-restored	% Contribution to dissimilarity
Spartina alterniflora	3.14	0.45	23.4
Salicornia sp.	1.78	0.02	10.0
Rubus sp.	0.00	1.73	9.7
Phragmites australis	0.00	1.41	6.4
Ascophyllum nodosum	1.27	0.00	5.2

Each point on the ordination diagram corresponds to an individual quadrat (in the case of a quadrat ordination diagram) or an individual species (in the case of a species ordination diagram). The distances between the points on the diagrams are an approximation to the degree of similarity in terms of distribution within the quadrats. For example, in a quadrat ordination diagram two quadrats having exactly the same species at exactly the same abundance would occupy the same point. In a species ordination diagram, two species occurring at exactly the same abundance in the same quadrats would also occupy the same point. As quadrats or species distributions diverge the points on the respective diagrams become further apart. It is useful to look at groupings of points on the ordination diagrams to interpret trends in the community structure (Kent and Coker 1992).

The Hatches Harbor marsh vegetation data were analyzed by the ordination technique, Detrended Correspondence Analysis (DCA). Kent and Coker (1992) provide a detailed description of ordination techniques including DCA. The data set consists of 129 permanent vegetation quadrats sampled in 1997 and again in 2000 (92 quadrats in the tide-restricted marsh and 37 quadrats in the unrestricted marsh) with a total of 107 plant species. Each quadrat has a different species composition and abundance resulting in a very complex data set, but as noted from the DCA diagrams (Figs. 8a and 8b) this complexity of species and quadrats are arranged along a gradient. Quadrats with similar vegetation (in terms of species composition and abundance) are plotted close together, while dissimilar quadrats are far apart. Based on a species ordination (not shown here), we can interpret the clusters of quadrats in terms of the species composition. On the far right side of the ordination diagram (Fig. 8a) are quadrats composed of low marsh species (*e.g.*, *S. alterniflora*, water, macroalgae), to the lower left of this group are quadrats with high marsh vegetation species (*e.g.*, *S. patens*, *D. spicata*). By coding the quadrats based on their location within the marsh we see that the majority of these quadrats are located, as expected, in the unrestricted marsh. Towards the top and center of the diagrams there is a group of quadrats comprised of species typical of remnant dune communities (*e.g.*,

8a.



8b.

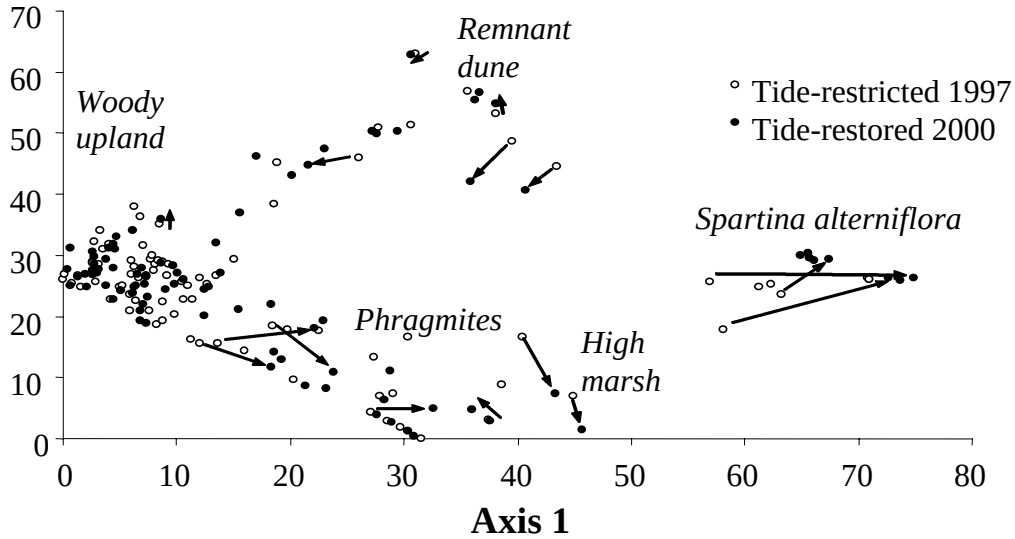


Figure 8. Ordination diagrams by Detrended Correspondence Analysis of vegetation plots from Hatches Harbor. Individual $1m^2$ plots are shown, with dominant vegetation types indicated for the different regions of the plots.

a. All vegetation plots from 1997 tide-restricted and unrestricted marsh and 2000 tide-restored and unrestricted marsh are shown.

b. For some individual plots time-trajectory lines (arrows) are drawn indicating vegetation change from 1997 to 2000 in the tide-restricted, then tide-restored marsh. Trajectories for only a few representative plots are shown. Each trajectory is for the same plot, but sampled in different years.

Deschampsia, *Cladonia* spp.*)* and another cluster of quadrats in the lower center is comprised of *Phragmites australis* and herbaceous species (*e.g.*, dead *Phragmites*, *Typha*). On the very left hand side there is a large grouping of quadrats primarily composed of woody and non-wetland species. The coding of the quadrats indicates that the clusters to the left are mostly composed of quadrats from the tide-restricted marsh. In terms of documenting temporal trends in vegetation, ordination techniques, like DCA, are especially valuable tools (Austin 1977). Fig. 8b is an ordination diagram of individual vegetation plots from the tide-restricted marsh in 1997 and the tide-restored marsh in 2000. We have plotted time trajectories (1997 pre-restoration to 2000 post-tidal restoration) for several individual vegetation plots and it is clearly demonstrated that the vegetation is changing as expected. As noted, some of the plots dominated by *S. alterniflora* have shifted quite dramatically toward the right of the plot, indicating wetter conditions. It is also encouraging that *Phragmites*-dominated plots are moving toward the right, again indicating a conversion toward typical salt marsh vegetation. The remnant dune and woody upland plots are changing little and especially not toward wetter, more saline conditions because of the modest opening of the dike in 2000. We expect to detect more dramatic changes or time-trajectory shifts following our 2002 growing season monitoring. Application of multivariate vegetation analysis techniques, like ordination, will be instrumental in documenting these changes and better understanding the processes associated with vegetation recovery.

Canonical Correspondence Analysis (CCA; ter Braak 1987), another ordination technique, evaluates relationships between environmental variables and floristic composition. Basically, CCA combines the vegetation data and associated environmental data collected for each quadrat into one ordination. As monitoring programs are initiated at several salt marshes within the Seashore, CCA can be applied to better understand why the vegetation at one site may differ from another site (*i.e.*, What environmental variables are influencing the vegetation patterns at each site?).

PART TWO
The Vegetation Monitoring Protocol

SUMMARY

The salt marsh vegetation monitoring protocol (Table 3) recommends sampling vegetation community composition and abundance with permanent plots using the point intercept method. Prior to sampling each $1m^2$ plot, all species present within the plot should be noted, and then using the point intercept method, the number of "hits" per species are recorded for each of 50 points. Sampling should be conducted in late summer or early fall. The study areas should be defined and if appropriate, divided into marsh segments (*e.g.*, tide-restricted, unrestricted, upstream, downstream, *etc*.). At least 20 vegetation plots are required per marsh segment to detect differences in community composition and abundance. Permanent quadrats should be arranged in transects and spaced a minimum of 10-20m apart to maintain independence. Transects should be randomly located within each marsh segment with the first permanent plot randomly located and subsequent plots systematically placed along each transect. Additional environmental parameters that can be recorded include water table level, soil salinity, soil sulfide concentration, height of indictor species such as *Phragmites australis*, and elevation of the permanent plots.

