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PATTERNS OF POPULATION STRUCTURE AND PRODUCTIVITY IN  
SALTMARSH SPARROWS

BY

JENNIFER WALSH

Baccalaureate Degree (BS), University of New Hampshire, 2007

THESIS

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

Master of Science

in

Natural Resources

December, 2009

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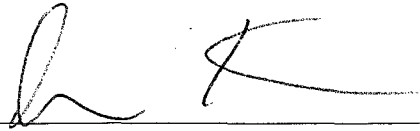
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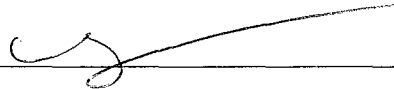
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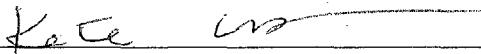
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## ACKNOWLEDGMENTS

U.S Fish and Wildlife Services and New Hampshire Sea Grant generously provided funding for this project. In addition, the College of Life Sciences and Agriculture provided a summer fellowship. I would like to thank my co-advisors, Dr. Kimberly Babbitt for her guidance and input and Dr. Adrienne Kovach for her invaluable mentorship and encouragement. Also, a special thanks to my committee member, Kate O'Brien for her advice and for all of her assistance and unwavering enthusiasm during countless hours of nest searches. I would like to thank the refuge biologists for their help in coordinating sampling efforts, including Nancy Pau (Parker River National Wildlife Refuge) and Suzanne Paton (John H Chafee National Wildlife Refuge). Thanks to Biodiversity Research Institute (Gorham, ME), specifically Oksana Lane, for making our sampling efforts possible and for sharing memorable advice and stories over long road trips. I would like to thank Dr. David Berlinsky for the use of his lab and for his years of support and guidance. A special thanks to Carolyn Schmitz, whose dedication to early morning fieldwork helped make data collection possible. Thanks to those who helped with DNA extraction in the lab including Justine Deming and Melanie Schroer. I'd like to thank the Hubbard Genome Center for the use of their capillary sequencer, and Jobriah Anderson for running the samples. I would also like to thank my parents and sister for their support and my fellow lab members for their advice and willingness to listen to and synthesize countless questions and ideas. Thanks to Erin Smith, for her tremendous listening skills and pep talks. Thank you to Marc Emond, David Moore, Sarah, Dorn and Andreas Cox, and Annalisa and Joel Wild-Miller at Tuck-Away farm for the home made

meals and kind words of encouragement. Lastly, a special thanks to my extended New Hampshire family, dogs included, for their endless kindness and positive reinforcement.

## TABLE OF CONTENTS

<b>ACKNOWLEDGMENTS</b> .....	<b>iii</b>
<b>LIST OF TABLES</b> .....	<b>vii</b>
<b>LIST OF FIGURES</b> .....	<b>viii</b>
<b>ABSTRACT</b> .....	<b>ix</b>
<b>INTRODUCTION</b> .....	<b>1</b>
<b>GENETIC VARIATION AND POPULATION CONNECTIVITY IN A SALT MARSH BREEDING PASSERINE, THE SALT MARSH SPARROW (<i>AMMODRAMUS CAUDACUTUS</i>)</b> .....	<b>6</b>
ABSTRACT.....	6
INTRODUCTION.....	7
METHODS.....	10
Study System and Sample Collection.....	10
DNA Extraction and Microsatellite Analysis.....	13
Population Structure and Connectivity.....	14
Patterns of Dispersal and Source/Sink Dynamics.....	15
RESULTS.....	17
Microsatellite Analysis.....	17
Population Structure and Connectivity.....	18
Patterns of Dispersal and Source/Sink Dynamics.....	24
DISCUSSION.....	27
Population Structure and Connectivity.....	27
Patterns of Dispersal and Source/Sink Dynamics.....	29
Conservation Implications.....	31
<b>NESTING SUCCESS AND DENSITY OF BREEDING SALT MARSH SPARROWS (<i>AMMODRAMUS CAUDACUTUS</i>) IN FIVE NEW ENGLAND SALT MARSHES</b> .....	<b>34</b>
ABSTRACT.....	34
INTRODUCTION.....	34
METHODS.....	36

Study System.....	36
Adult Abundance and Point Counts.....	38
Nest Monitoring and Survival.....	39
<b>RESULTS.....</b>	<b>40</b>
Adult Abundance and Point Counts.....	40
Nest Monitoring and Survival.....	40
<b>DISCUSSION.....</b>	<b>47</b>
Adult Abundance and Point Counts.....	47
Nesting Success.....	48
Conservation Implications.....	50
<b>CONCLUSION.....</b>	<b>52</b>
<b>LITERATURE CITED.....</b>	<b>55</b>
<b>APPENDIX A.....</b>	<b>60</b>
<b>APPENDIX B.....</b>	<b>72</b>



