

# Maine's Most Pristine Wetlands: Implementing a Long-Term Monitoring Plan



*The Great Heath, T18 MD BPP*

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## **ABSTRACT**

Wetlands fulfill a variety of ecosystem functions and human values. Consequently, their health is integral to the health of natural communities and human societies. Ecological Reserves are state lands that have been set aside by an act of the Maine State Legislature for the purposes of maintaining natural community types in their natural conditions, protecting Maine's biological diversity, as a benchmark against which biological and environmental change may be measured, and as sites for scientific research and long-term environmental monitoring.

The Maine Natural Areas Program (MNAP) administers an inventory and monitoring program for Ecological Reserves and is currently conducting monitoring in forested natural communities, including forested wetlands. In order to ensure that the program goals are being met, a protocol must also be developed to monitor the status and trends of open wetlands on Ecological Reserves. Current monitoring protocols for open wetlands in the Northeast were assessed for their relevance to MNAP's monitoring goals and a new wetland monitoring protocol is recommended here.

Several common ecological indicators were considered for inclusion in the proposed protocol. Vegetation characteristics and soil pore salinity were selected as key indicators based on their ability to meet MNAP's monitoring goals, and their ease of implementation. Transect/quadrat sampling methods for the chosen indicators were developed and field tested on four undisturbed wetlands representing a range of biodiversity. Species-area curves were calculated to determine the appropriate sampling intensity. PerMANOVA tests were run using PC-ORD to determine the degree of difference between an individual wetland over time. Protocols for monitoring with remote sensing imagery and on-site photographic "sampling" provide a permanent record of the site at the time of sampling and as a benchmark to support monitoring in the future.

If implemented, this long-term wetland monitoring program will document wetland change over time, and provide reference data for comparison with wetlands not located within Ecological Reserves.

## **1.0 INTRODUCTION**

The mission of the Maine Natural Areas Program (MNAP) “is to ensure the maintenance of Maine's natural heritage for the benefit of present and future generations and facilitate informed decision-making in development planning, conservation, and natural resources management (Maine Natural Areas Program, 2005).” The Program's success relies upon consistent and objective methods to collect, organize, and interpret information. Designation of Ecological Reserves was enabled by an act of the Maine Legislature (Chapter 592, MSRA Section 13076) in 2000. As of 2009, Maine has designated approximately 84,000 acres of Ecological Reserves within 16 public land units managed by the Maine Department of Conservation (DOC). In addition, the Maine Department of Inland Fisheries and Wildlife (IFW) manages 11,000 acres designated as Ecological Reserves. As specified in the legislation, the purposes of the reserves are:

1. to maintain one or more natural community types or native ecosystem types in a natural condition and range of variation and contribute to the protection of Maine's biological diversity
2. as a benchmark against which biological and environmental change may be measured,
3. as a site for ongoing scientific research, long-term environmental monitoring and education
4. to protect sufficient habitat for those species whose habitat needs are unlikely to be met on lands managed for other purposes (Maine Natural Areas Program, 2005)

A long-term Ecological Reserve monitoring plan was developed by a multi-disciplinary committee in 2003 (Cutko, 2003). This plan has been applied to state Ecological Reserves as well as more than 100,000 acres of other Maine lands managed in compatible ways by The Nature Conservancy and the Appalachian Mountain Club. The 2003 plan described a monitoring protocol for forested areas, including forested wetlands on Ecological Reserves. The forest monitoring protocol is based on the USDA Forest Service’s Forest Inventory and Analysis Program (FIA), which allows data from Ecological Reserves to be compared to a large data set for forests all over Maine. This protocol uses permanent plots on randomly placed transects and measures a variety of vegetation and forest structure characteristics as they change through time. The first round of sampling on all forested reserves is scheduled for completion during the summer of 2010 (Cutko, 2009).

Wetlands play important roles in ecosystem function, water resource management, and provide many other ecosystem services, thus serving a critical role on Ecological Reserves. One quarter of Maine is covered by wetlands (USFWS National Wetlands Inventory, 2009), approximately 8% of the area in

Ecological Reserves is open wetland, and 40 of the 104 natural community types in Maine are open wetlands (Maine Natural Areas Program, 2005). It is vital that these communities be monitored so that detrimental activities or trends over time can be documented and addressed rapidly if they arise. Information collected on a sample of “pristine” wetlands will serve as a baseline for evaluating the health of impacted and unprotected wetlands, and to ensure that quality habitat is being conserved. Thus, wetland monitoring is essential to MNAP’s mission and protection of Ecological Reserves.

Several other state and federal agencies and non-profit organizations are conducting wetland monitoring and water quality assessments in New England. Table 1 in the following section summarizes the variables included in their monitoring protocols. However, protocols developed to meet other agencies’ objectives are not able to meet MNAP’s monitoring goals and abilities. Specifically, these protocols focus on one-time wetland health/status assessment instead of long-term monitoring, are designed for wetlands with standing water, use expensive equipment that MNAP cannot easily acquire, and/or require more site visits than are feasible for MNAP’s Ecological Reserve monitoring efforts.

The development of a new wetland monitoring plan, therefore, is needed to protect and manage Ecological Reserves in Maine. This report documents efforts to develop such a plan.

## **2.0 BACKGROUND**

Wetland monitoring is not a new endeavor. Several governmental agencies and nonprofit organizations are conducting wetland monitoring in the Northeast (Neckles and M.Dionne, 2000; Northern Ecological Associates Inc., 2001; Maine DEP Bureau of Land and Water Quality, 2005; Neckles et al., 2009). During the summer and fall of 2009, a literature review, personal interviews, and field visits were conducted to determine how existing monitoring protocols could guide the development of a wetland monitoring protocol for MNAP. Several documents provide summaries of wetland monitoring parameters and variables in use around this region (Northern Ecological Associates Inc., 2001; U.S. EPA, 2002a; Faber-Langendoen, 2006). Water chemistry, water table depth, soil texture/organic content, plant species, percent cover, and presence of species of concern emerged as the most useful monitoring variables.

An effort was made in the development of a new wetland monitoring protocol to choose variables and sampling techniques that will allow MNAP to share and compare data with other agencies. However, the wide variety of methods already in use and the differing goals of the monitoring protocols made this difficult. This proposed protocol concentrates on the vegetative variables, due to the ease and relatively low cost of vegetation sampling, and their relevance to MNAP's mission and goals.

Two studies have been conducted to determine the statistical power of current wetland monitoring field techniques in the region (Konisky et al., 2006; James-Pirri et al., 2007). These studies and the protocols associated with them (Neckles and M.Dionne, 2000), as well as the National Park Service's (NPS) Northeast Temperate Network's (NETN) draft freshwater wetland monitoring protocol (Neckles et al., 2009), guided the development of the field sampling methodology for this protocol. James-Pirri et al. (2007) determined that 20 randomly located one meter-square vegetation plots were sufficient to detect change in the vegetation community in salt marshes over time, regardless of the size of the marsh or permanency of the plots. Konisky et al. (2006) used one-way analysis of variance (ANOVA) to test for significant differences between monitoring variables compiled from several projects over multiple years. These findings have guided, to a large degree, the monitoring protocol proposed here.

## **2.1 WETLANDS ON ECOLOGICAL RESERVES**

In creating a wetland monitoring protocol for MNAP, the first step was to define and identify the types of wetlands that exist within Ecological Reserves. The U.S. Fish and Wildlife Service (USFWS) describes wetlands as lands where "saturation with water is the dominant factor determining the nature of soil



development and the types of plant and animal communities living in the soil and on its surface” (Cowardin, 1979). According to this definition, wetlands also must have one or more of the following attributes: “(1) at least periodically, the land supports predominantly hydrophytes; (2) the substrate is predominantly undrained hydric soil; and (3) the substrate is nonsoil and is saturated with water or covered by shallow water at some time during the growing season of the year” (Cowardin, 1979).

The Cowardin classification scheme, developed for the National Wetland Inventory (NWI), arranges wetlands into systems, subsystems, and classes. Classes relate to the dominant vegetation and substrate type. Wetlands on Ecological Reserves were mapped and summarized according to the Cowardin classification in order to facilitate the selection of appropriate long-term monitoring sites. Although MNAP’s mission refers more specifically to the maintenance of natural community types, because of the much wider availability of data and maps using the Cowardin system, it has been used as a proxy for natural community types for this proposed protocol. This proposed protocol focuses solely on the estuarine and palustrine, emergent and scrub-shrub wetland types in the Cowardin classification and reflects the diversity and abundance of estuarine and palustrine wetlands in the state of Maine.

This proposed wetland monitoring protocol represents the minimum input recommended in order to meet MNAP’s monitoring goals and to provide a scientifically based, cost-effective method for monitoring change in open wetlands on Ecological Reserves. A variety of methods have been evaluated based on ease of implementation, efficiency in achieving monitoring goals, and compatibility with MNAP’s current monitoring efforts and skills. The methods have been field tested: preliminary evaluation of the data suggests that this protocol provides statistical rigor and will achieve MNAP’s monitoring goals.

In accordance with the purpose of the Ecological Reserve System, this protocol will help determine if natural communities are being maintained in their natural condition and if their biological diversity is being protected. In addition, this protocol will establish benchmarks against which to assess biological and environmental change, and contribute to scientific research and current monitoring efforts on Ecological Reserves. The basic field sampling plan is presented below, including modifications for application in salt marshes. Recommendations for the both the expansion and reduction of monitoring efforts are also discussed.

### **3.0 PROPOSED PROTOCOL GOALS AND OBJECTIVES**

The goals for the field sampling protocol include:

- 1) Establishing baseline information against which ecological changes in open wetlands on Ecological Reserves may be measured over time.
- 2) Collecting representative information for Reserves that can be compared with information on impaired or degraded wetlands.

To accomplish these goals, the following objectives will be addressed:

- 1) Collect a set of attributes for each study wetland, including natural community type, Cowardin classification, location, general description, and water regime. These factors will help match impacted wetlands with a similar reference wetland on an Ecological Reserve.
- 2) Determine status and trends in both the plant species richness and percent cover of each plant species in each study wetland.
- 3) Collect data on pore-water salinity in salt marshes.
- 4) Visually monitor the wetland through permanent photographic plots.
- 5) Provide recommendations for monitoring with remote sensing.

#### **4.0 DEVELOPMENT OF THE FIELD SAMPLING PROTOCOL**

Ideally, wetlands on Ecological Reserves are protected from outside threats associated with human development, altered hydrology, and invasive species encroachment. However, land use changes and hydrological alterations near Ecological Reserves can potentially affect even protected wetlands.

Monitoring protected wetlands can help differentiate changes caused by local factors from changes due to regional alterations of hydrology and climate for all wetlands in a region. Looking at which plants change most dramatically might also help identify which species may be in need of special protection or management (e.g. removal of invasive species). Therefore, the following monitoring questions guided the protocol development:

- 1) Does a wetland differ significantly from itself at another point in time?
- 2) If a wetland shows significant change overall, then what species have increased or decreased in relative abundance and contributed the most to the change?
- 3) How much does an impacted wetland differ from a similar reference wetland?
- 4) If an impacted wetland is different from a reference wetland, which species have contributed the most to that difference?

#### **4.1 SELECTION OF MONITORING VARIABLES**

During the summer of 2009, a survey of wetland inventory and monitoring protocols from northeastern New England was conducted to identify variables and indicators that are already in use in the region.

Ideally, MNAP's wetland monitoring protocol should be compatible with other monitoring efforts.

Detailed descriptions of many common wetland variables and indicators are available elsewhere (Neckles and M.Dionne, 2000; Neckles et al., 2009) so a brief discussion and justification of inclusion or omission will be presented here. Vegetation, water chemistry, hydrology, macroinvertebrates, algae, nekton, birds, and sediment deposition were found to be the most common indicators across protocols (Table 1). Each of these indicators was examined in order to determine its appropriateness for use in MNAP's protocol.

Table 1: Parameters and variables for wetland monitoring in use by multiple agencies and non-profit organizations, after (Northern Ecological Associates Inc., 2001).