The protocol presented is a minimum for monitoring vegetation in salt marshes. One of the goals of presenting a model protocol is to inspire commonality among the sampling programs in disparate geographical areas and to promote comparisons among datasets over space and time. However, this is a prototype protocol and as such is amenable to modifications to accommodate individual monitoring efforts. This protocol should serve to stimulate monitoring of salt marsh vegetation communities to provide long-term, quantitative datasets to help evaluate the status of wetland resources over time and responses to human-induced or natural habitat changes.

Table 3. Protocol for monitoring vegetation in salt marsh habitats. The protocol addresses spatial and temporal (long-term), sampling frequency, parameters of interest, and additional environmental data.

Sampling Gear	$1\ m^2$ quadrat, point (50 pts) intercept method
Season	Late summer, early fall
Annual frequency	1-5 yr interval depending on monitoring questions
Sampling design	Random with systematic placement of permanent quadrats
Number of samples	$\geq$20 samples per marsh segment
Vegetation parameters	Species composition and abundance as estimated by point intercept
Environmental data	Ground water, soil salinity, soil sulfide, elevation, GIS maps

PROTOCOL

Site Selection

The marsh of interest should be defined and boundaries delineated. Depending on the monitoring questions being addressed, it may be appropriate to employ a BACI (before, after, control, impact) study design. For example, at the Hatches Harbor marsh, the marsh was divided into two separate areas (tide unrestricted and tide-restricted). Vegetation was sampled in each area "before" tidal restoration and then "after" tidal restoration. Monitoring of each area will continue for the long-term. When addressing questions not related to a specific impact (tidal restoration in the case of Hatches Harbor), a reference marsh area may not be necessary.

Sample Location

Fig. 9 provides an example of how vegetation permanent plots should be established within a marsh. This figure will help guide the following discussion on segmentation of the marsh, transect layout, and permanent plot location along transects.

Segmentation of the Marsh

Once the study marshes are identified it may be necessary to systematically divide the area into segments to adequately sample the marsh. For example, if there is a clearly defined gradient of vegetation from the downstream end of the marsh to the upstream end, then the marsh should be divided into segments. This is the case at Herring River where there is a clear gradient from *Spartina*-dominated marsh at the downstream end to freshwater-brackish marsh toward the upstream portion of the estuary. A marsh could also be segmented into tide-restricted and tide-unrestricted. In a system like Nauset Marsh that appears to have a fairly uniform distribution of vegetation throughout, segmenting the marsh would not be appropriate. Thus, depending on overall variation in vegetation throughout the system (this variation is usually responding to a salinity or tidal range gradient), the number of segments in the study marsh can range from no segments to several.

Transect Layout

Vegetation will be sampled in $1m^2$ plots that are systematically located along transects. It is important to locate transects in a random manner, but it is also important to intersperse the sampling effort throughout each marsh segment. To accomplish this, each segment is then divided into equal-sized sections, and transects (usually one or two) are randomly located within each section. Using this method, several transects (usually four to five) will be interspersed and randomly located within each marsh segment.

Transects should traverse the main gradient (*e.g.* elevation) from the creek bank to upland edge of the marsh. The starting point for each transect is randomly located along the creek bank. The random location of the starting point for each transect is selected by

measuring the total distance of the creek bank within each segment, and then dividing that distance by the total number of sections for that segment. Usually, four or five sections per marsh segment are sufficient. A random number is then chosen that ranges from 0 to the total distance of the section, and a transect is placed at the distance indicated by the random number within that section. This process is then repeated for each section until all transects are randomly located within the marsh segment. If more transects are needed to fulfill the minimum vegetation plot requirement (*i.e.* 20 plots per marsh segment), then the marsh segment should be divided into more sections, and the process repeated. These measurements are best done from aerial photography. Random numbers can be generated from a spreadsheet using the random number function or can be chosen from a random number table commonly found in the appendix of statistical texts.

There is no definitive number of transects that should be established per marsh segment; however a few guidelines are suggested. Each transect should be at least 10m apart, to maintain independence of the replicate plots, and transects should cover an area that is representative of the marsh segment.

Permanent Plots along Transects

Once the starting location of each transect has been randomly located the first permanent plot of each transect should also be randomly located. The first plot should be randomly located within the low marsh zone. Measure the width of the low marsh and then place the plot at the distance selected by the random number (0 being on the edge of the bank). For example, if the low marsh zone is 5m wide, a random number between 0-5 would be selected. After the first plot is located, all subsequent plots are then systematically placed, at least 10m apart, along the length of the transect. All transects within a marsh segment should be parallel to each other (*i.e.*, should run along the same compass heading) for ease in re-locating plots. If there is no discernable low marsh zone, or if the vegetation zone at the creek bank is very broad, more than 10m, then the first plot should be picked by a random number between 0 and 10.

The number of transects within each segment and the spacing of plots along each transect will be variable depending on the area of the marsh. Regardless of the size of the area a minimum of 20 plots are required for each marsh segment. For example, if the marsh is 8-9 hectares in area, then 4 transects, with 40m spacing between plots along each transect would be appropriate. For smaller marshes, 20m spacing between plots may be necessary. However, all plots should be at least 10m apart to maintain independence of the replicate plots.

Marking Permanent Stakes

Each plot should be marked with a stake labeled clearly with transect and plot number. Construction of permanent stakes is at the discretion of the investigator. Some may prefer to install metal stakes (*e.g.*, rebar) or cement monuments to mark each plot;