## List of Tables

<b>Table 1.</b> Genetic diversity of Saltmarsh Sparrows from 9 marshes in the northeastern U.S. Marsh code refers to site labels in figure 1 and N is the number of individuals sampled. Observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosities, $F_{IS}$ , number of alleles and allelic richness for each population are averaged across 10 microsatellite loci.....	12
<b>Table 2:</b> Pairwise $F_{ST}$ values for 9 Saltmarsh Sparrow populations. P values < 0.0014 are significant (after a Bonferroni correction) and are indicated in bold.....	20
<b>Table 3:</b> Dispersal patterns and sex ratio of migrants in five Saltmarsh Sparrow population clusters.....	26
<b>Table 4:</b> Maximum numbers of Nelson’s and Saltmarsh Sparrows observed at a single point. Columns include number of points at each site surveyed, the maximum number of Saltmarsh and Nelson’s Sparrows observed, the number of individuals that could not be identified as Nelson’s or Saltmarsh sparrows (Sharp-tailed species), and the maximum number of individuals (Nelson’s or Saltmarsh) identified at a single point.....	41
<b>Table 5:</b> Number of Saltmarsh Sparrow nests monitored, % failed, and outcome (predated or flooded) on five marshes.....	43
<b>Table 6:</b> Nest success models for Saltmarsh Sparrows- five marshes in New England...	44
<b>Table 7:</b> Probability of Saltmarsh Sparrow chicks surviving the duration of a 26-day nesting cycle. Survival estimates were calculated in MARK using the Site*T model....	45

## LIST OF FIGURES

<b>Figure 1:</b> Location of marshes where Saltmarsh Sparrows were sampled. Marshes are labeled by a marsh code described in Table 1.....	11
<b>Figure 2:</b> Isolation by distance in Saltmarsh Sparrows: a. with all populations, the results are not significant. b. With Chapman's Landing removed.....	21
<b>Figure 3:</b> Genetically similar population groupings and dispersal barriers in 9 Saltmarsh Sparrow populations. Five clusters identified by SAMOVA are circled ( $F_{ct}=0.007$ , $P<0.0001$ ) and the first, second and third order barriers identified by Barrier are drawn as lines and labeled.....	22
<b>Figure 4:</b> Principle Component Analysis of pairwise $F_{ST}$ values for 8 populations of Saltmarsh Sparrows (2 Long Island sites combined). Cumulatively, axis one and two account for 68.5% of the variation detected in the data set.....	23
<b>Figure 5:</b> Location of Saltmarsh Sparrow point count surveys and nest monitoring efforts. Marsh location code names: Furbish marsh (RCF), Fairhill marsh (FH), Chapman's Landing (CL), Hampton marsh (HB), and Parker River (PR).....	37
<b>Figure 6:</b> Linear regression shows a significant correlation between continuous marsh area and the average density of birds in a marsh.....	42
<b>Figure 7:</b> Daily survival rates for Saltmarsh Sparrow chicks plotted over a 26-day nesting cycle.....	46

## ABSTRACT

### PATTERNS OF POPULATION STRUCTURE AND CONNECTIVITY IN SALTMARSH SPARROWS

By

Jennifer Walsh

University of New Hampshire, December, 2009

The Saltmarsh Sparrow (*Ammodramus caudacutus*) is one of the few species globally that is exclusively restricted to coastal wetlands. Despite the high vagility characteristic of avian species, the highly patchy distribution of tidal marshes can often lead to fine scale genetic structure in salt marsh obligates. To elucidate patterns of population structure, we investigated the degree of genetic differentiation among nine Saltmarsh Sparrow populations along the northeastern coastline of the United States. Although overall  $F_{ST}$  values were small (0.008), population substructuring was detected along with a positive correlation between geographic distance and genetic differentiation, suggesting that *Ammodramus caudacutus* follow an isolation by distance model. However, Chapman's Landing was a distinct outlier despite its close proximity to other sampled marshes, indicating that additional factors other than geography play a role in genetic structuring. To identify patterns of source/sink dynamics, we implemented assignment tests from the software program GENECLASS2 using an exclusion method. Results from the assignment tests indicate that Parker River, in Newburyport, MA, is a source population. Findings from the genetic analyses were combined with field data collected on nesting success and density of breeding adults to correlate overall productivity of the sites sampled with results from assignment tests. Furthermore, results from point count surveys indicate a positive correlation between marsh size and the

density of breeding adults ( $P= 0.0148$ ,  $R^2=0.8954$ ), with the most birds observed at points located in Parker River. Results from our nesting study indicate that nesting success is variable among sites, and that the cause of chick mortality also varies. Despite this the percent of failed nests is comparably similar among four of the five sites surveyed. Our results offer new insight for conservation strategies, including information on population clusters, data on population trends, and the identification of source/sink dynamics.