Parameter and Variable	GPAC	USFWS	DEP	EPA	NETN	MNAP
<b>Hydrology</b>						
Water chemistry	<input type="checkbox"/>		■	■	■	
Wetland elevation	■			■		<input type="checkbox"/>
Water table depth	<input type="checkbox"/>	■		■	■	
Tidal signal (pattern of water level change)	■	■				
Extent of tidal flooding	<input type="checkbox"/>					
<b>Soils and Sediments</b>						
Soil water salinity	■	■				■
Soil texture/organic content	■	■		■		
Sediment/organic accretion on surface	<input type="checkbox"/>					
Sediment/organic loss from surface	<input type="checkbox"/>					
<b>Vegetation</b>						
Species	■			■	■	■
Percent cover	■			■	■	■
Presence of species of concern	■	■	■	■	■	■
<b>Nekton</b>	<input type="checkbox"/>	<input type="checkbox"/>	■			
<b>Invertebrates</b>		■	■			
<b>Birds</b>	<input type="checkbox"/>	<input type="checkbox"/>				<input type="checkbox"/>

■ Primary variable

Secondary variable

GPAC = Global Programme of Action Coalition for the Gulf of Maine (Neckles and M.Dionne, 2000)

USFWS = Salt Marsh Restoration Monitoring Plan (Northern Ecological Associates Inc., 2001)

DEP = Maine Department of Environmental Protection (Maine Department of Environmental Protection Bureau of Land and Water Quality, 2005)

EPA = Methods for Evaluating Wetland Condition: Introduction to Wetland Biological Assessment (U.S. EPA, 2002a), (U.S. EPA, 2002b)

NETN = Protocol for Monitoring Freshwater Wetlands in National Parks of the Northeast Temperate Network (Neckles et al., 2009)

MNAP = Maine Natural Areas Program Proposed Wetland Monitoring Protocol

After exploring the suitability of many common wetland indicators, vegetation emerged as the most appropriate sampling variable. MNAP already focuses on vegetation and natural communities and has the equipment and expertise to carry out vegetation monitoring without much additional training or skill development. The proposed vegetation sampling is quick and inexpensive, can serve as an indicator of change in the other wetland variables, and it is related to specific wetland functions.

Vegetation sampling could also contribute to the application of Floristic Quality Assessment methods currently being developed for New England by the EPA, the New England Interstate Water Pollution Control Commission (NEIWPC), and others (Strout, 2009). Data collected through this proposed protocol may be used to calculate the Floristic Quality Index (FQI) for Reserve wetlands and allow more cross-agency comparison of wetlands. Once completed, this index should also be considered for inclusion in the protocol.

Changes in the hydrology, soil, and water chemistry can cause changes in plant communities, and because plants migrate slowly, seasonal anomalies are less likely to obscure longer term trends. Since plants often integrate the effects of hydrology and soil in wetlands, they can provide a primary and long term indication of wetland disturbance and impact. One possible confounding factor in long-term vegetation monitoring in wetlands is that change over time is a natural part of wetland communities, and normal hydrarch succession may mask or be mistaken for change due to human impact or climate change-induced stresses (Neckles et al., 2009). Thus, differentiating natural succession in wetlands from negative impacts is critical to the development of a monitoring protocol.

While a monitoring plan based solely on vegetation without concurrently measuring hydrology and water chemistry cannot determine exactly what factors contribute to changes in vegetation, enough is known about the successional stage, nutrient, pH, and hydrologic needs of many plants that interpretations of wetland health can be made based on plant sampling. Changes in vegetation composition noted in wetlands, however, could be used to initiate a more intensive and expensive monitoring of hydrology or water chemistry.

To measure hydrology, permanent wells would need to be installed and long-term water level measurements taken by volunteers, agency personnel, and/or with expensive data loggers. Wells must be installed, maintained, and flushed 24 hours before samples are tested (Neckles et al., 2009). Sampling would need to be done much more frequently than the 10 year interval currently planned for the

Ecological Reserve monitoring program. A simple water level staff gauge installed at each wetland would be unlikely to survive intact without maintenance during the 10 year sampling interval. Measurement would require multiple visits throughout the field season, as well as calibration to precipitation.

Measuring water chemistry presents challenges very similar to measuring hydrology (Neckles et al., 2009). In addition, MNAP does not currently own or have funds to procure water chemistry testing instruments. The possibility of borrowing instruments to measure pH, conductivity, temperature, and dissolved oxygen from the Maine Department of Environmental Protection (DEP) was explored. However, their equipment is too large to carry into remote sampling sites, is not meant for sampling in wetlands without open water, and would not be available for most of the field season.

While many of the water chemistry parameters are beyond the scope and budget of this monitoring protocol, soil pore water salinity can be determined from a simple field measurement and provides important clues to changing hydrology in salt marshes. In addition, only a few of the open wetlands on Ecological Reserves are salt marshes, and the refractometer needed to measure salinity can be easily borrowed from another agency. Field testing confirmed the ease and speed of this sampling method. Therefore, soil pore water salinity is the sole water quality parameter recommended for inclusion in this protocol.

Macroinvertebrates, macroalgae, and nekton have well developed protocols through the Maine DEP (Maine DEP Bureau of Land and Water Quality, 2005), but they require standing water and incur significant costs for sample identification in labs (Bouchard, 2009). Similarly, bird abundance, species richness, and behavior surveys are time-consuming, and do not directly answer the monitoring questions because of the many factors that affect bird behavior and abundance (i.e. habitat and food sources outside the sample wetlands, conditions along migration routes, and regional population trends).

Soil accretion is an important factor in estuarine wetlands because rising seas may inundate low areas unless sediment accumulates to maintain the relative elevation. Research conducted in several East Coast tidal marshes suggests that organic and inorganic sediment accretion in both low marshes and high marshes is “keeping up” with sea level rise (Bricker-Urso et al., 1989). Rather than applying expensive and time-consuming efforts to the few Maine tidal Ecological Reserves, inferences may be made about these Reserves from studies conducted elsewhere.

#### **4.2 SAMPLING DESIGN**

Monitoring protocols tend to be designed for determining either status or trends. Status is the condition or health of a resource at a particular time, while trends are directional changes in a resource through time (Neckles et al., 2009). Status is usually best determined by sampling many plots once, while studying trends benefits from sampling the same plots through time. Although MNAP is interested in the health/status of open wetlands on Ecological Reserves, these wetlands were chosen for inclusion in the Ecological Reserve Program because of their pristine character and assumed health. Therefore, monitoring for trends is the main focus of this protocol, and the proposed sampling design reflects that focus.

#### **4.3 SAMPLING POPULATION**

Every sampling design needs to identify its target population. The population of interest here includes emergent and scrub-shrub wetlands in estuarine and palustrine habitats on Ecological Reserves. Deepwater habitats such as lakes are already monitored by the Maine DEP, and forested areas, including forested wetlands, are monitored under the existing *Ecological Reserves Monitoring Plan* created in 2003 (Cutko, 2003). This proposed plan, therefore, compliments existing Maine Natural Areas Program protocols and management activities.

#### **4.4 STUDY SITE SELECTION**

Wetland habitats in Ecological Reserves were identified using the National Wetland Inventory (NWI) (USFWS National Wetlands Inventory, 2009). The relative abundance of wetland types of interest to the protocol was examined at three scales: within the state, within the Ecological Reserve system, and within the proposed sample wetlands. This multi-scale approach mimics the protocol MNAP employs to consider new land for inclusion in the Ecological Reserve system. Sample wetlands were selected to approximate the relative abundance at the state and Reserve scales.

The current NWI layers for Maine were mapped at 1:80,000 from imagery acquired in 1986 (U.S. Fish and Wildlife Service, 2002). Use of the now-outdated aerial photography used for NWI mapping could undermine the sample wetland selection process if proposed wetlands have changed considerably since then. Fortunately, because wetlands on Ecological Reserves experience minimal human impact, their boundaries probably have not changed much through recent years, although their wetland type may be shifting. Since field verification may reveal that proposed sample wetlands do not match their mapped

wetland type, adjustments to site selection and descriptive data are encouraged based on new information gathered in the field. Recommendations for updating the NWI maps are included in section 7.0.

Wetland types of interest with >1% areal extent in Maine and on Ecological Reserves were well represented in the sample sites (Table 2). On a statewide basis, PEM1 and PSS1 represented 78% of the wetlands of interest, with four classes (E2EM1, PSS3, PSS4 and PSS7) representing the remaining 21%. On sample wetlands, PSS1 and PSS7 represent 80% of the total area. Overall, 1,420 acres are included in proposed sample wetlands (Table 2). A more detailed breakdown of types of wetlands on specific reserves can be found in Table 3.

Table 2: Total area and relative abundance of each NWI wetland type summarized at three scales (state, Reserve, and proposed sample wetland). Sample wetlands for the proposed protocol were selected to approximate the relative abundance of wetland types at the state and Reserve scales. This selection of sample wetlands reflects changes made to the protocol in 2010. The Actual Sample Wetlands are those that were visited during 2010.

NWI Wetland Code	Statewide		Ecological Reserves		Proposed Sample Wetlands		Actual Sample Wetlands	
	Total Acres	% of wetlands of interest	Total Acres	% of wetlands of interest	Total Acres	% of wetlands of interest	Total Acres	% of wetlands of interest
<b>E2EM1</b>	<b>20,282</b>	<b>3%</b>	<b>2,022</b>	<b>17%</b>	<b>63</b>	<b>4%</b>	<b>63</b>	<b>7%</b>
E2EM2	1,589	<1%	1	<1%	-	0%		0%
E2EM5	646	<1%	51	<1%	-	0%		0%
E2SS1	99	<1%	5	<1%	-	0%		0%
PEM	128	<1%	*-	<1%	-	0%		0%
<b>PEM1</b>	<b>196,279</b>	<b>26%</b>	<b>1,810</b>	<b>15%</b>	<b>197</b>	<b>14%</b>	<b>85</b>	<b>9%</b>
PEM2	28	<1%	<1	<1%	-	0%		0%
PEM3	<1	<1%	-	0%	-	0%		0%
PEM5	39	<1%	-	0%	-	0%		0%
PSS	2	<1%	-	0%	-	0%		0%
<b>PSS1</b>	<b>394,584</b>	<b>52%</b>	<b>4,478</b>	<b>38%</b>	<b>469</b>	<b>33%</b>	<b>214</b>	<b>23%</b>
PSS2	299	<1%	-	0%	-	0%		0%
<b>PSS3</b>	<b>38,609</b>	<b>5%</b>	<b>291</b>	<b>2%</b>	<b>65</b>	<b>5%</b>	<b>51</b>	<b>5%</b>
<b>PSS4</b>	<b>47,513</b>	<b>6%</b>	<b>667</b>	<b>6%</b>	<b>35</b>	<b>2%</b>	<b>0</b>	<b>0%</b>
PSS5	101	<1%	-	0%	-	0%		0%
PSS6	66	<1%	-	0%	-	0%		0%
<b>PSS7</b>	<b>52,481</b>	<b>7%</b>	<b>2,363</b>	<b>20%</b>	<b>591</b>	<b>42%</b>	<b>540</b>	<b>57%</b>
Total	752,745		11,687		1,420		953	



Seven of the sixteen Department of Conservation (DOC) Ecological Reserves and four of the eleven Department of Inland Fisheries and Wildlife (IFW) Ecological Reserves contain proposed sample wetlands (Table 4, Figure 1). The Ecological Reserves not represented in this protocol were excluded because they do not contain sufficient open-wetland area. In addition, Scarborough Marsh, an IFW Reserve, has not been included, but could be in the future. This large salt marsh complex in southwestern Maine has many acres of estuarine emergent wetlands with persistent vegetation (E2EM1). Some monitoring is currently underway by the IFW in conjunction with restoration activities, and thus Scarborough Marsh has been excluded from this initial sample to avoid duplication of monitoring efforts. Once information about current monitoring is obtained, a portion of Scarborough Marsh not already being monitored may be included in this protocol.

Accessibility, wetland complex size, and level of disturbance were other factors considered in site selection. A report entitled *Sample Wetland Selection and a Vulnerability Assessment for Wetland Monitoring on Maine State Ecological Reserves* (Stone, 2009) explains in greater detail the methods used to select proposed sample wetlands.

Table 3: Wetland types are listed with the sample wetland(s) they occur in. The six more general wetland types presented in Table 2 can be further subdivided into more specific categories. These specific wetland types are listed with the sample wetlands they in which they occur. While PSS7 wetlands occupy a greater portion of sample wetlands than statewide wetlands, this is largely due to the one large, contiguous wetland on the Great Heath.