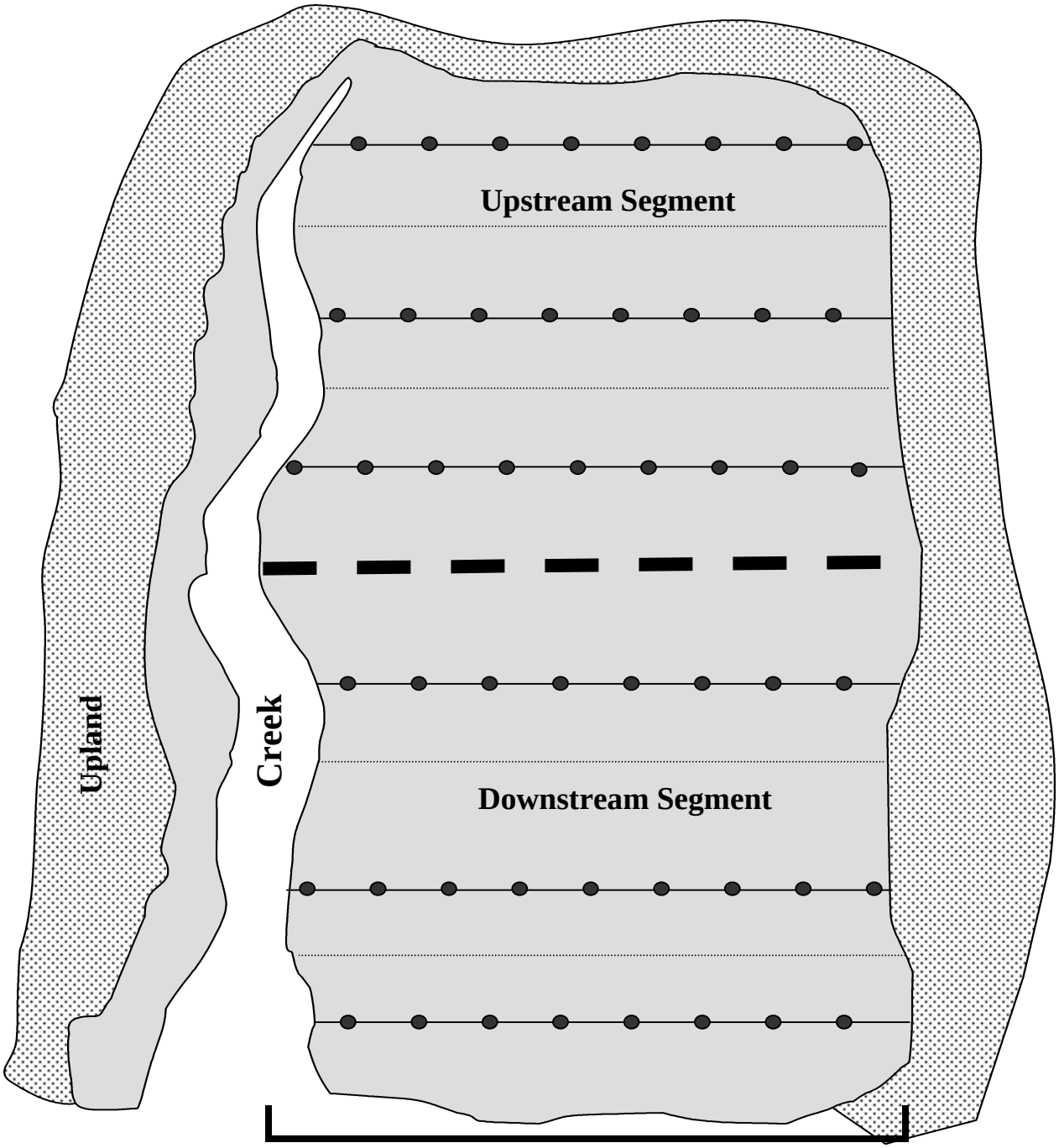


Figure 9. Sample marsh divided into upstream and downstream segments based on salinity distribution. Each segment was then divided into equal-sized sections (indicated by dashed lines) and a transect was randomly located, extending from the creek bank to upland, within each section. The first plot (near the creek bank) of each transect is randomly located, and all other plots are then systematically located along each transect. Note that each area contains at least 20 plots.

however, our experience suggests that 4 ft oak stakes (1 ft into the marsh sediment, 3 ft above marsh; about 1^2 inch) are adequate. When sampling is conducted some stakes may be missing due to ice damage or vandalism, but since the plots are systematically placed along a transect it is easy to re-locate a lost stake by running a meter tape from existing stakes or by using a GPS unit. As will be noted in the next section, a PVC groundwater well will be associated with all of the vegetation plots, or a sub-set. These wells can serve as excellent permanent plot markers.

Plot location and distance between plots should be carefully noted on a map so that plots can be re-located in future surveys in the event that stakes are missing. The coordinates (UTM coordinates) of all plots should be recorded, preferably with a GPS unit that has sub-meter accuracy.

Sampling Gear and Field Methods

Point Intercept Method

To sample each permanent vegetation plot the permanent marker (stake) is located. In order to sample vegetation that has not been trampled during the establishment of transects, the quadrat is offset 1m from the stake. Facing the direction of the transect (from the first plot towards the remaining plots of the transect) set the quadrat 1m to the right of the stake and orient the plot towards the direction of the transect. Be sure to maintain the same offset for all plots and record a detailed description of the offset (Fig. 10).

The sampling quadrat is shown in Fig. 11. A meter stick is placed on the marsh surface and then at 0, 25, 50, 75, and 100cm intervals along the meter stick, dowels ($\leq$3mm in diameter) are placed perpendicular to the meter stick. Each dowel is 1m in length and has a total of 10 marks, each spaced 11.1cm apart. In thick vegetation it may be necessary to weave the dowels through the vegetation.

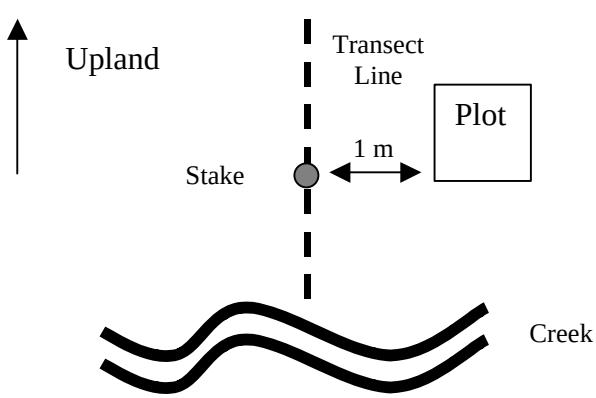
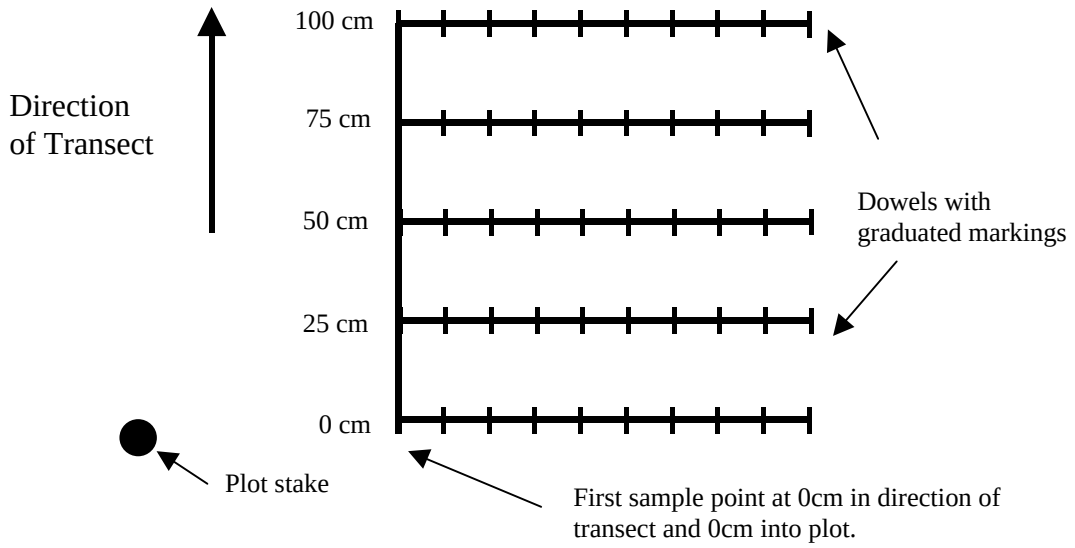


Figure 10. A schematic of the orientation of the sampling quadrat relative to plot stake.



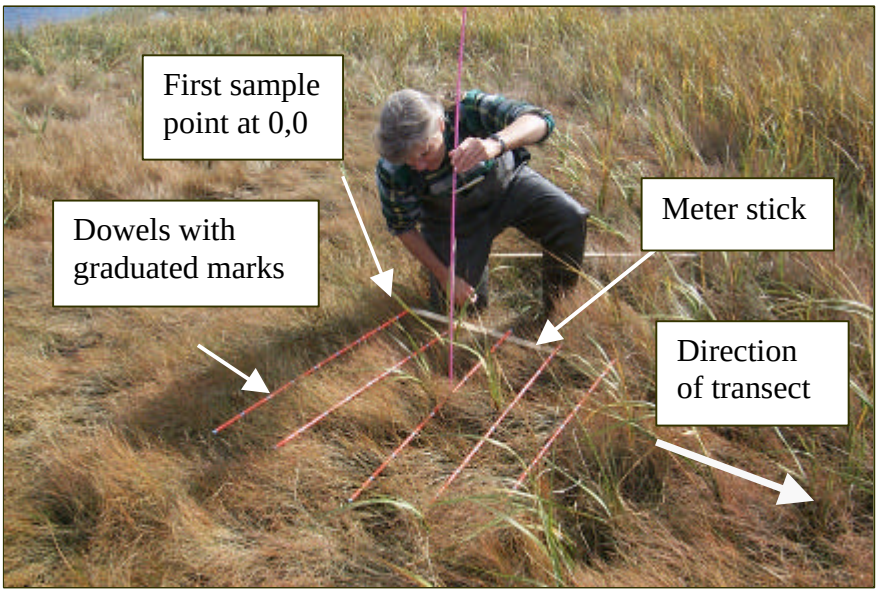


Figure 11. Schematic and photo of the sample quadrat and arrangement of dowels used in the point intercept method.