## **CHAPTER 1**

### **Introduction**

Genetics and wildlife conservation are two fields that are merging more frequently as the sensitivity of genetic markers to changes in natural populations increases. Recent advances in genetic techniques have allowed for a more sophisticated approach to analyzing both fine and broad scale genetic structure in populations. Specifically, the use of microsatellite markers in dispersal studies has become increasingly popular, with their use in genetic studies surpassing that of other molecular markers available (Zhang and Hewitt, 2003). The use of microsatellites in wildlife studies has a multitude of practical conservation implications. Molecular markers can be used to determine whether fragmented populations are displaying balanced rates of gene flow, and whether populations with decreased rates of dispersal warrant increased management (DeYoung, 2005). Maintenance of populations as genetic units or identifying populations as source stocks can also be achieved through genetic analysis (DeYoung, 2005). Characterizing patterns of dispersal and identifying source populations is important for maintaining connectivity and genetic diversity within less suitable and fragmented habitat patches.

Human development, combined with natural processes has led to highly fragmented habitats, creating barriers to dispersal for some organisms. The patchy nature of available habitat has led to the concept of metapopulations. A metapopulation is a group of

populations experiencing constant fluctuations in the colonization and extinction of local populations (Hanski and Simberloff, 1997). The concept of metapopulations includes the assumptions that there are noticeable differences between patch suitability for the habitat requirements of a species, and that patches are large enough to sustain panmictic local populations (Hanski and Simberloff, 1997). These differences in patch quality can affect the rates of dispersal of individuals between isolated patches. The difference in dispersal rates between subpopulations may manifest itself in source/sink dynamics. A source/sink metapopulation is defined as a metapopulation in which there are patches experiencing a negative population growth rate in the absence of immigration (sink) and patches in which the growth rate is positive (sources; Hanski and Simberloff, 1997). The source/sink metapopulation theory can be applied to natural populations, as areas with better quality habitat will support higher reproductive success in individuals, making these habitats sources due to their relatively high population productivity. Individuals from source areas immigrate to patches experiencing lower rates of reproductive success, a process known as the rescue effect (Brown and Kodric-Brown, 1977). Populations experiencing immigration without emigration, due to poor habitat quality, are population sinks. Identifying population sources and sinks is a key aspect of conservation biology. It is important to identify population sources, as species will be lost from the sink if the source is destroyed.

Salt marshes are unique ecotonal habitats characterized by high productivity and a large proportion of species endemism. Coastal wetlands play a significant role in coastal productivity, acting as nutrient sources and sinks and sites for nutrient transformation (Daiber, 1986). Tidal salt marshes act as sites for both detritus decomposition and the

transformation of indigestible plant matter into available resources for consumers; this is the major pathway of energy utilization in a marsh ecosystem (Gosselink and Mitsch, 1993). Additional attributes of salt marsh ecosystems include the provision of shelter and nutrients to fish and shellfish (Gosselink and Mitsch, 1993).

Despite the ecological importance of tidal wetlands, anthropogenic stressors, both historical and current, have led to the degradation of coastal habitat. Salt marsh disturbance began as early as the 18<sup>th</sup> century, through the conversion of natural habitat into managed land for livestock grazing (Bertness, 2004). More recent impacts stem from the influx of settlement in coastal areas. At the end of the last century, it was approximated that 37% of the world's population was living within 100 km of the coast (Greenberg, 2006a). This vulnerability to coastal development has led to the fragmentation of salt marsh habitat; and as a result, a high percentage of the supported endemic species are classified as endangered or as species of conservation priority (Greenberg, 2006a).

The Saltmarsh Sparrow is a salt marsh obligate, and one of only two passerines, globally, that nest exclusively in salt marshes (Greenberg, 2006b). The breeding range of the Saltmarsh Sparrow extends from Maine to Virginia (Greenlaw and Rising, 1994) with an estimated 90% of the breeding population focused in the Northeast (Hodgman et al., 2002). Due to its limited range and reliance on a patchily distributed habitat, the Saltmarsh Sparrow is considered globally vulnerable to extinction (IUCN Red List criteria; Birdlife International, 2004) and a species of conservation priority (U.S Fish and Wildlife Service, 2008).

Saltmarsh Sparrows are promiscuous, displaying a mating system that is

uncommon in passerines (DiQuinzio, 2001). With this promiscuous mating system, Saltmarsh Sparrows do not form pair bonds or defend territories (DiQuinzio, 2001). The breeding season of the Saltmarsh Sparrow extends from early June to mid-August (Greenlaw and Rising, 1994). Females construct nests using marsh grasses, and build the nest a few centimeters off of the ground; nest building takes approximately four days (Greenlaw and Rising, 1994).