Wetland Type	Proposed Sample Wetland	Acres	Total Acres	Percent
E2EM1P	Back River South W*	26	63	4%
	Back River North N*	37		
PEM1	Rocky Lake	9	197	14%
	Cutler North larger	13		
	Duck Lake	62		
	Salmon Brook	7		
	Spring River Lake	85		
	St. John Pond	19		
	Mattagodus	2		
PSS1	Cutler North	19	469	33%
	Duck Lake	10		
	Mattagodus	25		
	Mattawamkeak	114		
	Rocky Lake	160		
	Salmon Brook	22		
	Spring River Lake	102		
	St. John Pond	17		
PSS3	Cutler North	16	64	5%
	Killick Pond*	34		
	Spring River Lake	14		
PSS4	Salmon Brook	34	37	2%
	Spring River Lake	3		
PSS7	Cutler North	41	591	57%
	Cutler South	51		
	Great Heath	499		
Total			1421	

Table 4: Eight DOC Ecological Reserves and five IFW Ecological Reserves have proposed sample wetlands located within their boundaries. Some Reserves (i.e. Back River, Cutler, Salmon Brook) contain more than one sample wetland. Often these sample wetlands are in non-adjacent sections of the Reserve. In the case of Back River, two large, continuous, but oddly-shaped wetlands were divided

Sample Wetlands	Acres	Wetland Type	Acres
Back River South E*	26	E2EM1P	26.0
Back River South W*	37	E2EM1P	37.0
Back River North N*	20	E2EM1P	20.0
Back River North S*	15	E2EM1P	15.0
Bigelow Preserve	48	PEM1E	1.5
		PEM1/SS1E	24.9
		PSS4E	3.8
		PSS1E	3.7
		PSS4B	6.6
Cutler South	68	PSS7Ba	58.4
		PSS1E	9.6
Cutler North	261	PEM1E	148.2
		PSS1E	37.7
		PSS3/1Ba	30.8
		PSS7BA	44.0
Duck Lake	25	PSS7BA	25.1
Great Heath	499	PSS7BA	499.1
Killick Pond*	34	PSS3/4Ba	34.0
Machias River Watershed*	95	PEM1/SS1E	7.1
		PSS1/3E	20.7
		PSS1F	33.8
		PSS3/1E	8.3
		PSS7Ba	25.4
Mattagodus*	194	PEM1F	1.4
		PSS1F	192.1
Mattawamkeag*	297	PEM1E	4.5
		PSS1E	292.5
Rocky Lake	229	PSS1/3E	31.2
		PSS1/EM1E	21.7
		PSS1E	161.7
		PSS1F	14.5
Salmon Brook North	16	PEM1Eb	6.4
		PEM1FB	1.4
		PSS1/EM1EB	7.8
Salmon Brook South	119	PEM1E	15.3
		PSS1/3E	65.8
		PSS4Ba	38.2
Spring River Lake	410	PEM1E	253.8
		PSS1E	137.0
		PSS3/1E	6.3
		PSS3E	7.5
		PSS4/1E	3.0
		PSS4E	1.0
St. John Pond	171	PEM1E	42.2
		PSS1/3E	18.3
		PSS1E	110.7
<b>Total</b>	<b>2,564</b>		<b>2,564</b>

into two sample wetlands in order to facilitate transect layout and increase the amount of estuarine wetlands in the sample.

\*Indicates Ecological Reserves administered by the IFW.

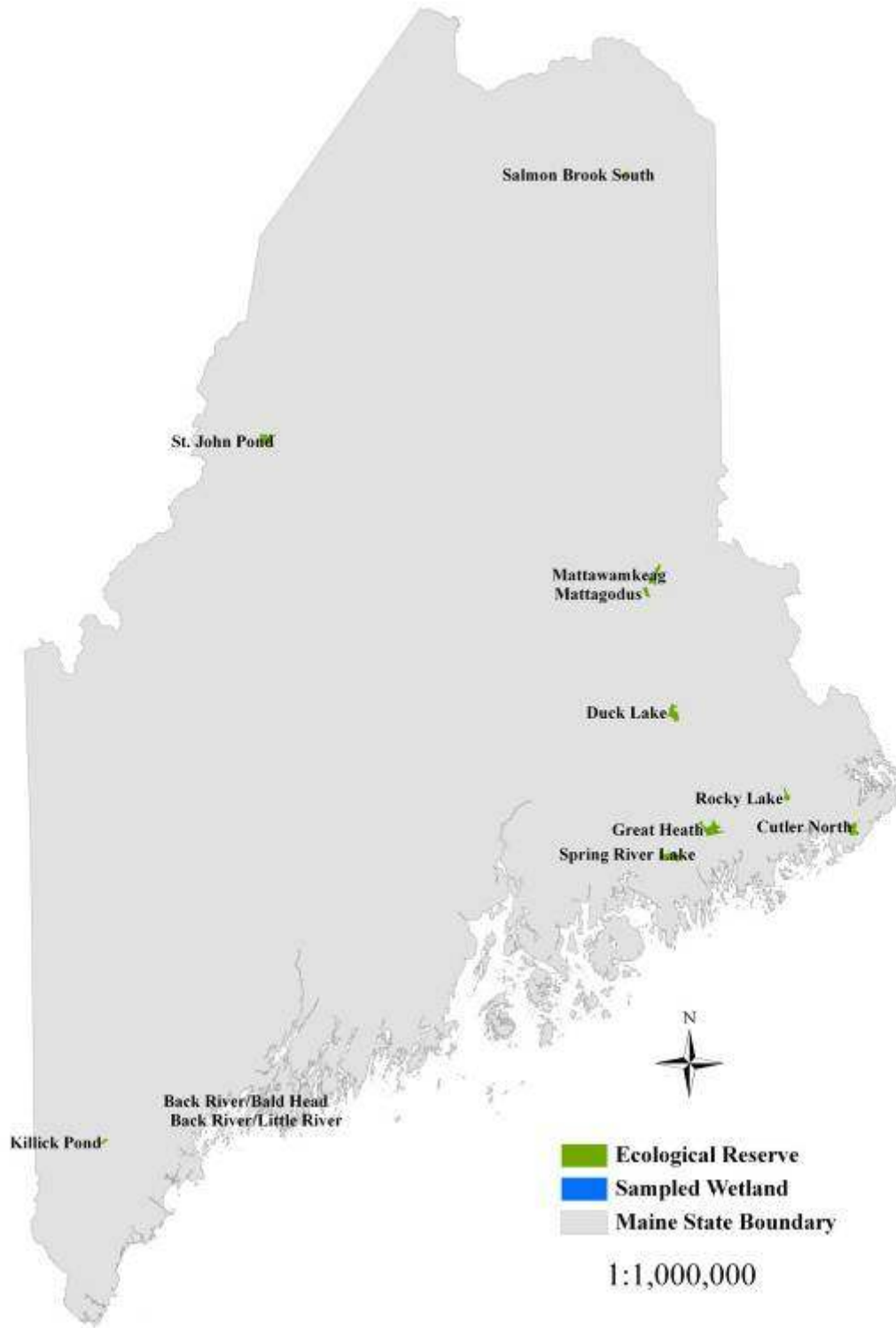


Figure 1: Map of Maine showing the locations of proposed sample wetlands on Ecological Reserves.

#### 4.5 SAMPLING DENSITY

Once the study sites were established, the number of plots to be sampled within each site (i.e. sampling density) was determined. Previous studies of species-area curves compiled for salt marshes in the Northeast show that 20-1m<sup>2</sup> randomly located quadrats per wetland capture the species richness of an area (Neckles and M.Dionne, 2000). Species-area curves derived for freshwater emergent wetlands in Acadia National Park by the Northeast Temperate Network also suggest that 20 quadrats are sufficient (Neckles et al., 2009).

It is essential to consider the statistical power desired in the sampling design. James-Pirri et al. conducted a power analysis to determine the number of plots needed in New England salt marshes to support a one way Analysis of Similarities (ANOSIM). This test was used to determine if there was a difference in salt marsh vegetation communities over time. The power analysis confirmed that 20 plots are enough to meet the desired power of 0.9 at  $\alpha=0.5$ , regardless of the size of the wetland or permanence of the plots (2007).

Sampling conducted in three wetlands on Ecological Reserves during the summer of 2009 confirmed that 20 plots were adequate for capturing much of the species richness, but 30 plots may be more appropriate for attaining optimal power (Figure 2). At Bald Head, 20 plots captured less than 80% of the species richness. Even in a sampling unit as large as the Great Heath, with a 50 meter plot interval, 20 plots captured 84% of the observed species richness while 30 plots captured 94% of the species richness (Figure 3).

Further study should be done on the Ecological Reserve wetland plots to confirm the validity of 30 plots per wetland and test the reproducibility of the sampling techniques. One way to accomplish this would be to sample the same wetlands again in 2010 and to run the test to see if it indicates that the wetlands are significantly different. Significant change would not be expected in one year under normal circumstances, so if significant change is indicated that might suggest a need to revise the sampling protocol. An appropriate modification cannot be predicted now, but possibilities include increasing the number of quadrats, choosing another method for assessing the relative abundance of vegetation, and adding or changing variables.

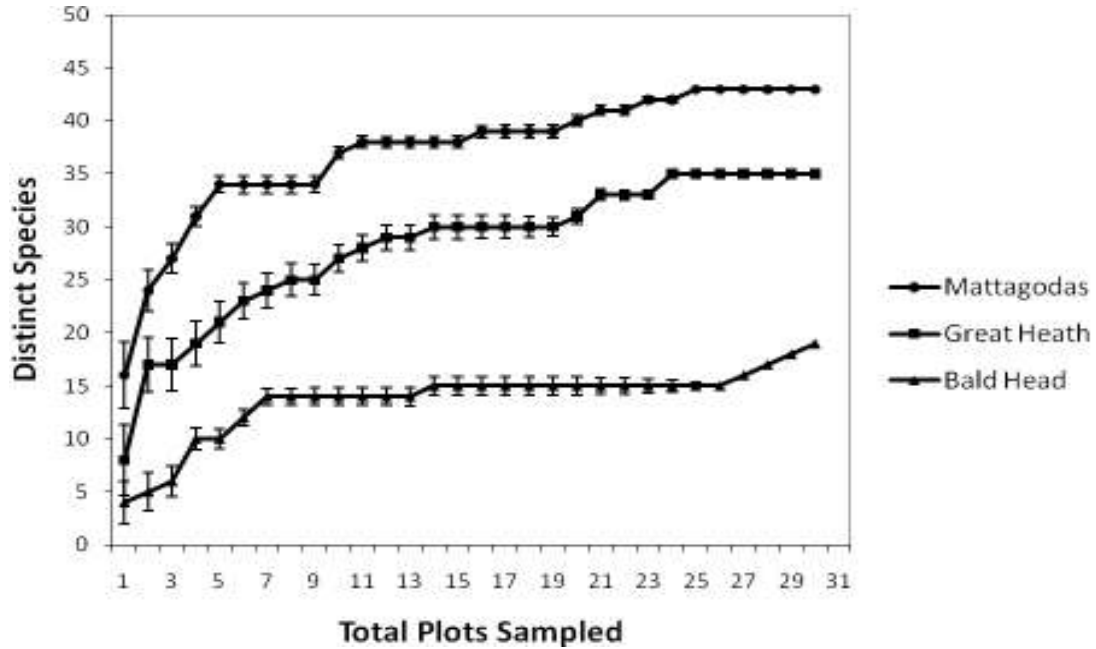


Figure 2: Species-area curves for three wetlands sampled during the summer of 2009 (number of species  $\pm$  1 standard error). The plots were located on two Great Heath, three Mattagodus and three Bald Head transects. The Y axis, distinct species, refers to the cumulative number of plant species observed. This data supports the appropriateness of sampling 30 plots per wetland.

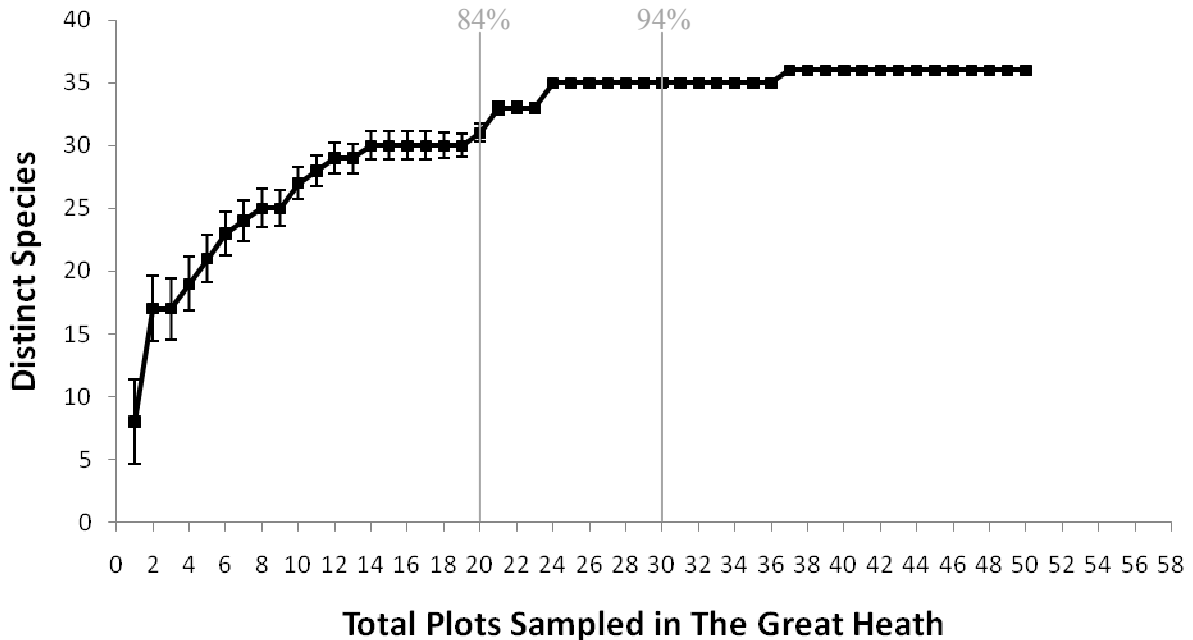


Figure 3: This species-area curve for the Great Heath shows distinct species tallied on three consecutively sampled transects (number of species  $\pm$  1 standard error). This curve shows that 20 plots captured 84% of the observed diversity of the site, 30 plots captured 94%, while sampling 50 plots adds only one species.