Thus, the $1m^2$ quadrat is divided into a grid of 50 evenly spaced points. A list of all species within the sample quadrat is then recorded on the data sheet for that plot (Table 4 provides a sample data sheet for vegetation sampling). A thin rod ($\leq$3mm in diameter) is then held vertical to the first sampling point and lowered through the vegetation canopy to the sample point on the ground. All of the species that touch the rod are recorded as a "hit" on the data sheet for that point. Categories other than plant species, such as

Table 4. Example of a field data sheet for the point intercept method.

Marsh _____Field Crew _____Date _____

Transect & Plot number _____Record species, first row is for points 1-25, second row is for points 26-50.

Point

SPECIES	Total Tally	1 26	2	3	4	5 30	6	7	8	9	10 35	11	12	13	14	15 40	16	17	18	19	20 45	21	22	23	24	25 50
1.																										
Species # 1 -- pts. 26-50	XXXX																									
2																										
Species # 2 -- pts. 26-50	XXXX																									
3																										
Species # 3 -- pts. 26-50	XXXX																									
4																										
Species # 4 -- pts. 26-50	XXXX																									
5																										
Species # 5 -- pts. 26-50	XXXX																									
6																										
Species # 6 -- pts. 26-50	XXXX																									
7																										
Species # 7 -- pts. 26-50	XXXX																									
8.																										
Species # 8 -- pts. 26-50	XXXX																									
9.																										
Species # 9 -- pts. 26-50	XXXX																									

Table 5. Cover type categories to be included in the point-intercept salt marsh vegetation program.

Live vascular plants (herbaceous and shrubs) identified by species

Standing dead vascular plants identified by species (*e.g.*, *S. alterniflora* dead). This category only includes standing dead (attached) plants that are from a previous year's growth. There may be some dead leaves from this year's growth (*e.g.*, the ends of leaves or leaves that are being replaced by new growth, *etc*.). If you are sure these dead leaves are from the current growing season, then record as live.

Macroalgae identified by species. This category generally includes the rockweeds (*e.g.*, *Fucus*, *Ascophyllum*). Microalgae (*e.g.*, diatom mats) and fine filamentous algae are not included in this category.

Bare. Includes mud, sand, microalgae cover, *etc*. These are areas that are not flooded with water and are devoid of standing live, standing dead, or macroalgae. There can be a thin film of surface water within the bare category.

Water. Permanent standing water is identified in plots that are partly within a creek, ditch, marsh pool, or flooded panne.

Wrack/Litter. Wrack is material that has floated into the plot. This is generally dead (not attached) plant material, but could also be trash. Litter is dead plant material that is highly decomposed and is no longer attached.

Trash. Items such as logs, old piers, tires, *etc*.

Rock. Boulders or rocks can be found on the surface of northern New England marshes.

NOTES:

- If an intercept point has standing water that is covering a bare mud bottom, this point should be recorded as standing water. It is assumed that the bottom is bare and there is no need to record this.
- If macroalgae or submerged aquatic vegetation are hit at the intercept point in a standing water habitat, then both the plant and water should be recorded.
- If a plot is at the edge of a marsh pool (water), *Spartina* overhangs the water, and the intercept point hits the *Spartina* and water, then both *Spartina* and water should be recorded.

"water", "bare ground", "wrack or litter," and others are also recorded if they are "hit" by the rod. Table 5 provides definitions of cover type categories that should be included. After the first point is completed, the process is repeated for all remaining points in the sampling quadrat until all 50 points have been sampled. The total number of hits per species for each plot is then tallied on the data sheet (Table 4). Sampling of all marsh segments should occur within the same time frame (within 1-2 weeks of each other) and occur when the marsh surface is not flooded so that tidal waters do not conceal vegetation. This method is ideal for marshes with low vegetation as shown in Fig. 11. However, the method has been successfully used in marshes with taller vegetation canopies, like *Phragmites* and *Typha* marshes, or marshes dominated by shrubs (*e.g.*,

Iva). The observer needs to be careful to use a long rod and to look up to determine if higher vegetation touches the rod.

Associated Environmental Variables

Water Table Level

Water table level provides information on the amount of waterlogging or drainage that is occurring in a marsh. Water table level is an important parameter to use when attempting to understand why vegetation is changing. Water table level is measured using ground water wells. It is recommended that a water table level well be placed in association with each vegetation sampling plot. However, in some marsh study designs with numerous distinct segments or if numerous salt marshes are being monitored, then it may not be logistically feasible to include a well with each vegetation plot. Investigators may elect to establish a water table level wells at alternate vegetation plots, or even a less frequent arrangement.

For water table level and soil salinity, approximately 20-30 sample stations could be visited within a low tide period. Sampling can be accomplished by one person, but teams of people are always recommended when conducting field work.

Construction and installation of the wells is outlined below. Groundwater wells can also be purchased from hydrological supply companies.

Materials

- 1.5 inch (4 cm) interior diameter, schedule 40, PVC Tubes (comes in 10 ft lengths and can be purchased at home goods stores)
- PVC caps to fit the tubes. Two caps (rounded preferably) are required for each well
- ¼ inch drill bit
- Meter sticks
- Black medium tip permanent markers
- Mallets to pound wells into ground and blocks of wood to place on well top when wells are pounded
- All weather copier paper for field data sheets

Groundwater Well Fabrication

- Cut PVC into 70 cm lengths (4 wells per 10 ft of tube), 10 cm will be aboveground, 60cm will be belowground.
- Drill ¼ inch holes in the belowground section of the well (along the 10–60cm length of the well). Drill enough holes to allow water to percolate into the well. The top of the well is the 0-10cm section that has no drill holes, the bottom of the well is the section with the drill holes. To prevent surface water from entering the well the top 0-10cm section of the well is left intact.

- Place a cap on the bottom of each well. Well bottoms should fit snugly, but do not need to be glued.
- Draw a line 10cm down from the top of the well. In the field, this line will serve as a guide for how deep the well should be driven into the peat.
- The remaining caps are for the top of the wells.
- Drill a ¼ inch hole in the center of the remaining top well caps. These caps are used to prevent rainwater from entering the well. A hole is drilled in the center of the top cap for venting.
- Well top caps are installed in the field

Well Installation (refer to Fig. 12)

- Locate vegetation plot stake.
- Place groundwater well 1m away from the plot stake in the direction of the transect and pound well into the marsh.
- Pound well until only 10cm of well is above ground and all drill holes are below the marsh surface. Use 10cm mark on well as a guide.
- Label top cap (cap with center drill hole) with plot identification number. The well number will be the same as the plot stake number.
- Place top cap loosely on well top. Do not jam the cap onto the well top. These caps must be removed to measure the water table level.