As ground nesting birds, Saltmarsh Sparrows have adapted to ensure nesting success in a habitat experiencing tidal fluctuations. Nest site selection appears to be based on vegetation types. The females choose areas where the vegetation is taller and denser for nest construction (Gjerdrum, 2005). Saltmarsh Sparrows may be using vegetation type as cues to indicate both substrate elevation and tidal flow when selecting nesting sites (DiQuinzio, 2002). Research has also shown that female Saltmarsh Sparrows display synchrony with tidal cycles as they, on average, build nests within three days after a tidal flood (Shriver, 2007). The initiation of nest construction immediately after a high tide increases the probability that nesting would be completed before the next flood (with spring flood tides occurring every 28 days; Shriver, 2007).

As salt marsh obligates, Saltmarsh Sparrows must inhabit an environment that experiences tidal fluctuations, habitat fragmentation and the increasingly problematic effects of climate change and sea level rise. It is estimated that 50% of the available breeding habitat for Saltmarsh Sparrows has been lost over the past 300 years to wetland draining and ditching (Greenlaw and Rising, 1994). Despite the increasing concerns for Saltmarsh Sparrow conservation, there is little information on population dynamics. Information on the dispersal and structure of Saltmarsh Sparrow populations would



contribute to the determination of management units. Genetic analysis can provide insight about population structure and dynamics, allowing for a more informed approach to conservation and consideration in the formation of future management plans.

In this study, application of the above genetic techniques in combination with models of daily survival rates and field estimates of adult abundance is used to provide insight into the ecological patterns characteristic of this species. The following chapters aim to characterize patterns of genetic structure in Saltmarsh Sparrows and to combine data on productivity and dispersal to identify possible source populations.

**The objectives of my thesis research were to:**

1. Assess population structure and identify genetically similar population clusters
2. Characterize patterns of dispersal and source/sink dynamics
3. Compare relative abundance of breeding adults between marsh complexes
4. Determine nesting success and fledgling survival rates

## CHAPTER 2

### GENETIC VARIATION AND POPULATION CONNECTIVITY IN A SALT MARSH BREEDING PASSERINE, THE SALTMARSH SPARROW (AMMODRAMUS CAUDACUTUS)

#### Abstract

Despite the high vagility characteristic of migratory avian species, behavioral mechanisms and habitat fragmentation may lead to reduced connectivity and patterns of genetic substructuring. The Saltmarsh Sparrow (*Ammodramus caudacutus*) is a saltmarsh obligate that breeds along the Northeast coast of the U.S. and is exclusively restricted to patchily distributed wetland habitat. Field observations of site fidelity in Saltmarsh Sparrows combined with the fragmentation of salt marsh habitat may lead to patterns of fine scale genetic structure. To elucidate patterns of population structure, we investigated the degree of genetic differentiation among nine Saltmarsh Sparrow populations in the northeastern United States. Although overall  $F_{ST}$  values were small (0.008), population substructuring was detected along with a positive correlation between geographic distance and genetic differentiation, suggesting that *Ammodramus caudacutus* follow an isolation by distance model. We also identified inland marshes that displayed high levels of genetic differentiation despite their close geographic proximity to other sampling locations, suggesting that additional mechanisms, besides geography, influence population genetic structure. Our results offer new insight for conservation strategies,

specifically the assessment of potential management units, as we highlight five population clusters based on genetic similarity.

### **Introduction**

Gene flow among geographically fragmented populations acts as a cohesive force that maintains genetic connectivity and increases variation within populations (Allendorf and Luikart, 2007). Thus, characterizing patterns of dispersal and population connectivity is a fundamental component of developing and implementing effective management systems for species of conservation priority. Natural populations are often described as hierarchical, with multiple levels existing both regionally and locally. Thus, the identification of population substructuring can be useful in the determination of appropriate management units (Allendorf and Luikart, 2007). Furthermore, identifying the degree of connectivity between geographically separated subpopulations can highlight dispersal patterns, including the identification of sources and sinks. Individuals from source areas immigrate to patches experiencing lower rates of reproductive success (sinks), a process known as the rescue effect (Brown and Kodric-Brown, 1977). Therefore, if the source population is lost, the sink populations can no longer sustain a viable population size. This is often seen in cases of patchy populations, where high dispersal rates between sites that are spatially separated create homogenous populations (Scheiman et al., 2007).

The classification of the above processes in natural populations is increasingly important in light of habitat degradation and fragmentation, especially in ecosystems subject to anthropogenic stressors. Fragmented populations often experience reduced rates of migration, leading to subsequent loss of genetic diversity (Frankham et al., 2002).