## **5.0 RECOMMENDATIONS FOR FIELD SAMPLING PROCEDURES**

Sampling techniques were field-tested on four open wetlands on Ecological Reserves during the summer of 2009. These test sites included Little River salt marsh, The Great Heath, Mattagodus Circumneutral Fen, and Bald Head salt marsh. Based on field testing, recommendations regarding site selection, GIS database development, quadrat location and analysis, and supplementary procedures are proposed below.

For each selected wetland, sampling quadrats should be placed on three stratified-random transects, that is, transects located randomly within drainage strata. A quadrat interval should be calculated so that a total of 30-1m<sup>2</sup> quadrats fit onto the three transects. The GPS coordinates of the transect endpoints should be located on the ground, and species richness and percent cover of vegetation assessed on each of the 30 plots. In salt marshes, the salinity of soil pore water should also be sampled. Photographic plots should be established for each transect. The data will be entered into a wetland monitoring database to support subsequent statistical analysis.

### **5.1 GIS DATABASE AND MAPPING**

A base map provides the foundation for monitoring activities (Neckles and M.Dionne, 2000). Recording information on physical and cultural features, location of the site and the plots within the site, and ecological condition is essential. Prior to the field visit, a GIS database and map containing the following features should be compiled: Ecological Reserve boundary, NWI wetlands/sample wetland area, roads, railroads, and other cultural features. Rare plants and mapped communities from MNAP's Plant Centroid and S Precision layers should also be noted. Where necessary, driving directions and other access information should be included. Transects should be mapped and labeled with GPS coordinates, and these coordinates uploaded to the GPS unit.

GIS data layers for transect locations should be generated, maintained, and updated as needed. Layers should include, but are not limited to: original transects, actual endpoints of the field transects for each sampling year, and "tracks" showing the route actually walked along the transect. Base maps must be maintained both digitally and in hard copy so that they can be consulted and updated as necessary before and after each field sampling event. Applicable GIS data layers can be found in Maine's online GIS archive at: <http://megis.maine.gov/catalog/> and on the MNAP server.

## **5.2 TIMING OF SAMPLING AND SAMPLING INTERVAL**

Vegetation sampling in wetlands should be conducted during the growing season at the time of peak standing biomass of dominant herbaceous species, that is, mid-July through August in Maine (Diers and Roman, 2000; Neckles et al., 2009). For salt marshes, sampling must be conducted as close to low tide as possible (Neckles et al., 2002). If feasible, sampling after extreme periods of drought or large precipitation events should be avoided.

Each wetland should be sampled once during each monitoring cycle. To complement the Ecological Reserve forest monitoring protocol, ideally wetland sampling in a given reserve could be conducted during the same year that the adjacent forest plots are sampled; that is, a 10-year sampling interval, which meets the long-term goal of this protocol. However, at least three years of data are required on a specific wetland before trends can be assessed (Neckles et al., 2009). Thus, a five-year sampling interval would be preferable, because it could detect change more rapidly. Furthermore, while the longer sampling interval is appropriate for monitoring forests with long-lived trees, a shorter sampling interval may be more appropriate for wetlands with more herbaceous and shrubby vegetation.

## **5.3 TRANSECT LAYOUT**

Field transects are recommended instead of a simple random plot layout in order to make sampling more efficient, to make plots easier to locate in the field without permanent markers, and to ensure that the entire wetland gradient from channel to upland is included in the sample. Three transects should be identified for each wetland. Transects should be randomly selected during the first round of sampling and be considered permanent for any sampling thereafter. Transect end points should be marked and located with GPS units to  $\pm 10$  feet accuracy (using a GPS point averaging function available on many standard navigating GPS receivers).

Permanently marking plots with a post or stake is not recommended because the long sampling interval and the unstable nature of wetland substrates reduce the likelihood that a marker will remain in place until the next sampling event. In addition, staking permanent plots may not add enough statistical power to warrant the extra effort and expense of installation (James-Pirri et al., 2007).

Using a stratified-random sampling design maximizes statistical power with field sampling efficiency (James-Pirri et al., 2007). Stratification will be accomplished using NWI data, and National Agriculture Imagery Program (NAIP) imagery in ArcGIS. Step-by step instructions for creating and dividing



transects in ArcGIS 9.3.1 are located on the G: drive at the MNAP in Projects>>Ecoreserves>>Wetland Monitoring>>How To Guides, in a file named “How to Create Wetland Transects in ArcMap 9.doc.” (Figure 4). Whenever possible, only one straight line should be used to describe the axis. If the drainage changes direction considerably then two axes at different angles may be applied.

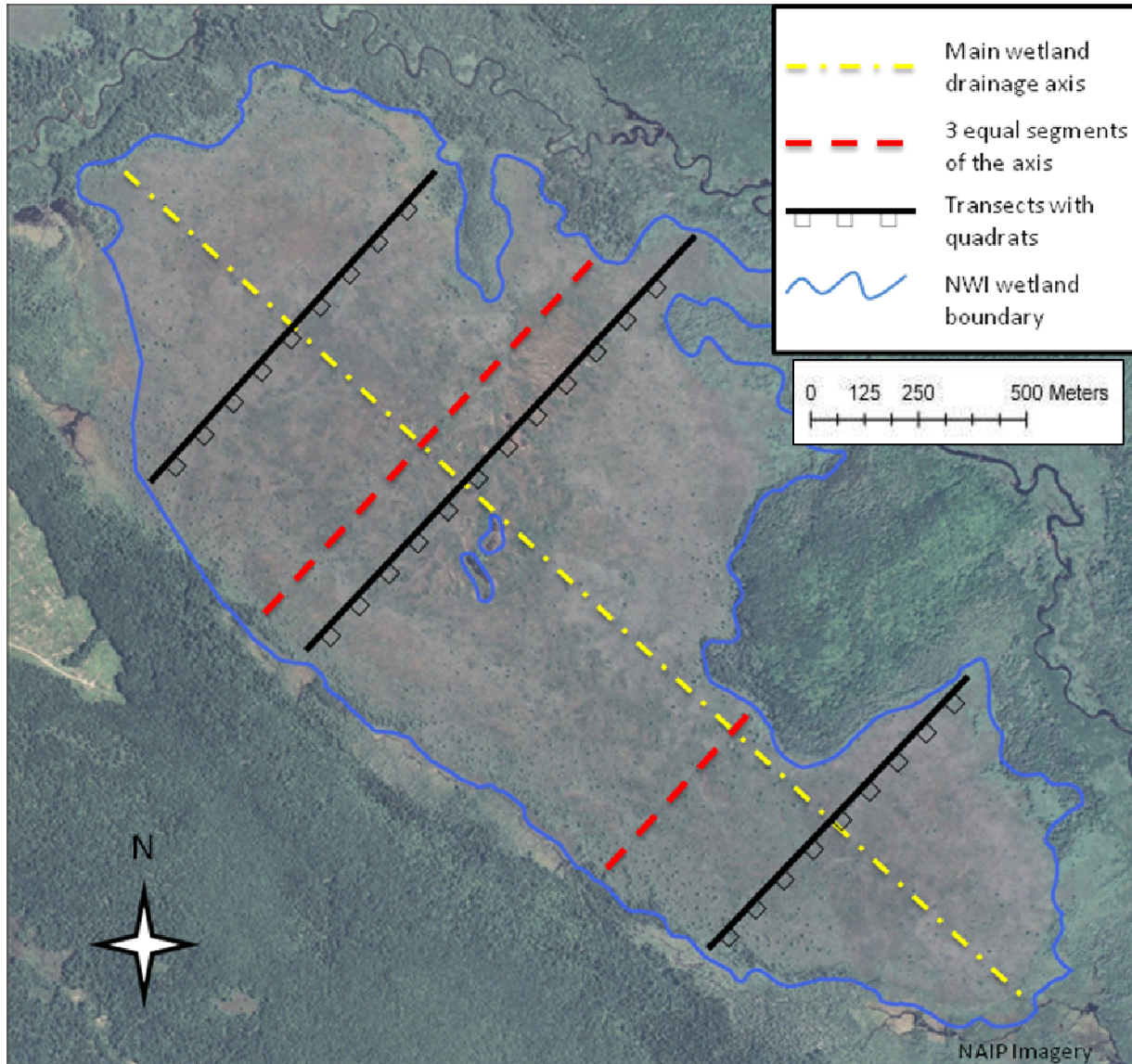


Figure 4: This stratified-random transect layout for a wetland in the Great Heath shows the main drainage axis (yellow dashed line) of the wetland, three equal segments of the axis (red dashed lines), the randomly placed transects (black solid line) located in each segment, and the quadrats (not to scale) laid out at equal intervals so that 30 quadrats fit on the three transects. Transects are located in the field using GPS units. The aerial photo is NAIP imagery (USDA Farm Service Agency, 2009) and the NWI wetland boundary is also included (USFWS National Wetlands Inventory, 2009).

The main drainage axis in the sample wetland should be identified and 30 points placed evenly along the line (Figure 4). Points 1-10, 11-20 and 21-30 will each be considered their own segment. A random number calculator can be used to select a point in each of the three segments that will become the intersection of the field sampling transect. Transects should be drawn perpendicular to the main drainage axis, and should intersect the line at the randomly chosen point. If the Ecological Reserve includes both sides of the drainage, then the transect should span the drainage up to the upland on either side. If MNAP does not have permission to access both sides of the drainage, then the transect should start at the drainage and end at the upland.

Once all three transects are laid out, then the plot distribution can be determined. By adding the lengths of all three transects and dividing by 29 ( $n-1$ ), a quadrat interval for that wetland can be calculated (to the closest meter) so that 30 quadrats fit evenly-spaced on the transects. The number of plots per transect need not be constant, so long as there are 30 plots per wetland and the quadrat interval is equal. If the quadrat interval using this method is less than five meters, then the wetland should be broken into additional segments and transects added until a quadrat interval of five meters or more is achieved.

GPS coordinates for the ends of each transect should be determined in ArcMap using the Calculate Geometry function in the attribute table. These points can also be derived from the transect layer as it is being uploaded to the GPS unit. Once the points are uploaded to the GPS unit, they are ready to use for the location of transects in the field.

Once the GPS coordinates have been used to navigate to the approximate start of each transect, some adjustments can be made on the ground. The transect should start either at a point close to the upland but still distinctly in the wetland with a minimum of upland species encroaching, or the transect should start at the edge of the drainage channel within the wetland. In either case, the first plot should be randomly located within the “edge” community. If the starting point is modified significantly in the field, then a new GPS location for the actual transect starting point and end point should be recorded, and the transect layer altered to reflect this change in ArcMap.

All GPS locations recorded in the field should be based on point averaging, that is recording and averaging 200-300 instantaneous measurements over a 3-5 minute interval. Many navigational GPS units have an option for point averaging. This option provides a much higher locational accuracy than a single

instantaneous point location. Optimal conditions for GPS point collection include a minimum of 4 satellites overhead, clear skies, and little to no winds.

#### 5.4 VEGETATION SAMPLING IN QUADRATS

Meter-square quadrats should be sampled using a modified Braun-Blanquet cover-abundance scale with eight cover classes as follows: 1 (<1%), 2 (1-5%), 3 (6-10%), 4 (11-25%), 5 (26-50%), 6 (51-75%), 7 (76-95%), 8 (96-100%). The modifications from the original Braun-Blanquet scale are that the 6-25% category has been split into two classes, and the 96-100% category has been split out from the >75% class. This allows for finer-scale observations as recommended by U.S. Geological Survey (USGS) researcher Hilary Neckles (Neckles, 2009). One meter-square plots were chosen because they are a standard, efficient, manageable size for estimating accurate cover classes, and are already in use in other vegetation monitoring protocols in the region, including MNAP's forest monitoring protocol (Neckles and M.Dionne, 2000; Cutko, 2003; Neckles et al., 2009). Each species in a plot should be recorded and its percent cover estimated using the previously mentioned cover classes.

Other cover types, such a **bare ground, dead vegetation, wrack, macro algae, woody debris, rock** and **open water** will also be included. **Bare ground** includes any mineral soil that is visible beneath the stems and leaves of live vegetation. **Dead vegetation** is any dead plant material that is not wrack. If dead vegetation is laying on mineral soil, this should be counted as dead vegetation and not bare ground. Only mineral soil counts as bare ground. In a bog, dead, soggy or trampled peat should be counted as dead vegetation, not bare ground, because there are many feet of peat on top of the original substrate. **Wrack** includes dead vegetation that has floated to its current location. **Woody debris** includes any large woody wrack or fallen trees within the plot. **Rock** includes exposed bedrock and clasts larger than cobble size. Open water with **macro algae** floating in it can be classified as both cover types. When there is open water above mineral soil, only the open water is recorded.

#### 5.5 SOIL PORE WATER SALINITY

Salinity should be measured in each quadrat on the salt marsh sites (Back River Ecological Reserve). Water should be extracted from within the quadrat using a soil pore-sipper from depths between five and twenty centimeters (Neckles and M.Dionne, 2000). A paper filter or a filter on the tip of the sipper should be used to strain the sample so that soil particles do not interfere with the function of the refractometer. Salinity should be recorded in parts per thousand and analyzed separately from the vegetation data. If

open water is present in a plot, then the salinity of that surface water also may be sampled, and should be recorded separately.