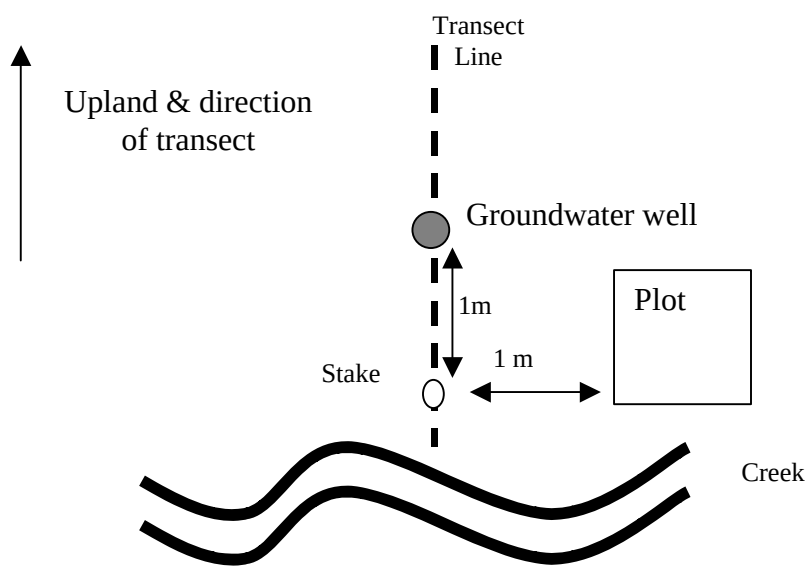


Figure 12. Schematic showing the location of groundwater well relative to stake and vegetation plot.

Sampling Procedure (refer to Fig. 13)

- Water table level should be measured within 2 hours of low tide (from 2hrs before to 2hr after low tide).
- Sampling should occur throughout the growing season, perhaps at 10 day to 2 week intervals
- Record well number
- Remove well cap
- Insert the meter stick into well (0mm end first) until the meter stick barely touches the water surface. By peering into the well as the meter stick is lowered you will be able to see the surface tension of the water break as the meter stick reaches the water surface.
- Record the measurement off the meter stick at the top of the well (Measurement A in Table 6).
- Record the height of the well from the marsh surface (Measurement B in Table 6). This measurement is important because the well could move from ice flows, freezing/thawing, trampling, vandalism, *etc.*
- The height of the well from the marsh surface is subtracted from the total distance of the top of the well to the water level. This will give the distance of the water level below the marsh surface. This calculation will be done back in the office and should not be done in the field. The above two numbers are all that is required to be recorded in the field.
- If the well is dry (no water in the well at all), record "dry" on the data sheet
- If the marsh surface is flooded, measure the depth of the water from the marsh surface to the water surface.
- Replace the top cap. Be sure not to jam the cap onto the well top.

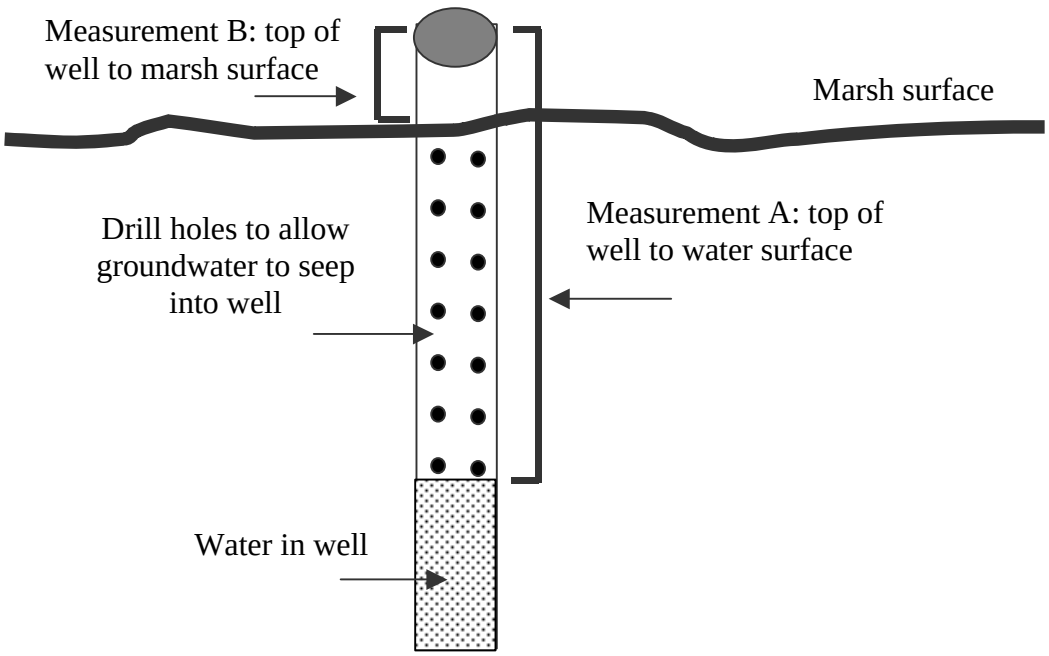


Figure 13. Schematic of groundwater well in place in the marsh

Soil Water Salinity

In addition to water table level, soil water salinity is an important factor controlling the patterns of salt marsh vegetation. It is not appropriate to sample soil water salinity from water within the groundwater wells for several reasons. First, most useful measurements will be from the of portion sediment that has the most active roots and rhizomes. This is generally from the marsh surface to 10-15 cm deep. The groundwater wells are integrating soil water from the surface to a greater depth (60cm). Second, water collected within the groundwater wells tends to stratify over-time, with denser high salinity water near the bottom of the well and fresher water near the surface of the well. The well could be pumped dry before each sampling event, allowed to fill, and then the water in the well sampled for salinity; however, the process of filling could take several hours (although filling is quite rapid for some wells, depending on soil porosity). To avoid these problems with sampling water from the groundwater wells, a soil probe in recommended for collecting soil water.

Materials for Soil Salinity

- Soil probe, constructed of stainless steel tubing (gas chromatograph tube, 0.065 in inner diameter, 0.085in outer diameter), cut to about 70cm length, with one end crimped and slotted to allow entry of soil water (Fig. 14)
- 10-15ml plastic syringe, or larger volume syringe up to 5ml.
- 5cm length of plastic tubing to attach the soil probe to the syringe.
- Salinity hand-held refractometer
- Filter paper (cut-up coffee filters are fine)
- Plastic squeeze bottle with freshwater to rinse and calibrate refractometer
- Data sheets and pencils (Table 6)

Soil Probe Fabrication

- Make 3 – 4 slits approximately 5mm apart and 2.5cm from one end of the metal tubing. The slits can be made with a roto-tool or a fine blade hacksaw. The slits are to allow water to be drawn up into the tube (Fig. 14)
- Close the end of the metal tube (nearest to the slits) by crimping with pliers.
- Attach a short length of plastic tubing to the uncrimped end of the metal tubing.
- Attach the syringe to the other end of the plastic tubing
- Make sure that water can be drawn up into the tubing by pulling the plunger on the syringe
- Mark increments of 15cm, 30cm, and 45cm on the metal tube with tape so that depth of the soil salinity sample can easily be determined.

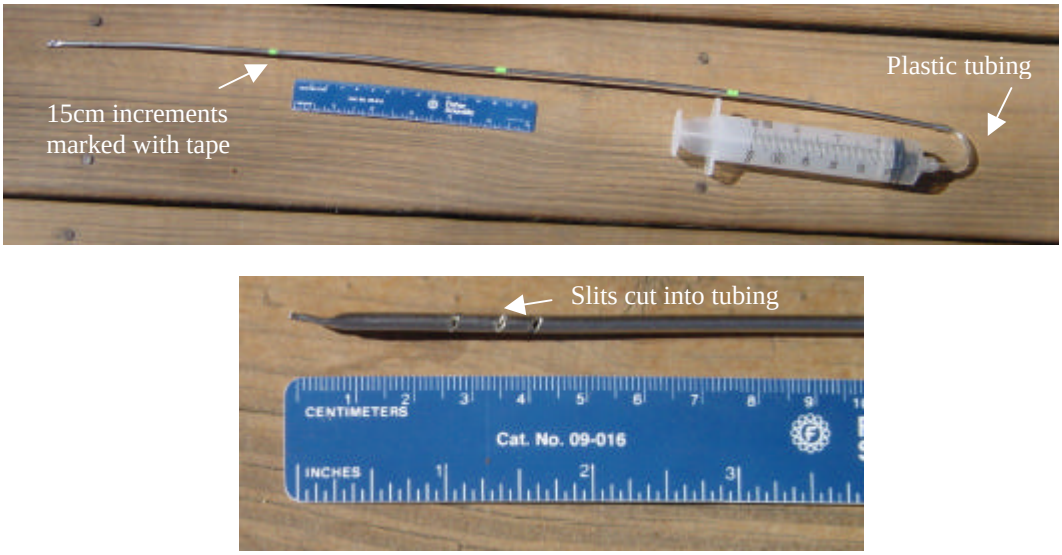


Figure 14. Photograph of a soil probe used to sample soil water salinity.