The identification of source populations is important for maintaining connectivity and genetic diversity within less suitable habitat patches.

Coastal wetlands are an ecotonal system inhabited by a variety of salt marsh specialists suited for a range of extreme abiotic conditions including daily fluctuations in salinity and tidal cycles (Greenberg, 2006a). Coastal areas are some of the most heavily settled areas globally, with 37% of the world's population living within 100 km of the coast at the end of the last century (Greenberg, 2006a). Increasing anthropogenic pressures, however, such as coastal development, has led to the fragmentation and loss of marsh habitat. In New England, human development has resulted in the loss of 80% of coastal wetlands (Bertness et al., 2002). As a result, a high percentage of the supported endemic species are endangered or of conservation priority (Greenberg, 2006a). Because habitat fragmentation can restrict movement between populations, the loss of coastal habitat may inhibit the dispersal of salt marsh obligates and act to reduce gene flow among populations.

Globally, the Saltmarsh Sparrow (*Ammodramus caudacutus*) is one of only two passerine species found exclusively in salt marshes (Greenberg, 2006b). The breeding range of the Saltmarsh Sparrow extends from Maine to Virginia (Greenlaw and Rising, 1994) with an estimated 90% of the population breeding in the northeastern coast of the United States (Hodgman et al., 2002). Due to the limited range of this species, and its exclusive habitat requirements, the Saltmarsh Sparrow is listed as a species of conservation priority (U.S. Fish and Wildlife Service, 2008) and is considered globally vulnerable to extinction (IUCN Red List criteria; Birdlife International, 2004). Despite the conservation status of this species, information on the impacts of coastal

fragmentation on population structure is limited. Information on genetic substructuring and the identification of genetically similar population clusters can provide insight in the formation of appropriate management units in Saltmarsh Sparrow populations.

Population structure is expected to be more pronounced in sedentary organisms, in comparison to birds, which are characterized by high vagility (Avise, 2004). However, many avian species are characterized by site fidelity (Wheel Wright and Mauck, 1998) and mating systems (Woxvold, 2006; Bouzat, 2004) that can lead to genetic structuring. Habitat disruption and patchy distribution further contribute to population structure by reducing migration between breeding populations (Frankham et al., 2002; Chan, 2006). Observations of site fidelity in Saltmarsh Sparrows coupled with their exclusive reliance on a highly patchily distributed ecosystem may lead to fine scale patterns of genetic structure. To elucidate these patterns of genetic structure in this coastally restricted avian species, we investigated the degree of connectivity between breeding populations of Saltmarsh Sparrows.

In this study, we used microsatellite analysis to assess the level of genetic variability and patterns of dispersal between Saltmarsh Sparrow populations sampled from nine marsh complexes along the northeastern coastline of the United States. Our main objective was to characterize patterns of population substructuring. We predicted that the fragmented nature of wetland habitat combined with behavioral characteristics of Saltmarsh Sparrows would result in fine scale genetic structure among sampled populations. We also predicted that larger, more continuous marshes would act as population sources for smaller, more isolated patches. Individual and population based analyses were used to: (i) characterize population genetic structure among sampled

populations; (ii) identify genetically similar population clusters that can provide insight in the formation of management units; (iii) determine whether the degree of genetic differentiation is explained by geographic distance; (iv) characterize patterns of dispersal and source/sink dynamics.

## **Methods**

### **Study System and Sample Collection**

We sampled Saltmarsh Sparrows during June and July of 2006 to 2008 at multiple subsites within nine marshes along the northeastern coast of the U.S. within the northern half of the species' breeding range. Study marshes were located in Wells, ME (Rachel Carson NWR), Scarborough, ME (Rachel Carson National Wildlife Refuge, NWR), Hampton, NH, Rye, NH, Stratham, NH, Newburyport, MA (Parker River NWR), Narragansett, RI (John H. Chafee NWR), Shirley, NY (Wertheim NWR) and Oceanside, NY (Figure 1). Most sites were sampled in one year; Rhode Island and Parker River were sampled in two and three years, respectively (Table 1). At each site, we deployed two to six 12-m mist nets with size 36 mm mesh to capture a target sample of 50 birds from each site. Blood samples (30-50  $\mu$ l) were drawn from the cutaneous ulnar vein using a non-heparinized capillary tube and stored at room temperature on Whatman filter cards for later genetic analysis. The location of mist net deployment was recorded using GPS.