## **5.6 PHOTOGRAPHIC RECORD**

Photographic records of wetlands can offer valuable insight into change over time, provide a visual reference for changes detected by statistics, and provide a permanent record of site conditions. Photo sampling stations are common in ecological monitoring, especially in monitoring restoration projects (Northern Ecological Associates Inc., 2001). At least five photographs should be taken at each photo station with a high-resolution digital camera. These should include looking forward along the transect from starting point to end point, looking directly back along the transect, looking perpendicular to the transect in both directions, and an oblique photo of the quadrat. Transect end points should be photo stations, and one or more quadrats near the center of each transect should also be chosen as additional photo stations. Oblique photos of other quadrats may be included. Photos should be labeled with date, transect number, quadrat number, and compass direction. Photo stations should be marked on the Ecological Reserve Wetland Monitoring Data sheets (Appendix 1), enabling them to be revisited during subsequent sampling events.

## **5.7 SUPPLEMENTAL MONITORING DATA**

In addition to sampling the aforementioned variables, qualitative data should be recorded at each site. The data sheets (Appendix 1) include space to sketch each transect. This can facilitate data management and comparison by giving a better picture of where an individual plot fits into the wetland as a whole. Upland areas, channels, obvious topographical changes, landmarks, natural community boundaries, and photo points should be recorded.

## **6.0 DATA COMPILATION AND ANALYSIS**

### **6.1 DATA MANAGEMENT**

All monitoring data collected on Ecological Reserves will reside with MNAP. Field Data should be recorded on the MNAP Ecological Reserves Wetland Monitoring Field Form and MNAP Ecological Reserves Salt Marsh Monitoring Field Form (Appendix 1) developed for this protocol, and entered into a database developed for this purpose. Wetland Site Survey Summaries should be written and/or updated after each sampling visit, and the Summary Table filled in to indicate the sampling progress on each wetland.

### **6.1.2 PHOTO MANAGEMENT**

Images recorded for the photo sampling protocol must be labeled and filed appropriately. Since the field GPS units MNAP uses (Garmin Oregon 550 ®) have photo capabilities, and the GPS coordinates are recorded with the photo, this makes taking photos in the field even easier and more useful. The challenge is to make that data useful once it's been downloaded to a PC as well. In BaseCamp®, Garmin's GPS management software, you can view the photos and some of their metadata (<http://www.garmin.com/garmin/cms/us/onthetrail/basecamp>). In order to assign coordinates to the photos, though, the program matches the time they were recorded with the time on a GPS Track. If the tracking function wasn't turned on there's no way to figure out their coordinates in BaseCamp. Another downside to this is that some of the coordinates' precision is lost.

Other photo management programs are available. Opanda® is available free online in a trial version. Using Opanda, the original, more precise, GPS coordinates and direction the photo was taken in are visible for each photo. However, in the trial version there is no way to easily transfer that data to an Excel file or other data management system. Coordinates cannot be copied and pasted and only parts of the information (e.g x coordinates, but not y coordinates) are available for export. The professional version costs around \$90, and allows many more options for data management (<http://www.opanda.com/>).

In addition, staff at MNAP are currently developing a photo storage system for the entire agency, and this may prove useful to this protocol when it is completed.

### **6.2.1 STATISTICAL ANALYSIS**

Summary statistics can be computed and graphs generated based on these data with Microsoft Excel, PC-ORD, or another statistics program. The comparison of two wetlands or of a single wetland at two different points in time can be made using multivariate statistical software that can run a non-parametric procedure for detecting differences, such as a one-way Analysis of Similarities (Clarke and Warwick, 1994).

Testing for wetland similarities and differences based on protocol variables involves three steps: 1) a Euclidean distance similarity index is calculated (Krebs, 1999); 2) the index then is used to create a similarity matrix to allow for the objective identification of plots that are more or less similar; and 3) an analysis of similarities randomization test (ANOSIM) is then applied to the matrix to test for significant differences between groups of sample plots (Clarke and Warwick, 2001; Clarke and Gorley, 2006).

ANOSIM is just one example of this type of statistical test and is a one-way non-parametric analog to MANOVA (Clarke and Green, 1988). This test can be run with software available from Primer-e (<http://www.primer-e.com>). Another example is the perMANOVA test from PC-ORD (McCune and Mefford, 2006). Excellent explanations of these tests are provided in Roman et al. (2001) and James-Pirri et al. (2007) respectively. The original article describing the PerManova test is “A new method for non-parametric multivariate analysis of variance.pdf.”

For wetlands that are significantly different, it is useful to know which species contributed most to the differences. This can be calculated by taking the square of the difference of one species between wetland 1 and wetland 2 over the square of the sum of the differences of all species between wetland 1 and wetland 2 (Figure 5). Species or cover types with the highest ratio contributed most to the dissimilarity (Roman et al., 2001).

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

Where;

D = Distance

C<sub>1i</sub> = cover of species i in marsh 1

C<sub>2i</sub> = cover of species i in marsh 2

Figure 5: Formula for determining how much an individual species contributes to any observed differences, after Roman et al., 2001.

A permutation-based MANOVA (PerMANOVA) test to detect significant differences in cover or species after (Anderson, 2001), was applied to data from the Mattagodus Circumneutral Fen using PC-ORD (McCune and Mefford, 2006). The test requires two groups of data; for this purpose the groups were defined by time. Since only one actual set of data had been collected at Mattagodus when this protocol was being field tested in July, 2009, the second set was created by altering the initial data. During the first test run, the data were modified manually, increasing or decreasing about a quarter of the values by one cover class. The result was 60 plots (30 for each time-group) by 37 species. This test showed no significant difference ( $p=1.0$ ) over time. During the second test run, the about half of the cover classes were increased or decreased by one or more value to simulate a considerable change in vegetation

composition over time. This test indicated that there was a significant difference ( $p=0.013$ ) in vegetation over time.

Additional statistics and information about PerMANOVA can be found in Appendix 2, which is the output from PC-ORD's PerMANOVA test. These results suggest that small yearly variation and observer errors should not confound the test, while larger changes in vegetation will be detected. Thus, PC-ORD is recommended as an analysis tool for this protocol. An example of how to enter wetland data in PC-ORD to run the recommended test is given in Appendix 3.

### **6.2.2 FURTHER RESULTS**

During the summer of 2010 wetlands in the Great Heath, Mattagodus, and Bald Head were re-sampled, and their data compared to the 2009 sampling data using PC-ORD's PerMANOVA test. Data was prepared for analysis by entering it into an Excel spreadsheet with formatting compatible with PC-ORD. Only species cover class information was used in this initial analysis. Species-cover class data was entered for both sampling events (2009 and 2010).

The Great Heath was the first wetland to be re-sampled. The 2010 transects closely approximated the 2009 transects, but as is evident through comparison of the various GIS layers, the transects did not exactly overlap. The p-value for this initial PerMANOVA was 0.0008. This value indicated that there is almost no chance that the difference in the data "caused" by time is a product of random variation. Therefore, the Great Heath 2009 IS SIGNIFICANTLY DIFFERENT than the Great Heath 2010.

Since, in our professional opinion, the Great Heath has not actually changed significantly in the short time between sampling events, we decided to try and clean up the data with the hope that removing some of the less common species would increase the p value. The original article that describes the PerMANOVA test (Anderson, 2001) noted that some data manipulation is appropriate. For example, the author indicated that any species which only occurred once in his study was exempt from the data analysis. We applied this to the Great Heath data by discarding species that occurred only once in one year, or up to 3 times at a very low percent cover in only one of the years. If an entire plot (especially a plot located at the end of the transect) was obviously quite different than the rest of the plots, then it too was discarded. Twenty-one species total were discarded. Nineteen species were discarded as previously described, non-sphagnum bryophytes were discarded because of inconsistent sampling techniques between the two years, and POL sp was discarded because of suspected identification issues. In addition, the end-plots for each transect were discarded for both years to avoid inconsistencies because of the inclusion of upland species.

The p-value for this second PerMANOVA test was 0.1716. Since, for our purposes, any p-value above 0.1 indicates no significant change, this indicates that with cleaner data, the Great Heath is no different in 2009 than in 2010.

After re-sampling Mattagodus the same process was repeated. Five species with low occurrences in both 2009 and 2010 were discarded. Several species recorded for the first time in 2010 were discarded. They likely represent the increased area covered by the newly designed 2010 transects, and not actual differences between the years. The species AST BOR and AST NEM from 2010 were combined under AST spp from 2009, and the category *Carex* spp. from 2009 was discarded because there is no way to match unknown species between years. In addition, one plot (the most upland-influenced plot) was removed from the 2010 data to equalize the number of transects between years. The p value for this test was 0.0004, twice as significant as the first Great Heath test.

Bald Head data was also entered and altered. Eleven species with low occurrences were discarded. *Carex palacea* was removed, although it occurred in several 2009 plots, because it was never found in 2010, probably due to identification errors. Wrack was also removed because of inconsistent sampling techniques. In addition, plots 5 and 13 (chosen randomly) were removed from 2009 data to make the number of plots in each year equal. The test of this data resulted in p=0.0002.

One additional wetland was also tested. The wetland along the East Machias River in the Rocky Lake Ecological Reserve had been sampled in a preliminary attempt at wetland monitoring on EcoReserves in 2003. Data from the 2003 plots located most similarly to the 2010 plots was entered. Twenty-one species with low occurrences were removed, in addition to several species only found in 2003. An entire 2003 transect located in a heath was removed, because no similar community was included in 2010. The westernmost 7 plots of the southern 2003 transect were also removed. This brought the total plot count for each year to 30. The p value for this test was 0.0006. This was unsurprising for such a difference in time and sampling method.

After examination of the PerMANOVA process, it was hypothesized that perhaps removing too much of the intra-wetland diversity could reduce the amount of inter-wetland variability the test allowed. Another set of Bald Head data was prepared for testing, this time with no species removed. However, the p-value for this test was still 0.0002.

In conclusion, the PerMANOVA results indicating vegetation changes between 2009 and 2010 may be explained by a few factors:



- Despite the use of GPS to place accurately place transects, even slight differences in plot placement may affect results, particularly at the heterogeneous plots near wetland/upland transition zones at the ends of transects.
- 2009 was a very wet summer, and 2010 was a very dry summer. Differences in rainfall likely impacted vegetation in the two years, with some species (e.g., *Calopogon tuberosus*) much more abundant in the wet year than in the dry.
- Alternatively, these results indicate that the PerMANOVA statistical test may be too sensitive for detecting broad changes in wetlands sampled through this method. Use of a different, perhaps less-sensitive, statistical analysis tool should be explored.

## **7.0 MONITORING WITH REMOTE SENSING**

While monitoring the change in extent of wetland communities through remote sensing is not practical for MNAP to complete in-house, the National Wetland Inventory (NWI) can be a valuable resource. This national database covers about 60% of the lower 48 states, including the entire state of Maine (USFWS National Wetlands Inventory, 2009). Wetlands are mapped according to the Cowardin classification scheme and the resulting GIS data layers are available to the public at no cost. The current NWI layers for Maine were mapped at 1:80,000 from imagery acquired in the mid 1980s (U.S. Fish and Wildlife Service, 2002).

Under a new USFWS program, revision of NWI maps throughout the U.S. is underway. New NWI map updates will increase the mapping scale to 1:40,000 or better. This will greatly improve the accuracy of the wetland mapping and provide two dates for change-detection analysis. These NWI updates will take many years to complete for the entire lower 48 states and they may not be available in Maine for several years. However, according to USFWS, coastal wetlands and wetlands near urban areas will be given priority. Central Maine is listed at medium wetland development risk, and the rest of Maine has low development risk (U.S. Fish and Wildlife Service, 2002). When these updates become available, they should be incorporated within MNAP's database.

In the meantime, NWI is willing to partner with state agencies that need more frequent mapping updates for specific areas. It is recommended that MNAP contract with NWI to update wetland maps on Ecological Reserves every 10 years and request that NWI use USDA National Agriculture Imagery

Program (NAIP) imagery as the base imagery (Morrissey, 2009). The NAIP program collects very high resolution (1m visible and near IR bands) imagery annually on a statewide basis (USDA Farm Service Agency, 2009). These layers then can be used to run change detection analysis tools with GIS software on all open wetlands located in Ecological Reserves, supplementing those wetlands already being monitored on the ground.

Following field verification, wetlands with a high degree of change in their shape, area, or wetland classification detected through this process can be added to the field monitoring protocol, or targeted for additional monitoring or ecological indicators to better understand the nature of the change. By using NWI's expertise already developed over years of wetland mapping, MNAP can maximize the information they obtain for wetland monitoring through remote sensing at minimum cost.