Sampling Procedure

- Sampling should coincide with groundwater well sampling. Always measured within 2hrs of low tide.
- Calibrate (zero) hand-held salinity refractometer with fresh water (tap is okay) before EACH field day.
- At a location near the groundwater well, insert the soil salinity probe (crimped end downward) 15cm into the sediment (Tape can be used to mark 15cm). The plastic syringe is attached to the top of the probe. Carefully withdraw the plunger to collect soil water.
- Once several milliliters (just a few drops) of water have been withdrawn into the syringe, detach it from the probe. If the marsh is dry at 15cm, then insert the probe deeper (30cm, then 45cm) until soil water is collected. Record the depth that soil water was collected. Record dry if no soil water was collected at 45cm.
- Place a piece of filter paper over the nozzle of the syringe. Depress the syringe plunger and let the water pass through the filter paper and onto the glass plate of the refractometer.
- Read and record the soil water salinity (Table 6). The station location for the soil salinity is the same as the water table level station and vegetation plot.
- Clean-up. Discard (never re-use) the filter paper. Using water from the groundwater well or a nearby creek, rinse silt and sediment from the probe by drawing up water into the syringe. Discard all the water in the syringe and probe before sampling the next station. Rinse refractometer with freshwater; dry refractometer.
- SAMPLING FREQUENCY: Soil salinity should be sampled in conjunction with groundwater sampling (10-14 day intervals during the growing season).

Table 6. Example of a field data sheet for water table level and soil salinity monitoring.

Water Table Level & Soil Salinity Monitoring

SITE _____ DATE _____

Data Collector(s) _____

Note: *If water is below marsh surface indicate water table level with a negative sign in "Depth Column". If water is on the marsh surface, write "surface" in Column A, measure water depth and record depth with a positive sign in "Depth" column.*

Plot No.	Time	A. Top of Well to Water (cm)	B. Top of Well to Marsh (cm)	Depth to Water Table (A-B)[1]	Salinity (ppt)	Depth if other than 15 cm

[1] calculate in lab.

Soil Sulfides (an optional monitoring variable)

Under waterlogged and anaerobic soil conditions sulfide concentrations can rise to levels that are toxic to root metabolism, often inhibiting nitrogen uptake and plant growth (Howes et al 1986, Koch *et al.* 1990). When restoring salt marshes by re-introducing tidal flow it is suggested that soil porewater sulfide be monitored as a measure to help understand why vegetation patterns are changing. For example, if tidal flow is re-introduced to a wetland site and the soils become waterlogged then high sulfide levels could result. Sulfide toxicity from waterlogged soils can stress *Phragmites* growth, while *Spartina alterniflora* is more tolerant of high sulfide (Chambers *et al.* 1998).

Field and laboratory methods for total sulfides in salt marsh porewaters are described in detail by Portnoy and Giblin (1997) and will only be summarized here.

Materials

- Soil probe (same probe as used for salinity as described above), constructed of stainless steel tubing (gas chromatograph tube, 0.065 inch inner diameter, 0.085inch outer diameter), cut to about 70 cm length, with one end crimped and slotted to allow entry of soil water.
- 10-15 ml plastic syringe, or larger volume syringe up to 50 ml.
- Small volume pipette.

Sampling Procedures (Field and Lab)

- Sampling should coincide with groundwater well and soil salinity sampling. Always measured within 2 hrs of low tide.
- At a location near the groundwater well, insert the soil salinity probe (crimped end downward) 15 cm into the sediment (Tape can be used to mark 15cm). The plastic syringe is attached to the top of the probe. Carefully withdraw the plunger to collect soil water. Be certain that air is purged from the probe and syringe prior to sampling using a three-way valve.
- Once several milliliters of water have been withdrawn into the syringe, detach it from the probe. If the marsh is dry at 15cm, insert the probe deeper until soil water is collected. Record the depth that soil water was collected.
- In the field, the porewater sample is collected from the syringe with a pipette and discharged into 2% zinc acetate and stored on ice. Volume of the pipette depends on expected sulfide concentration, but 0.1 ml is often appropriate.
- Sulfide is determined colorimetrically after Cline (1969).
- SAMPLING FREQUENCY: Soil sulfide should be sampled at least monthly during the growing season in conjunction with groundwater and soil salinity sampling.

Data Management

Data should be recorded on standardized data sheets (Tables 4 and 6). After sampling vegetation, the total number of hits per species per plot should be tallied and entered into a spreadsheet program. We will briefly describe the data entry format for a non-parametric permutation procedure aimed at assessing similarity. Once data are entered into the spreadsheet it can be manipulated into the appropriate format for other statistical programs (*e.g.* ordination procedures).

Vegetation data should be entered into a spreadsheet where the columns represent the species and the rows represent the individual sample plots. Column and row labels should be no more than 8 characters in length. It is necessary to have a complete list of all species that occur in all plots prior to data entry. The total tally of hits per species for each quadrat is entered next to the respective species on the list. If a particular species is absent from a sample quadrat its value is entered as zero ("0") in the spreadsheet. Table 7 illustrates the spreadsheet layout. Once the data are entered into the spreadsheet it should be verified against the field data sheets for accuracy. To reduce the importance of dominant species the percent cover data can be coded according to the Braun-Blanquet cover scale (0, 1: <1-5%, 2: 6-25%, 3: 26-50%, 4: 51-75%, 5: 76-100%). Conversion to cover ranks is strongly suggested for similarity testing and ordination procedures.

Table 7. Example of spreadsheet layout for vegetation data that will be analyzed by ANOSIM. The number in each cell represents the total tally of hits (out of a maximum of 50 hits) for that species in that plot. T1-P1 = Transect 1, plot 1; Dis_spic = *Distichlis spicata*; Phr_aust = *Phragmites australis*.

Species	Dis_ spic	Phr_aust	Spa_alte	Bare	Water
T1-P1	15	36	43	3	5
T1-P2	2	1	0	42	37
T1-P3	39	8	27	8	2
T2-P1	0	0	0	24	15
T2-P2	0	2	0	5	0

Data Analysis Techniques

Non-parametric permutation testing procedures can be effectively used to evaluate dissimilarity or similarity in vegetation communities between marshes or between sample years. ANOSIM, part of the PRIMER statistical package (Plymouth Routines In Multivariate Research, Carr 1997) is just one example of a non-parametric test, similar to multivariate analysis of variance (MANOVA) but without the generally unattainable assumptions (Clarke and Warwick 1994, Carr 1997). Non-parametric permutation similarity procedures use a similarity metric and we suggest using Euclidean Distance as our sample size estimate was based on this same metric (see Fig. 5).