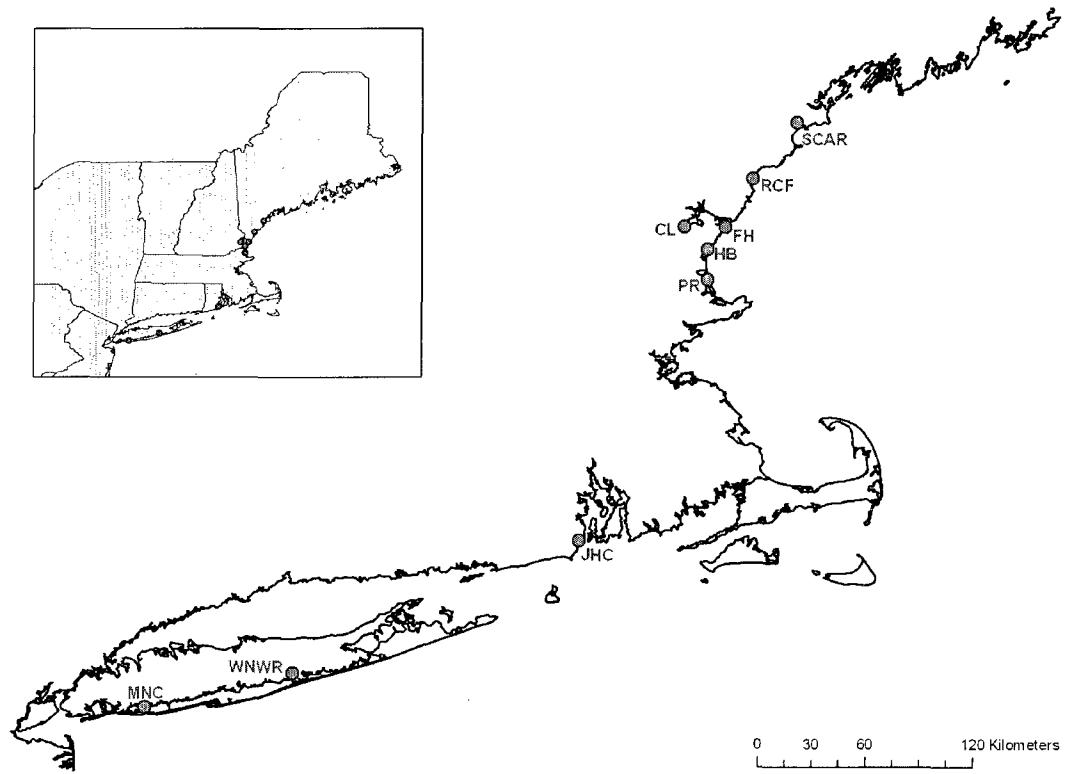


Figure 1: Location of marshes where Saltmarsh Sparrows were sampled. Marshes are labeled by a marsh code described in Table 1.

**Table 1: Genetic diversity of Saltmarsh Sparrows from 9 marshes in the northeastern U.S. Marsh code refers to site labels in figure 1 and N is the number of individuals sampled. Observed ( $H_o$ ) and expected ( $H_E$ ) heterozygosities,  $F_{is}$ , number of alleles and allelic richness for each population are averaged across 10 microsatellite loci.**

Sampling Location	Marsh code	N	$H_o$	$H_E$	$F_{is}$	# of alleles	Allelic richness	# of private alleles
Spurwink- Scarborough, ME (Rachel Carson NWR)	SCAR	40	0.786	0.826	0.048	10.6	8.71	2
Furbish- Wells, ME (Rachel Carson NWR)	RCF	64	0.774	0.801	0.034	11.3	8.51	1
Chapman's Landing- Stratham, NH	CL	30	0.790	0.803	0.016	9.6	8.43	1
Fairhill- Rye, NH	FH	35	0.808	0.797	-0.014	9.8	8.42	0
Hampton Beach- Hampton, NH	HB	45	0.784	0.799	0.019	10.2	8.38	1
Parker River- Newburyport, MA (Parker River NWR) (sampled in 2006,2007 and 2008)	PR	72	0.794	0.816	0.026	12.7	8.82	6
John H Chafee NWR- Narragansett, RI (sampled in 2007 and 2008)	JHC	53	0.756	0.793	0.046	11.1	8.5	2
Wertheim NWR- Shirley, NY	WNWR	29	0.717	0.772	0.072	9	7.87	1
Marine Nature Center- Oceanside, NY	MNC	19	0.768	0.772	0.007	8.2	8.01	2
<b>Total</b>	<b>Total</b>	<b>387</b>						