## **8.0 ESTIMATED COST OF IMPLEMENTATION**

Sampling along each transect will take approximately 1.5 hours, and most wetlands can be sampled in approximately one day, including access time. Preparation, data entry and statistical analysis will each take about two hours, or 0.75 days. Round trip driving times to each site from the MNAP office in Augusta vary widely, from two hours to a full day. Trips can usually be combined with visits to other sample wetlands or sites for other projects to minimize actual transportation costs. The projected number of staff-days necessary to implement the first round of field sampling and analysis is 50. In 2010 the actual time spent to monitor seven of the proposed sample wetlands, plus additional protocol development and database design was closer to 45. After figuring in the "fully burdened" cost (including benefits and overhead) of two-person sampling teams consisting of one professional staff and one field intern, the total estimated staffing cost of the proposed protocol is \$14,681.25.

## **9.0 RECOMMENDATIONS**

Current sample wetlands may be excluded from this protocol if they are no longer conducive to sampling or are no longer considered a wetland type of interest. In addition, recommendations for a pared-down set of sample wetlands are included in Appendix 4. Reducing the number of wetlands sampled will not affect the local application of monitoring, but may reduce ability of this protocol to generalize statewide trends and provide an adequate number of reference wetlands. Additional sample wetlands may be added at any point.

A monitoring interval of five years is recommended, but a ten-year interval is acceptable if there are budget and time constraints. The highest level of efficiency will likely be realized if all or most of the wetlands can be sampled within a single season.

Finally, once the Floristic Quality Assessment for New England is developed it should be considered for inclusion in this protocol.

## **10.0 SUMMARY**

Ecological monitoring is an important and frequently used tool for land managers. Monitoring provides a formal reason and standard procedures for observing ecosystems that might otherwise go unvisited or with details unnoticed. These observations can serve multiple purposes including fulfillment of legal obligation, resource inventory, change detection, cataloging of human impact, comparison of impacted and reference sites, and increased knowledge of the ecosystem.

Monitoring wetlands is especially important because their dominant herbaceous and shrubby vegetation can change more rapidly than long-lived forest trees and because of how quickly changing water quality and hydrologic characteristics can impact a wetland. Healthy wetlands provide numerous functions and values for humans, as well as all other non-human members of their communities.

This proposed protocol will help ensure that Ecological Reserves are fulfilling their legislated purposes stated in the introduction. Monitoring vegetation on open wetlands will allow MNAP to confirm that natural community types and native ecosystem types are being protected in their natural conditions, thereby protecting habitat for many species. The monitoring data can be used to measure change within reference wetlands, and also will be available for comparison to impacted wetlands.

The three-tiered approach (monitoring through remote sensing, field sampling, and photographic sampling) of this proposed protocol can provide information at a variety of scales, and tools for sharing this data with a range of scientific and lay audiences. In addition, it provides ecologists and land managers a chance to become more familiar with the land they are protecting, and to be better informed guardians of Ecological Reserves.

## REFERENCES

- Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, 26:32-46.
- Bouchard, R. Personal communication. June 11, 2009.
- Bricker-Urso, S., S. W. Nixon, J. K. Cochran, D. J. Hirschberg and C. Hunt. 1989. Accretion Rates and Sediment Accumulation in Rhode Island Salt Marshes. *Estuaries*, 12:300-317.
- Clarke, K. R. and R. N. Gorley. 2006. PRIMER v6: user Manual/Tutorial. In PRIMER-E (ed.). Plymouth Marine Laboratory, Plymouth, UK.
- Clarke, K. R. and R. H. Green. 1988. Statistical design and analysis for a 'biological effects' study. *Marine Ecology Progress Series*, 92:205-219.
- Clarke, K. R. and R. M. Warwick. 1994. Similarity-based testing for community patten: the 2-way lay-out with no replication. *Marine Biology*, 118:167-176.
- Clarke, K. R. and R. M. Warwick. 2001. Change in marine communities: and approach to statistical analysis and interpretation. In PRIMER-E (ed.). Plymouth Marine Laboratory, Plymouth, UK.
- Cowardin, L. M., V. Carter, F. C. Golet, E. T. LaRoe. 1979. Classification of wetlands and deepwater habitats of the United States. U. S. Department of the Interior, Fish and Wildlife Service, Washington, D.C. 131pp.
- Cutko, A. 2003. Ecological Reserves Monitoring Plan. Maine Department of Conservation Natural Areas Program.
- Cutko, A. Personal communication. 6 June 2009.
- Diers, T. and C. Roman. 2000. Ecosystem Indicator: Vegetation. p 712-14 in H.A. Neckles and M.Dionne, Editors. *Regional standards to identify and evaluate tidal wetland restoration in the Gulf of Maine*. Wells National Estuarine Research Reserve Technical Report, Wells, ME.
- Faber-Langendoen, D., J. Rocchio, M. Schafale, C. Nordman, M. Pyne, J. Teague, T. Foti, and P. Comer. 2006. *Ecological Integrity Assessment and Performance Measures for Wetland Mitigation*. NatureServe, Arlington, Virginia.
- James-Pirri, M.-J., C. T. Roman and J. F. Heltshe. 2007. Power analysis to determine sample size for monitoring vegetation change in salt marsh habitats. *Wetlands Ecology and Management*, Volume 15, Number 4:335-345.
- Konisky, R. A., D. M. Burdick, M. Dionne and H. A. Neckles. 2006. A Regional Assessment of Salt Marsh Restoration and Monitoring in the Gulf of Maine. *Restoration Ecology*, Vol. 14, No. 4:pp. 516-525.
- Krebs, C. J. 1999. *Ecological methodology*. Addison Wesley Longman Inc, Menlo Park, CA.
- Maine DEP Bureau of Land and Water Quality. Monitoring and Assessment. Retrieved December 12, 2009, from <http://www.maine.gov/dep/blwq/monitoring.htm>
- Maine Department of Environmental Protection Bureau of Land and Water Quality. Monitoring and Assessment. Retrieved December 12, 2009, from <http://www.maine.gov/dep/blwq/monitoring.htm>
- Maine Natural Areas Program. Retrieved November 20, 2009, from <http://www.maine.gov/doc/nrimc/mnap/>
- McCune, B. and M. J. Mefford. 2006. PC-ORD. Multivariate Analysis of Ecological Data. MjM Software, Glenden Beach, Oregon, USA.
- Morrissey, L. Personal communication. 21 October 2009.

- Neckles, H. A. Personal communication. 1 July 2009.
- Neckles, H. A., M. Dionne, D. M. Burdick, C. T. Roman, R. Buchsbaum and E. Hutchins. 2002. A Monitoring Protocol to Assess Tidal Restoration of Salt Marshes on Local and Regional Scales. *Restoration Ecology*, Vol. 10 No. 3:pp. 556-563.
- Neckles, H. A., A. T. Gilbert, G. R. Guntenspergen, N. P. Danz, T. Hollenhorst, A. Little and J. Olker. 2009. Protocol for Monitoring Freshwater Wetlands in National Parks of the Northeast Temperate Network. U.S. Geological Survey.
- Neckles, H. A. and M. Dionne (eds.). 2000. Regional standards to identify and evaluate tidal wetland restoration in the Gulf of Maine. Wells National Estuarine Research Reserve Technical Report, Wells, ME.
- Northern Ecological Associates Inc. 2001. Salt Marsh Restoration Monitoring Plan for Ditch-Plugging Efforts in New England Marshes. U.S. Fish and Wildlife Service Gulf of Maine Program.
- Roman, C. T., M.-J. James-Pirri and J. F. Heltshe. 2001. Monitoring Salt Marsh Vegetation. Cape Cod National Seashore, Wellfleet, MA.
- Stone, E. 2009. Sample Wetland Selection and a Vulnerability Assessment for Wetland Monitoring on Maine State Ecological Reserves. University of Vermont.
- Strout, K. personal communication. June 15, 2009.
- U.S. EPA. 2002a. Methods for Evaluating Wetland Condition: Introduction to Wetland Biological Assessment.
- U.S. EPA. 2002b. Methods for Evaluating Wetland Condition: Using Vegetation To Assess Environmental Conditions in Wetlands.
- U.S. Fish and Wildlife Service. 2002. National Wetlands Inventory: A Strategy for the 21st Century.
- USDA Farm Service Agency. Imagery Products, NAIP Imagery. Retrieved December 12, 2009, from <http://www.fsa.usda.gov/FSA/apfoapp?area=home&subject=prog&topic=nai>
- USFWS National Wetlands Inventory. NWI Home. Retrieved December 12, 2009, from <http://www.fws.gov/wetlands/>

APPENDIX 1: ECOLOGICAL RESERVES WETLAND MONITORING FIELD FORMS

<b>MNAP Ecological Reserves Wetland Monitoring Field Form 4.0</b>				
Transect Summary			Date	
Site Name	Time In		Out	
Transect    1   2   3   4	Start	UTME		
Sheet        1   2   3   4	End	UTMN		
Recorders	End	UTME		
Directions to Site	End	UTMN		
GPS Accuracy				
Compass Bearing			# plots:	
Transect Length (m)			plot interval:	
Degree of flooding: permanent, semi-permanent, seasonal, intermittent, temporary, saturated				
Nearest body of water				
Historic conditions, alterations and impacts				
Size of site				
General Wetland Type				
<p>Sketch transect. Indicate start and end points, orientation, plots, photo points with direction and photo numbers, landmarks, high water marks, and the boundaries of vegetation zones (e.g. upland, high marsh, low marsh, streams, and open water) Measure distances in meters from the start point.</p>				
<p>Additional Notes, including the location of a permanent photo plot if established. Incidental observations of macro-invertebrates, birds, and mammals including tracks, scat, and other sign</p>				

**MNAP Ecological Reserves Wetland Monitoring Field Form 4.0**  
 Percent Cover Date

Site Name Transect 1 2 3 4 Sheet 1 2 3 4

Cover Classes: 1 (<1%) 2 (1-5%) 3 (6-10%) 4 (11-25%) 5 (26-50%) 6 (51-75%) 7 (76-95%) 8 (96-100%)

GEN SPE	1	2	3	4	5	6	7	8	9	10	x-tra plot
Cover Low (<0.5m)											
Cover Mid (0.5-2m)											
Cover High (2-5m)											
open water											
bare ground/rock											
SPHAGNUM spp.											
LICHEN spp.											
1											
2											
3											
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38											
Photo #											

**MNAP Ecological Reserves Salt Marsh Monitoring Field Form 4.0**

Transect Summary Date

<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td colspan="5">Site Name</td> </tr> <tr> <td>Transect</td> <td>1</td> <td>2</td> <td>3</td> <td>4</td> </tr> <tr> <td>Sheet</td> <td>1</td> <td>2</td> <td>3</td> <td>4</td> </tr> <tr> <td colspan="5">Recorders</td> </tr> <tr> <td colspan="5">Directions to Site</td> </tr> </table>	Site Name					Transect	1	2	3	4	Sheet	1	2	3	4	Recorders					Directions to Site					<table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td colspan="2">Time In</td> <td>Out</td> </tr> <tr> <td rowspan="2">Start</td> <td colspan="2">UTME</td> </tr> <tr> <td colspan="2">UTMN</td> </tr> <tr> <td rowspan="2">End</td> <td colspan="2">UTME</td> </tr> <tr> <td colspan="2">UTMN</td> </tr> <tr> <td colspan="3">GPS Accuracy</td> </tr> <tr> <td colspan="2">Compass Bearing</td> <td># plots:</td> </tr> <tr> <td colspan="2">Transect Length (m)</td> <td>plot interval:</td> </tr> </table>	Time In		Out	Start	UTME		UTMN		End	UTME		UTMN		GPS Accuracy			Compass Bearing		# plots:	Transect Length (m)		plot interval:
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GPS Accuracy																																																
Compass Bearing		# plots:																																														
Transect Length (m)		plot interval:																																														

Time of Low Tide
Nearest body of water
Historic conditions, alterations and impacts
Size of site
General Wetland Type

Current Weather
Recent weather

Sketch transect. Indicate start and end points, orientation, plots, photo points with direction and photo numbers, landmarks, high water marks, and the boundaries of vegetation zones (upland, high marsh, pools, pannes, low marsh, streams, and tidal flats). Measure distances in meters from the start point.

Additional Notes, including the location of a permanent photo plot if established.  
 Incidental observations of macro-invertebrates, nekton, birds, and mammals including tracks, scat, and other sign



# MNAP Ecological Reserves Salt Marsh Monitoring Field Form 4.0

Percent Cover and Salinity Plot Data Date

Site Name

Transect 1 2 3 4 Sheet 1 2 3 4

Cover Classes: 1 (<1%) 2 (1-5%) 3 (6-10%) 4 (11-25%) 5 (26-50%) 6 (51-75%) 7 (76-95%) 8 (96-100%)

GEN SPE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	extra-plot
pore water salinity																
dominant height																
open water																
live vascular plants																
dead vascular plants																
macroalgae																
wrack																
woody debns																
rock																
bare ground																
1																
2																
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## APPENDIX 2: OUTPUT FROM PC-ORD'S PERMANOVA TEST.