To determine individual species that contribute to any observed differences detected between marshes or between years we suggest the following procedure:

$$1 - \frac{D}{D_{max}} = 1 - \frac{(C_{1i} - C_{2i})^2}{\Sigma\ (C_{1i} - C_{2i})^2}$$

Where;
D = Distance
C_{1i} = cover of species i in marsh 1
C_{2i} = cover of species i in marsh 2

Ordination techniques, such as detrended correspondence analysis (DCA), which has also been discussed in Part One of this protocol, can also be performed on the data. Ordination techniques are part of the family of exploratory data analyses that enable researches to formulate ideas about community structure as well as casual relationships between variation in vegetation and environmental factors (Kent and Coker 1992).

If additional data are collected, such as the height of *Phragmites australis*, these data can be analyzed by Analysis of Variance to determine if there are differences in height between marshes (unrestricted vs. tide-restricted) or between years (pre-restoration vs. post-restoration).

Groundwater well data, salinity data, and sulfide data are best analyzed by ANOVA to detect differences among marshes, seasons, and years.

Equipment List

Equipment necessary to conduct the minimum vegetation monitoring protocol is listed below. Additional gear will be necessary if other or different environmental parameters are to be included (*e.g.*, ground water level, soil salinity, sulfide). See protocol for equipment lists for these other methods.

<u>Essential Gear</u>
Map of transect and plot locations
Plot stakes
Meter tape to measure out plot locations
Compass to lay out transects
Meter Stick
4 dowels marked in 20cm increments
1 thin rod
Plant Identification guides
Waterproof notebooks / datasheets
Pencils
Permanent marker to label stakes

Personnel

At least 2 people are required to sample vegetation, one to place the bayonet and the other to record data. It is estimated that 2 people, who are familiar with salt marsh plant identifications, can sample approximately 10-20 plots per day. This does not include time spent re-locating plots that have lost their stakes. If plots need to be relocated it is suggested that this be done prior to sampling.

For water table level and soil salinity, approximately 20-30 sample stations could be visited within a low tide period. Sampling can be accomplished by one person, but teams of people are always recommended when conducting field work.

Literature Cited

Able, K.W., K.L. Heck, Jr., M.P. Fahay, and C.T. Roman. 1988. Use of salt-marsh peat reefs by small juvenile lobsters on Cape Cod, Massachusetts. *Estuaries* 11: 83-86.

Aubrey, D.G., and P.E.Speer. 1985. A study of non-linear tidal propagation in shallow inlet/estuarine systems. Part I: observations. *Estuarine, Coastal and Shelf Science* 21: 185-205.

Austin, M.P. 1977. Use of ordination and other multivariate descriptive methods to study succession. *Vegetatio* 35: 165-175.

Ayvazian S.G., L.A. Deegan, and J.T. Finn. 1992. Comparison of habitat use by estuarine fish assemblages in the Acadian and Virginian zoogeographic provinces. *Estuaries* 15: 368-383.

Bakker, J.P., H. Olff, J. H. Willems, and M. Zobel. 1996. Why do we need permanent plots in the study of long-term vegetation dynamics? *Journal of Vegetation Science* 7:147-156.

Barrett, N.E., and W.A. Niering. 1993. Tidal marsh restoration: trends in vegetation change using a Geographical Information System (GIS). *Restoration Ecology* 1: 18-28.

Bertness, M.D. 1999. *The Ecology of Atlantic Shorelines*. Sinauer Associates Inc., Sunderland, MA. 417 pp.

Bourn, W.S. and C. Cottam. 1950. Some biological effects of ditching tidewater marshes. Research Report 19. US Fish and Wildlife Service, Washington, DC.

Brush, T., R.A. Lent, T. Hruby, B.A. Harrington, R.M. Marshall, and W.G. Montgomery. 1986. Habitat use by salt marsh birds and response to Open Marsh Water Management. *Colonial Waterbirds* 9: 189-195.

Burdick, D.M., M. Dionne, R.M. Boumans, and F.T. Short. 1997. Ecological responses to tidal restorations of two northern New England salt marshes. *Wetlands Ecology and Management* 4: 129-144.

Burger, J., J.K. Shisler, and F.H. Lesser. 1982. Avian utilization on six salt marshes in New Jersey. *Biological Conservation* 23: 187-212.

Carr, M. R. 1997. PRIMER (Plymouth Routines in Multivariate ecological Research) User Manual. Plymouth: Plymouth Marine Laboratory, 27+ p.

Chambers, R.M., T.J. Mozdzer, and J.C. Ambrose. 1998. Effects of salinity and sulfide on the distribution of *Phragmites australis* and *Spartina alterniflora* in a tidal salt marsh. *Aquatic Botany* 62: 161-169.

Clarke, K.R. and Green, R.H. 1988. Statistical design and analysis for a 'biological effects' study. *Mar. Ecol. Prog. Ser.* 92:205-219.

Clarke, K. R. and Warwick, R. M. 1994. Change in marine communities: an approach to statistical analysis and interpretation. Plymouth: Plymouth Marine Laboratory, 114 p.

Cline, J.D. 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnology and Oceanography* 14: 454-458.

Dahl, T.E. 1990. Wetlands losses in the United States, 1780's to 1980's. United States Department of the Interior, Fish and Wildlife Service, Washington, DC. 21 pp.

Daiber, F.C. 1986. *Conservation of Tidal Marshes*. Van Nostrand Reinhold Co., New York.

Dean, R.G. 1979. Effects of vegetation on shoreline erosional processes, p. 415-426. *In* P.E. Greeson, J.R. Clark and J.E. Clark (eds.), Wetland Functions and Values: The State of our Understanding. American Water Resources Association, Minneapolis, MN.

Elzinga, C. L., Salzer, D. W., and Willoughby, J. W. 1998. Measuring and Monitoring Plant Populations. USDI, BLM National Applied Resources Center, Denver, CO; July 1998. BLM/RS/ST-98/005+1730. 492 p.

Floyd, D. A. and Anderson, J. E. 1987. A comparison of three methods for estimating plant cover. *Journal of Ecology* 75:221-228.

Godfrey, P.J., K. Galluzzo, N. Price, and J. Portnoy. 1999. Water Resources Management Plan, Cape Cod National Seashore, Massachusetts. National Park Service, Cape Cod National Seashore, Wellfleet, MA.

Green, R. H. 1979. Sampling Design and Statistical Methods for Environmental Biologists. J. Wiley & Sons. New York. 257 p.

Greig-Smith, P. 1983. *Quantitative Plant Ecology*. University of California Press, Berkeley. 359p.

Heck, K.L., Jr., K.W. Able, M.P. Fahay, and C.T. Roman. 1989. Fishes and decapod crustaceans of Cape Cod eelgrass meadows: species composition, seasonal abundance patterns and comparison with unvegetated substrates. *Estuaries* 12: 59-65.

Heck, K.L., Jr., K.W. Able, C.T. Roman, and M.P. Fahay. 1995. Composition, abundance, biomass, and production of macrofauna in a New England estuary: comparisons among eelgrass meadows and other nursery habitats. *Estuaries* 18: 379-389.

Hellings, S.E. and J.L Gallagher. 1992. The effects of salinity and flooding on *Phragmites australis*. *Journal of Applied Ecology* 29: 41-49.

Howes, B.L., J.W.H. Dacey, and D.D. Goehringer. 1986. Factors controlling the growth form of *Spartina alterniflora*: feedbacks between above-ground production, sediment oxidation, nitrogen and salinity. *Journal of Ecology* 74: 881-898.

Howes, B.L., P.K. Weiskel, D.D. Goehringer and J.M. Teal. 1996. Interception of freshwater and nitrogen transport from uplands to coastal waters: the role of saltmarshes, p. 287-310. *In*, K.F. Nordstrom & C.T. Roman (eds.), Estuarine Shores: Evolution, Environments and Human Alterations. John Wiley & Sons, NY.