## **DNA Extraction and Microsatellite Analysis**

DNA was extracted from blood samples using a DNeasy Blood Kit (Qiagen, Valencia, CA). Because some of our field sites were located within an overlap zone between the Saltmarsh Sparrow and a congener, the Nelson's Sparrow (*Ammodramus nelsoni*), we performed a genetic bar-coding assay on all samples to confirm the species identity of sampled individuals (Walsh et al, in prep; see appendix A). DNA was amplified using 11 microsatellite loci combined in 4 multiplexes: Aca01, Aca04, Aca05, Aca08, Aca11, Aca12, Aca17, Aca21 (Hill, 2008), Esc $\mu$ 1, Esc $\mu$ 6 (Hanotte et al., 1994) and As $\mu$ 15 (Bulgin, 2003). 12.5  $\mu$ l polymerase chain reactions contained 2 $\mu$ l of eluted genomic DNA, 0.3-0.8 $\mu$ M of each primer (labeled with Hex, Ned or Fam), 1.5-2.5mM MgCl<sub>2</sub>, 5X colorless buffer (Promega), 0.2mM of deoxyribonucleotides, and 0.1-0.2 units of Taq DNA polymerase (Promega). Optimized cycling conditions were as follows: 30-32 cycles of 94°C for 30 s, 53°-56°C for 45 s, 72°C for 1 min and a final extension step at 72°C for 5 min. Optimal annealing temperatures were 53°C for As $\mu$ 15, 55°C for Esc $\mu$ -1 and Esc $\mu$ -6 and 56° for Aca01, Aca04, Aca05, Aca08, Aca11, Aca12, Aca17 and Aca21. Amplified products were electrophoresed on an automated DNA sequencer (ABI 3130 genetic analyzer, Applied Biosystems, Foster City, CA) and individual genotypes were scored using PEAKSCANNER software (ABI). Positive controls were used in conjunction with the software program Allelogram (Morin et al., 2009) to standardize across electrophoretic runs. Alleles were binned manually based on the normalized raw scores generated by Allelogram.

We used the software program MICRO-CHECKER (Van Oosterhout, 2004) to check the data set for errors and to test for the presence of null alleles. We identified null

alleles in Aca21 and subsequently dropped this locus from the final data set. The data were tested for linkage disequilibrium using the randomization method implemented in the software program FSTAT (Goudet et al., 2002). To assess genetic diversity, unbiased estimates of expected and observed heterozygosities and allelic richness were calculated in FSTAT. Estimated  $F_{IS}$  values generated in FSTAT were used to test for deviations from Hardy-Weinberg equilibrium (significance testing was performed using 10,000 randomization steps with a Bonferroni adjustment).

### **Population Structure and Connectivity**

To characterize genetic differentiation among sampled populations, pairwise  $F_{ST}$  values were calculated using FSTAT. For sites that were sampled in multiple years (RI and PR), we used  $F_{ST}$  values to test for annual fluctuations in genetic variation. There were no significant differences in  $F_{ST}$  values when comparing the same site over multiple years, allowing us to combine multi-year data for the Parker River and Rhode Island sites. Significance testing was performed by the permutation method in FSTAT with a nominal level of 5/100 and a Bonferroni adjustment was applied to correct for multiple tests.

To investigate the presence of hierarchical structure, the software programs SAMOVA (Dupanloup, 2002) and BARRIER (Manni et al., 2004) were used to identify population clusters. The program SAMOVA uses an Arlequin input file in conjunction with a geographic file containing lat/long coordinates for the sample sites to cluster populations. SAMOVA is run multiple times on the same data set with variations in the number of groups of populations (K) specified. The most appropriate number of groups for the data set is achieved by maximizing the value for  $F_{CT}$ . We ran our data set in

SAMOVA for  $K=1-9$  clusters and compared  $F_{CT}$  values between each run to identify the most appropriate number of clusters for our data set.

We used the software program BARRIER, which implements the Monmonier algorithm to identify genetic barriers between adjacent populations. Unlike SAMOVA, which does not truly implement a geometric approach and thus can identify populations that are maximally differentiated, BARRIER only compares adjacent populations and is more suited for finding genetic barriers between sets of populations (Manni et al, 2004). First, second, and third order barriers were generated using ten per locus  $F_{ST}$  matrices calculated per locus in the program GENEPOP (Raymond and Rousset, 1995). We used overall  $F_{ST}$  values to generate a consensus of first, second, and third order barriers between all loci. A principle components analysis (PCA) is complementary to the Monmonier boundary plots generated in BARRIER, and thus both methods were used in conjunction to strengthen the interpretation of the results (Manni et al., 2004). A PCA was generated with 1000 permutations in the software program GenAlEx 6.1 (Peakall and Smouse, 2006). To test for a correlation between geographic distance and genetic distance we used a Mantel test with 1000 permutations, as implemented in GenAlEx 6.1.