The output from PC-ORD's PerMANOVA indicates that the groups (i.e. the two wetlands) were defined by time, and also lists the number of combined plots and number of species in those two wetlands. The high observed F value (64) and the low p-value (0.013000) both support rejecting the null hypothesis that the wetlands were not different.

```
***** PERMUTATION BASED MANOVA (PERMANOVA) *****
PC-ORD, 5.26
13 Nov 2009, 13:41:59
PERMUTATION-BASED NONPARAMETRIC MANOVA CALCULATED WITH METHOD OF:
ANDERSON, M. J. 2001. A NEW METHOD FOR NON-PARAMETRIC MULTIVARIATE
ANALYSIS OF VARIANCE. AUSTRAL ECOLOGY 26:32-46.
Mattagodus test
Groups were defined by values of: time
Main matrix has: 60 plots by 37 species
Distance measure = Euclidean (Pythagorean)

Evaluation of differences in species between groups.
Design: One-way
Randomization test of significance of pseudo F values
Number of randomizations: 4999
Random number seed: 5997 selected by time.
-----
Source d.f. SS MS F p *
-----
time 1 199.37 199.37 2.4306 0.013000
Residual 58 4757.5 82.025
Total 59 4956.8
-----

Statistics from randomizations
-----
F from randomized groups Number
F ----- > or =
Source Observed Mean Maximum S.Dev observed F p *
-----
time 2.43055 1.00641 4.21697 0.00109 64 0.013000
-----

* proportion of randomized trials with indicator value
equal to or exceeding the observed indicator value.
p = (1 + number of runs >= observed)/(1 + number of randomized runs)

Variance components estimated for random effects model (Model II)
Ignore variance components if you consider the factor to have fixed effects.
COMPONENTS OF VARIANCE
-----
Source Variance % of variation
time 3.9114 4.551
Residual 82.025 95.449
Total 85.937 100.000
-----
***** PerMANOVA finished *****
13 Nov 2009, 13:42:00
```

Figure 6: Output from PC-ORD's PerMANOVA test. Actual data from sampling Mattagodus Circum-neutral Fen was compared with manually changed data from the same wetland to mimic two sampling events.

### **APPENDIX 3: EXAMPLE OF THE MAIN AND SECOND DATA FRAMES FOR RUNNING PC-ORD'S PERMANOVA TEST.**

Data entry for use in PC-ORD is fairly straightforward. The software is able to import data from both Microsoft Excel and MS Access, two programs that MNAP currently uses. In the main data frame the 30 plots from both wetlands to be compared (whether it is a reference and impacted wetland or the same wetland at two different times) are in the left hand column (Figure 6). The species that occur in those wetlands are across the top. For this purpose they were given numbers. They could be labeled in various ways, as long as it is standard between wetlands being compared. Q indicates that it is quantitative data. The upper left hand corner states that there are 60 plots by 37 species. The numbers in the matrix are Braun-Blanquet cover class estimates for each species in each plot.

The second data frame differentiates the two wetlands (Figure 7). Once again the plots from both wetlands are in the left-hand column, and the variable that defines them is in the attribute column. In this case it is Time 1 and Time 2.

60 plots		37 species																																				
	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q			
	s1	s10	s12	s16	s17	s35	s37	s43	s44	s45	s46	s47	s48	s49	s50	s51	s52	s54	s57	s58	s59	s60	s61	s62	s63	s64	s65	s67	s68	s69	s72	s73	s74	s75	s76	s77	s78	
plot 1	3	2	2	2	2	0	2	1	5	3	5	1	2	2	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
plot 2	7	1	2	2	2	0	3	0	2	0	4	1	2	2	2	1	1	2	2	2	3	4	5	4	3	4	5	6	5	4	3	2	3	4	0	0	0	
plot 3	4	2	0	0	0	1	2	3	0	3	5	0	2	0	2	0	0	2	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
plot 4	5	2	0	0	1	0	3	2	0	4	4	0	0	0	1	1	1	0	2	0	0	1	0	5	4	3	4	5	6	7	6	5	4	3	0	0	0	
plot 5	6	2	0	1	0	0	4	3	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	2	2	1	1	2	5	0	0	0	0	0	0	0	0	
plot 6	1	2	0	0	0	0	3	3	0	4	0	0	0	0	0	0	1	0	0	0	0	0	0	3	0	0	0	2	5	0	0	0	0	0	0	0	0	
plot 7	0	0	0	0	0	5	0	0	4	0	0	0	0	0	0	1	0	0	0	0	0	0	4	0	1	0	0	4	0	0	0	0	0	0	0	0	0	
plot 8	0	2	0	0	0	0	3	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	7	0	0	0	0	0	0	0	0	
plot 9	0	0	0	0	0	1	3	0	0	7	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	6	0	0	0	0	0	0	0	0	0	
plot 10	0	0	0	0	0	0	1	1	0	6	0	0	0	0	0	1	0	0	0	0	0	0	2	0	2	2	0	0	0	0	1	0	0	0	3	0	0	
plot 11	0	3	0	0	0	0	2	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	5	0	4	0	0	1	0	0	0	0	0	
plot 12	0	0	0	0	0	2	1	0	7	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	0	6	0	0	0	0	0	0	0	0	0	
plot 13	0	0	0	0	1	0	3	1	0	5	0	0	0	0	0	0	0	0	0	0	0	1	0	4	0	0	0	7	0	0	0	0	0	0	0	0	0	
plot 14	0	2	0	0	0	0	4	3	0	1	0	0	0	0	0	0	0	1	0	0	0	0	3	0	0	0	7	0	1	0	0	0	0	0	0	0	0	
plot 15	0	4	0	0	0	0	4	4	0	4	0	0	0	0	0	1	0	1	0	0	0	6	0	3	0	0	1	5	0	0	0	0	0	0	0	0	0	
plot 16	2	0	0	0	0	4	4	0	4	0	2	0	0	0	0	0	1	0	0	0	0	2	2	0	1	2	1	5	0	0	2	0	0	0	0	0	0	
plot 17	6	2	0	0	0	0	3	2	0	2	6	0	0	0	0	2	0	2	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
plot 18	7	2	0	2	2	0	3	3	0	0	7	0	0	0	0	0	2	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
plot 19	6	3	0	2	1	1	4	3	4	0	2	1	3	0	0	1	3	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
plot 20	0	0	2	0	2	0	3	0	0	5	2	2	0	2	1	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	
plot 21	0	3	0	0	0	0	4	0	0	7	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	4	0	0	0	0	0	2	0	0	2	0	
plot 22	0	2	0	0	0	0	3	0	0	6	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	4	0	0	0	0	0	2	0	0	2	0
plot 23	0	1	0	0	0	0	3	0	0	3	0	0	0	0	0	0	0	0	0	0	1	0	0	3	0	0	2	0	5	0	0	0	0	0	0	2	1	
plot 24	5	3	0	0	0	4	0	0	6	0	0	0	0	0	0	0	1	0	0	0	1	0	2	0	0	0	4	0	0	0	0	0	0	2	0	0	2	0
plot 25	6	5	0	2	0	0	3	0	0	4	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	5	0	0	0	0	1	1	0	0	1	
plot 26	5	5	0	2	0	0	4	4	0	2	0	0	0	0	2	0	0	1	0	0	2	0	3	0	0	1	0	0	2	0	0	0	0	0	0	0	0	
plot 27	6	4	0	1	0	0	4	3	0	4	0	0	0	0	0	0	1	0	0	1	0	3	0	2	0	2	0	2	0	2	0	0	0	0	0	0	0	
plot 28	5	2	0	2	0	0	3	6	0	4	0	0	0	0	0	0	1	0	0	1	0	0	2	0	0	2	0	0	2	0	0	0	0	0	0	0	0	0
plot 29	6	2	0	0	0	0	3	3	0	5	6	0	2	0	0	1	0	0	0	0	2	0	0	0	2	0	0	2	0	0	0	0	0	0	0	0	0	0
plot 30	3	1	0	2	0	0	2	3	0	0	8	0	0	0	1	0	0	0	1	0	0	2	0	2	0	2	0	0	2	0	0	0	0	0	0	0	0	0
plot 1	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
plot 2	6	1	2	2	2	0	2	0	2	0	4	1	2	2	1	1	2	1	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
plot 3	4	1	0	0	0	1	2	3	0	3	5	0	2	0	1	0	0	0	1	0	0	0	1	1	1	1	0	0	1	0	1	0	1	0	1	0	1	
plot 4	1	1	0	0	1	0	4	2	0	4	4	0	0	0	1	1	1	0	2	0	0	1	0	1	1	1	0	1	1	0	1	0	1	0	1	0	1	
plot 5	2	1	0	1	0	0	4	3	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	2	2	1	1	2	5	0	0	0	0	0	0	0	0	
plot 6	3	2	0	0	0	0	5	3	0	4	0	0	0	0	0	1	0	0	0	0	0	3	0	3	4	5	6	5	6	7	8	7	8	6	7	8	6	
plot 7	4	0	0	0	0	3	0	0	4	0	0	0	0	0	0	1	0	0	0	0	0	6	0	1	0	0	7	0	0	0	4	0	0	0	4	0	6	
plot 8	5	3	0	0	0	0	3	0	0	5	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	7	0	0	0	5	0	0	7	0	0	5	0	7
plot 9	6	0	0	0	0	1	3	0	0	7	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	6	0	0	0	6	0	0	6	0	0	6	0	6
plot 10	6	0	0	0	0	1	1	0	3	0	0	0	0	1	0	0	0	0	0	0	2	0	2	2	0	0	0	1	0	0	5	3	1	5	0	0	1	5
plot 11	5	0	0	0	0	1	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	2	0	4	0	4	1	3	4	0	0	0	4	
plot 12	4	1	0	0	0	0	2	1	0	5	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	0	6	0	0	0	3	0	4	3	0	4	3	
plot 13	3	0	0	0	0	0	3	1	0	5	0	0	0	0	0	0	0	0	0	0	0	1	0	4	0	0	4	0	0	0	2	0	4	2	0	4	2	
plot 14	2	2	0	0	0	0	2	3	0	1	0	0	0	0	0	0	2	0	0	0	0	3	0	0	0	7	0	1	0	3	0	6	3	0	6	3	0	
plot 15	1	2	3	4	5	6	6	8	9	3	0	0	0	0	1	0	2	0	0	0	5	1	5	0	0	1	5	0	0	4	0	6	4	0	6	4	0	
plot 16	2	4	5	3	2	4	5	6	4	3	2	3	3	4	5	3	2	0	0	0	0	2	2	0	1	2	1	5	0	0	2	5	0	5	0	5	0	
plot 17	6	2	4	2	3	8	7	6	5	4	3	5	4	3	2	3	4	2	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0
plot 18	5	3	0	2	1	0	3	3	0	0	7	0	0	0	0	0	2	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
plot 19	6	4	0	2	2	1	4	3	4	0	2	1	3	0	0	1	3	0	1	0	0	0	0	0	0	4	0	4	0	0	0	0	0	0	0	0	0	0
plot 20	0	5	2	0	1	0	3	0	0	5	2	2	0	2	1	0	2																					

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Figure 8: The second data frame for running a PerMANOVA test in PC-ORD differentiates the two wetlands being compared.

**APPENDIX 4: COST ESTIMATES FOR IMPLEMENTING A FULL OR A REDUCED PROTOCOL.**

Table 5: Cost estimate of implementing the full protocol. Total staff costs as calculated for individual sample wetlands include one professional and one intern for the drive time and field days, one intern for the data entry, and one professional for the data analysis. Additional expenses do not include equipment that MNAP already owns, or the cost to run computers for data entry and analysis.