Intergovernmental Panel on Climate Change. 1995. Climate Change. *In* J.T. Houghton, L.G. Meira Filho, B.A. Callendfar, N. Harris, A. Kattenberg, and K. Maskell (eds.), The Science of Climate Change. Cambridge University Press, NY 572 pp.

Kennedy, K.A., and P.A. Addison. 1987. Some considerations for the use of visual estimates of plant cover in biomonitoring. *Journal of Ecology* 75: 151-157.

Kent, M. and P. Coker. 1992. *Vegetation Description and Analysis*: A Practical Approach. John Wiley & Sons.

Koch, M.S., I.A. Mendelsshon, and K.L. McKee. 1990. Mechanism for the hydrogen-sulfide-induced growth limitation in wetland macrophytes. *Limnology and Oceanography* 35: 399-408.

Krebs, C.J. 1989. *Ecological Methodology*. Harper Collins. 654 p.

McDonnell, M. J. 1997. A simple below-ground marker for permanent plots in urban and suburban environments. *Urban Ecosystems* 1:179-181.

Miller, W.R., and F.E. Egler. 1950. Vegetation of the Wequetequock-Pawcatuck tidal-marshes, Connecticut. *Ecological Monographs* 20: 143-172.

Neckles, H. A. and M. Dionne, editors. 2000. Regional standards to identify and evaluate tidal wetland restoration in the Gulf of Maine. (T. Diers and C. Roman. Ecosystem Indicator – Vegetation, p. 12-14). Wells National Estuarine Research Reserve Technical Report. Wells, ME.

Niering, W. A. and Warren, R. S. 1980. Vegetation patterns and process in New England salt marshes. *Bioscience* 30:301-307.

Nixon, S.W. 1982. The ecology of New England high salt marshes: a community profile. Technical report FWS/OBS-81/85. US Fish and Wildlife Service, Office of Biological Services, Washington, DC. 70p.

Nixon, S.W. and C.A. Oviatt. 1973a. Ecology of a New England salt marsh. *Ecological Monographs* 43: 463-498.

Nixon, S.W. and C.A. Oviatt. 1973b. Analysis of local variation in the standing crop of *Spartina alterniflora*. *Botanica Marina* 16: 103-109.

Pickett, S. T. A., S. L. Collins, and J. J. Arnesto. 1987. A hierarchical consideration of causes and mechanisms of succession. *Vegetatio* 69:109-114.

Poissonet, P. S., Daget, P. M., Poissonet, J. A., and Long., G. A. 1972. Rapid point survey by bayonet blade. *Journal of Range Management* 25:313.

Poissonet, P.S., J.A. Poissonet, M.P. Godron, and G.A. Long. 1973. A comparison of sampling methods in dense herbaceous pasture. *Journal of Range Management* 26: 65-67.

Portnoy, J.W., and A.E. Giblin. 1997. Effects of historic tidal restrictions on salt marsh sediment chemistry. *Biogeochemistry* 36: 275-303.

Portnoy, J.W., B. L. Nowicki, C.T. Roman, and D.W. Urish. 1998. The discharge of nitrate-contaminated groundwater from developed shoreline to marsh-fringed estuary. *Water Resources Research* 34: 3095-3104.

Portnoy, J, and M. Reynolds. 1997. Wellfleet's Herring River: the case for habitat restoration. *Environment Cape Cod* 1: 35-43.

Roman, C.T. 2001. Salt marsh vegetation, p. 2487-2490. *In* J.H. Steele, K.K. Turekian, and S.A. Thorpe (eds.), Encyclopedia of Ocean Sciences. Academic Press, London.

Roman, C.T., and N.E. Barrett. 1999. Conceptual framework for the development of Long-term monitoring protocols at Cape Cod National Seashore. Technical Report, USGS Patuxent Wildlife Research Center, Coastal Research Field Station, Narragansett, RI. 59p.

Roman, C.T., R.A. Garvine, and J.W. Portnoy. 1995. Hydrologic modeling as a predictive basis for ecological restoration of salt marshes. *Environmental Management* 19: 559-566.

Roman, C.T., N. Jaworski, F.T. Short, S. Findlay, and R.S. Warren. 2000. Estuaries of the northeastern United States: habitat and land use signatures. *Estuaries* 23: 743-764.

Roman, C.T., W.A. Niering, R.S., Warren. 1984. Salt marsh vegetation change in response to tidal restriction. *Environmental Management* 8: 141-150.

Roman, C.T., J. A. Peck, J.R. Allen, J.W. King, P.G. Appleby. 1997. Accretion of a New England (U.S.A.) salt marsh in response to inlet migration, storms, and sea-level rise. *Estuarine, Coastal and Shelf Science* 45: 717-727.

Roman, C.T., K.B. Raposa, S.C. Adamowicz, M.J. James-Pirri, and J.G. Catena. IN PRESS. Quantifying vegetation and nekton response to tidal restoration of a New England salt marsh. *Restoration Ecology*.

Sinicrope, T.L., P.G. Hine, R.S. Warren, and W.A. Niering. 1990. Restoration of an impounded salt marsh in New England. *Estuaries* 13: 25-30.

Smartt, P.F.M., S.E. Meacock, and J.M. Lambert. 1974. Investigations into the properties of quantitative vegetational data. I. Pilot study. *Journal of Ecology* 62: 735-759.

Smartt, P.F.M., S.E. Meacock, and J.M. Lambert. 1976. Investigations into the properties of quantitative vegetational data. II. Further data type comparisons. *Journal of Ecology* 64: 41-78.

Smith, E.P., Pontasch, K.W. and Cairnes, J., Jr. 1990. Community similarity and the analysis of multispecies data: a unified statistical approach. *Water Research* 24:507-514.

Stewart-Oaten, A., J.R. Bence, and C.W. Osenberg. 1992. Assessing effects of unreplicated perturbations: no simple solutions. *Ecology* 73: 1396-1404.

Stewart-Oaten, A., W.W. Murdoch, and K.R. Parker. 1986. Environmental impact assessment: "pseudoreplication" in time? *Ecology* 67: 929-940.

Sutherland, W.J., editor. 1996. *Ecological Census Techniques: a handbook*. Cambridge University Press, Cambridge, UK. 336p.

ter Braak, C. J. F. 1987. The analysis of vegetation-environment relationships by canonical correspondence analysis. *Vegetatio* 64: 69-77.

Tiner, R.W., Jr. 1984. Wetlands of the United States: current status and recent trends. US Fish and Wildlife Service, National Wetlands Inventory, Washington, DC. 59 pp.

Titus, J.G. 1991. Greenhouse effect and coastal wetland policy: How Americans could abandon an area the size of Massachusetts at minimum cost. *Environmental Management* 15: 39-58.

Underwood, A.J. 1992. Beyond BACI: the detection of environmental impacts on populations in the real, but variable, world. *J. of Experimental Marine Biology and Ecolog*y 161: 145-178.

Valiela, I., J.M. Teal, and W. Saas. 1975. Production and dynamics of salt marsh vegetation and the effects of experimental treatment with sewage sludge: biomass, production and species composition. *J. Applied Ecology* 12: 973-982.

Warren, R.S., and W.A. Niering. 1993. Vegetation change on a northeast tidal marsh: interaction of sea-level rise and marsh accretion. *Ecology* 74: 96-103.

Wolfe, R.J. 1996. Effects of open marsh water management on selected tidal marsh resources: a review. *J. American Mosquito Control Association* 12: 701-712.

Zar, J. H. 1999. *Biostatistical Analysis.* 4th Ed. Prentice Hall, New Jersey. 663 plus appendices.