Proposed Sample Wetlands	Prep time in days		Round trip drive time in days		Field time in days		Data entry and analysis time in days		Total Time in days		Staff Cost		Total Staff Cost
	Intern	Prof	Intern	Prof	Intern	Prof	Intern	Prof	Intern	Prof	Intern	Prof	Intern and Prof
Back River South W*	0.3	0.0	0.3	0.3	0.8	0.8	0.5	0.0	1.8	1.0	\$236.25	\$530.00	\$766.25
Back River North N*	0.3	0.0	0.3	0.3	0.8	0.8	0.5	0.0	1.8	1.0	\$236.25	\$530.00	\$766.25
Cutler South	0.3	0.0	1.0	1.0	0.8	0.8	0.5	0.0	2.5	1.8	\$337.50	\$927.50	\$1,265.00
Cutler North	0.3	0.0	1.0	1.0	1.0	1.0	0.5	0.0	2.8	2.0	\$371.25	\$1,060.00	\$1,431.25
Duck Lake	0.3	0.0	1.0	1.0	1.0	1.0	0.5	0.0	2.8	2.0	\$371.25	\$1,060.00	\$1,431.25
Great Heath	0.3	0.0	1.0	1.0	1.0	1.0	0.5	0.0	2.8	2.0	\$371.25	\$1,060.00	\$1,431.25
Killick Pond*	0.3	0.0	0.5	0.5	0.8	0.8	0.5	0.0	2.0	1.3	\$270.00	\$662.50	\$932.50
Mattagodus*	0.3	0.0	0.5	0.5	0.8	0.8	0.5	0.0	2.0	1.3	\$270.00	\$662.50	\$932.50
Rocky Lake	0.3	0.0	1.0	1.0	1.5	1.5	0.5	0.0	3.3	2.5	\$438.75	\$1,325.00	\$1,763.75
Salmon Brook	0.3	0.0	1.0	1.0	0.8	0.8	0.5	0.0	2.5	1.8	\$337.50	\$927.50	\$1,265.00
Spring River Lake	0.3	0.0	0.5	0.5	1.3	1.3	0.5	0.0	2.5	1.8	\$337.50	\$927.50	\$1,265.00
St. John Pond	0.3	0.0	1.0	1.0	1.0	1.0	0.5	0.0	2.8	2.0	\$371.25	\$1,060.00	\$1,431.25
<b>Protocol Development</b>									<b>12.0</b>	<b>0.5</b>	<b>\$1,620.00</b>	<b>\$265.00</b>	<b>\$1,885.00</b>
<b>Totals</b>	<b>3</b>	<b>0</b>	<b>9</b>	<b>9</b>	<b>11.25</b>	<b>11.25</b>	<b>6</b>	<b>0</b>	<b>29.25</b>	<b>20.75</b>	<b>\$5,568.75</b>	<b>\$10,997.50</b>	<b>\$16,566.25</b>

Additional Expenses	Units	Number	Cost per	Total
Lodging	rooms	14	\$80.00	\$1,050.00
	campsites	7	\$20.00	\$140.00
Food per diem	days	20	\$20.00	\$400.00
Vehicle	miles	3,000	\$0.45	\$1,350.00
Field Equipment (misc.)				\$50.00
PC-ORD Statistical Analysis Software	Single User License	1	\$300.00	\$300.00
Rite in the Rain copier paper	pkg of 200 sheets	1	\$30.00	\$30.00
<b>Total Cost Additional Expenses</b>				<b>\$3,320.00</b>

<b>Total Estimated Protocol Cost</b>	<b>\$19,886.25</b>
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Table 6: Cost estimate of sampling a reduced wetland monitoring protocol during the 2010 field season. Total staff costs as calculated for individual sample wetlands include one professional and one intern for the drive time and field days, one intern for the data entry, and one professional for the data analysis. Additional expenses do not include equipment that MNAP already owns, or the cost to run computers for data entry and analysis.

Proposed Sample Wetlands	Prep time in days		Round trip drive time in days		Field time in days		Data entry and analysis time in days		Total Time in days		Staff Cost		Total Staff Cost
	Intern	Prof	Intern	Prof	Intern	Prof	Intern	Prof	Intern	Prof	Intern	Prof	
Back River North N*	0.25	0	0.25	0.25	0.75	0.75	0.5	0	1.75	1.00	\$236.25	\$530.00	\$766.25
Cutler North	0.25	0	1.00	1.00	1.00	1.00	0.5	0	2.75	2.00	\$371.25	\$1,060.00	\$1,431.25
Duck Lake	0.25	0	1.00	1.00	1.00	1.00	0.5	0	2.75	2.00	\$371.25	\$1,060.00	\$1,431.25
Great Heath	0.25	0	1.00	1.00	1.00	1.00	0.5	0	2.75	2.00	\$371.25	\$1,060.00	\$1,431.25
Killick Pond*	0.25	0	0.50	0.50	0.75	0.75	0.5	0	2.00	1.25	\$270.00	\$662.50	\$932.50
Mattagodus*	0.25	0	0.50	0.50	0.75	0.75	0.5	0	2.00	1.25	\$270.00	\$662.50	\$932.50
Rocky Lake	0.25	0	1.00	1.00	1.50	1.50	0.5	0	3.25	2.50	\$438.75	\$1,325.00	\$1,763.75
<b>Protocol Development 2010</b>									<b>12.00</b>	<b>0.75</b>	<b>\$1,620.00</b>	<b>\$397.50</b>	<b>\$2,017.50</b>
<b>Database Design</b>									<b>0</b>	<b>3.00</b>	<b>\$0.00</b>	<b>\$1,590.00</b>	<b>\$1,590.00</b>
<b>Totals</b>	<b>1.75</b>	<b>0</b>	<b>5.25</b>	<b>5.25</b>	<b>6.75</b>	<b>6.75</b>	<b>3.5</b>	<b>0</b>	<b>29.25</b>	<b>15.75</b>	<b>\$3,948.75</b>	<b>\$8,347.50</b>	<b>\$12,296.25</b>

Additional Expenses	Units	Number	Cost per	Total
Lodging	Rooms	8	\$80.00	\$640.00
	campsites	2	\$20.00	\$40.00
Food per diem	days	10	\$20.00	\$200.00
Vehicle	miles	2,000	\$0.45	\$900.00
Field Equipment (misc.)				\$50.00
PC-ORD Statistical Analysis Software	Single User License	1	\$300.00	\$300.00
Rite in the Rain copier paper	pkg of 200 sheets	1	\$30.00	\$30.00
<b>Total Cost Additional Expenses</b>				<b>\$2,160.00</b>

<b>Total Estimated Cost of Wetland Sampling in 2010</b>	<b>\$14,456.25</b>
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If the cost of sampling this set of 18 wetlands is prohibitive, then a smaller sub-set could be sampled as funds allow. Removing Back River South E, Back River North S, Cutler South, Duck Lake, Killick Pond, Killick Pond, Mattawamkeag, Salmon Brook North from the field sampling protocol will save an estimated \$6,483.75 (for a projected total cost of \$13,868.75), while still retaining relative areas of wetland types in sample wetlands that mimic the statewide relative areas (Table 7). These recommendations also retain geographical variety in the sample wetlands and keep some of the most interesting and pristine wetlands in the protocol. Selection of sample wetlands is ultimately based on professional opinion, agency needs, and resource availability.

Table 7: Areas and percent areas of wetland types included under the pared-down sample wetland set described above.

Wetland Type	Sample Wetlands	Acres	Total	Percent of Sample Wetlands
E2EM1P	Back River South W*	37.0	57.0	3%
	Back River North N*	20.0		
PEM1/SS1E	Bigelow Preserve	24.9		
	Machias River Watershed*	7.1		
PEM1E	Bigelow Preserve	1.5	494.4	24%
	Cutler North	148.2		
	Salmon Brook South	15.3		
	Spring River Lake	253.8		
	St. John Pond	42.2		
PEM1F	Mattagodus*	1.4		
PSS1/3E	Machias River Watershed*	20.7		
	Rocky Lake	31.2		
	Salmon Brook South	65.8		
	St. John Pond	18.3		
PSS1/4E	Bigelow Preserve	7.7	856.6	41%
PSS1/EM1E	Rocky Lake	21.7		
	Bigelow Preserve	3.7		
PSS1E	Cutler North	37.7		
	Rocky Lake	161.7		
	Spring River Lake	137.0		
PSS1F	St. John Pond	110.7		
	Machias River Watershed*	33.8		
	Mattagodus*	192.1		
	Rocky Lake	14.5		
PSS3/1Ba	Cutler North	30.8	52.9	3%
PSS3/1E	Machias River Watershed*	8.3		
	Spring River Lake	6.3		
PSS3E	Spring River Lake	7.5		
PSS4/1E	Spring River Lake	3.0	52.6	3%
PSS4B	Bigelow Preserve	6.6		
PSS4Ba	Salmon Brook South	38.2		
PSS4E	Bigelow Preserve	3.8		
PSS4E	Spring River Lake	1.0		
PSS7BA	Cutler North	44.0	568.5	27%
PSS7BA	Great Heath	499.1		
PSS7Ba	Machias River Watershed*	25.4		



## APPENDIX 5: ROCKY LAKE STUDY WETLAND SAMPLING TRANSECTS

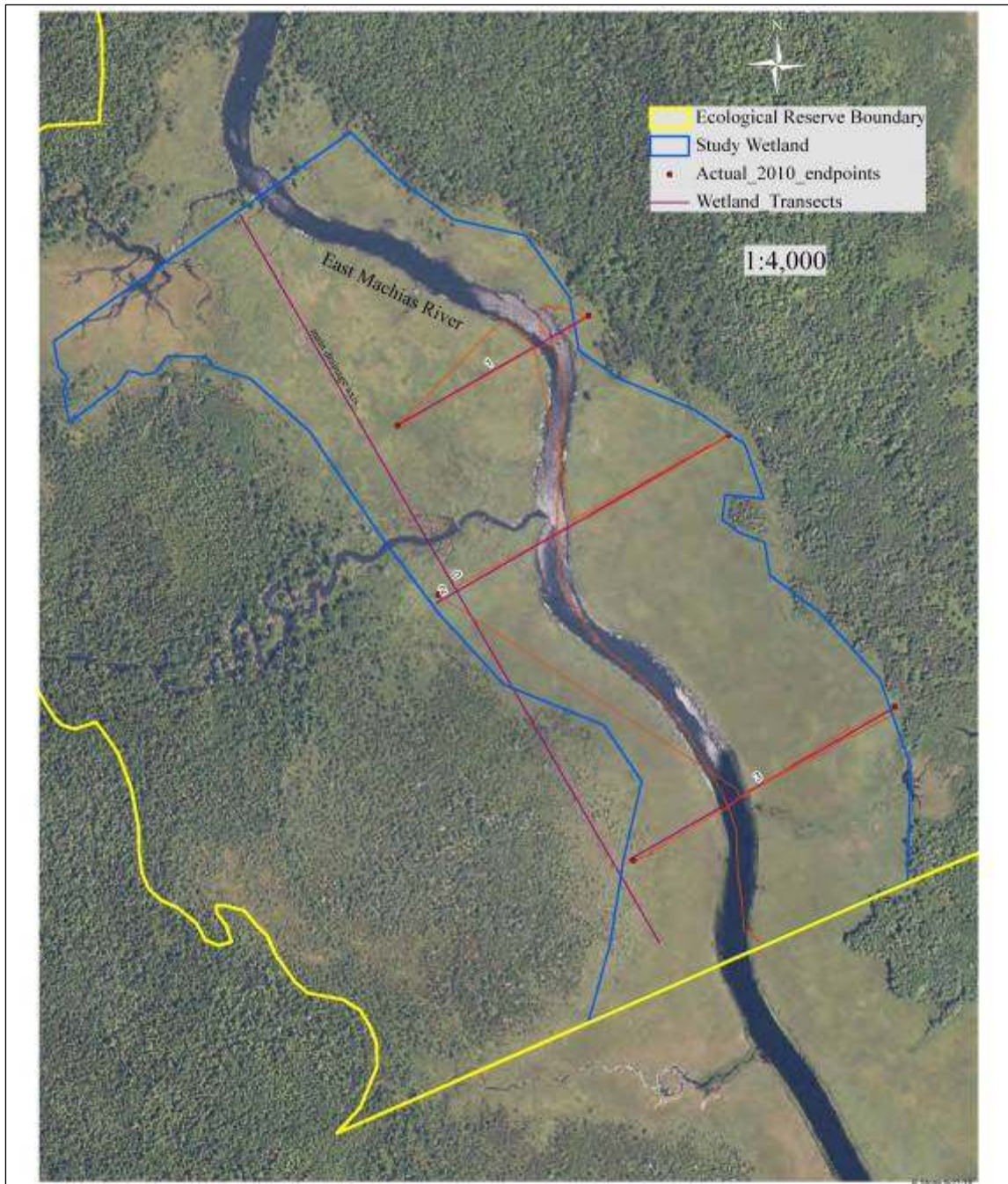


Figure 9: This map shows the sampling transect layout in ArcMap. The Rocky Lake Ecological Reserve Boundary is shown in yellow. The sample wetland boundary is in blue, and the ideal transects are in red. The orange line shows the actual track that field researchers followed while collecting data during the summer of 2010. As seen in the northern transect, poor GIS accuracy and human error can make it difficult to make mapped locations and field locations of transects match up. Also note that leaving the GPS unit on and referring to it often can improve field accuracy as seen in the southern two transects.

Project Budget

**Maine Natural Areas Program  
Wetland Monitoring on Ecoreserves  
FY2010 - FY2011 Q2**

<b>Expenses</b>		<b>Total Project</b>
<b>Personnel</b>	Admin	521.03
	Data Management	2,514.27
	Environmental Review	46.22
	Field Work	4,824.06
	Financial Management	335.06
	Project Meeting	559.19
	Project Report	326.69
	Technical Materials	872.23
	Train Interns	148.77
	Personnel Benefits	<u>1,350.36</u>
	<b>Total Personnel</b>	<b>\$11,497.88</b>
<b>All Other</b>		-
	4099 Misc Prof Fees	5,699.84
	4271 Other Transportation	31.82
	4273 Lodging	445.09
	4274 Meals & Grats	368.58
	4672 Rent State Vehicle	372.69
	6401 Grant Public/Private Org	<u>\$1,324.50</u>
	<b>Total All Other</b>	<b>\$8,242.52</b>
<b>Total Project Expenses</b>		<b>\$19,740.40</b>