### **Patterns of Dispersal and Source Sink Dynamics**

Assignment tests were used to characterize dispersal patterns among sampled populations and to detect the presence of source/sink dynamics. We used the software program GENECLASS 2 (Piry, 2004) to calculate partial Bayesian assignments. We used the Cornuet et al. (1999) resampling method to calculate assignment probabilities. The Cornuet et al. (1999) method uses a population's observed allele frequencies to simulate multilocus genotypes and then compares the probability that a sampled individual

originated from a population to the distribution of probabilities calculated through simulations. Because this method considers each individual separately, as opposed to comparing populations, it does not assume that all possible putative populations of origin have been sampled (Berry, 2004). Thus this approach is the most appropriate for our sampling methods, as we know that we have not sampled all of the Saltmarsh Sparrow populations in the northeast. Assignment probabilities were calculated using 10,000 simulations. Since our objective was to identify overall dispersal trends as opposed to detecting first generation migrants we chose the Cornuet et al. (1999) method over that of Paetkau et al. (2004).

We used the exclusion methods described in Manel et al. (2002) and chose a threshold value of 0.1 to assign individuals. When the individual genotype likelihood is below the chosen threshold, the population can be excluded as the origin of the individual; if all but one population can be excluded using this method, we can assign the remaining population as the population of origin (Manel et al., 2002). The ‘detect migrants’ option in GENECLASS2 was also applied to lend further support to our assignment results. We chose the likelihood-based test statistic  $L_h$ , as this is the most appropriate statistic when all putative source populations have not been sampled (Piry, 2004).

To test for the presence of sex-biased dispersal, males and females were compared using corrected assignment indices and  $F_{ST}$ -based tests implemented in GENECLASS 2 and FSTAT, respectively. To be consistent with the assignment tests, we grouped populations based on the 5 clusters identified in SAMOVA for all analyses of sex-biased dispersal. We tested for statistical significance in differences between  $F_{ST}$ ,  $F_{IS}$

and relatedness values for males and females using a randomization test implemented in FSTAT (Goudet, 2002). Significant differences in  $F_{ST}$ ,  $F_{IS}$ , and relatedness between males and females are indicative of sex-biased dispersal. If, for example, males are the dispersers and females display philopatry, the males should be less related to each other than the females. Based on our assignment tests (see above), we also determined what proportion of the individuals assigned as migrants were male or female for each population. We also calculated log transformed assignment indices ( $AI$ ) using GENECLASS. To do this, log values generated using the Paetkau (2004) frequency methods were corrected ( $AIC$ ) for a population effect by subtracting individual assignment probabilities from an overall probability averaged over the entire population (Favre et al., 1997). It is expected that corrected  $AI$  values for a population will average 0 and negative  $AIC$  values will be indicative of dispersing individuals, as immigrants into a population will have a lower assignment probability. Finally, to test for patterns of relatedness for each population, we estimated mean relatedness between males (MM), females (FF) and opposite-sex pairs (FM) using the Queller and Goodnight (1989) estimate as implemented in the software program SPAGeDi (Hardy and Vekemans, 2002).

## **Results**

### **Microsatellite Analysis**

We genotyped 421 individuals; 34 individuals were found to have Nelson's specific mitochondrial DNA and were removed from the data set (see appendix 1). Of the remaining samples (n= 387), 13 individuals (3.3%) had missing values for no more than

two loci. Individual loci were variably polymorphic, with the number of alleles for each locus ranging from 4 to 29. There were a total of 137 alleles and of those, 16 were found in only a single population (Table 1). Mean observed and expected heterozygosities ranged from 0.717-0.826 (see Table 1). There were no significant deviations from Hardy-Weinberg, and no linkage between loci.

### **Population Structure and Connectivity**

Small but significant differences in genetic divergence ( $F_{ST}$ ) were detected among sampled populations (Table 2), with values ranging from 0.0001 to 0.0240 and an overall  $F_{ST}$  value of 0.008. Chapman's Landing and Spurwink were the most differentiated populations by  $F_{ST}$ . Significant  $F_{ST}$  values indicated differentiation of Long Island and Rhode Island from the other populations. The smallest  $F_{ST}$  values occurred in the comparison of Parker River and Hampton to all other populations. This was true even in the comparison of Parker River to the sites in Long Island, despite the large geographic distance separating these sites. The two Long Island sites (WNWR and MNC) were combined based on small sample size; this was supported by small, non-significant  $F_{ST}$  values between the two sites (0.0015). Thus these locations were treated as one population for all subsequent analyses, including PCA and the Mantel test. To test for isolation by distance, Chapman's landing was an outlier ( $R^2 = 0.122$ ,  $P = 0.149$ ; Table 2) and was removed from the data. When a Mantel test was performed on the 7 remaining populations, we found that the degree of genetic differentiation was positively correlated with geographic distance ( $R^2 = 0.41$ ,  $P = 0.001$ ; Figure 2).

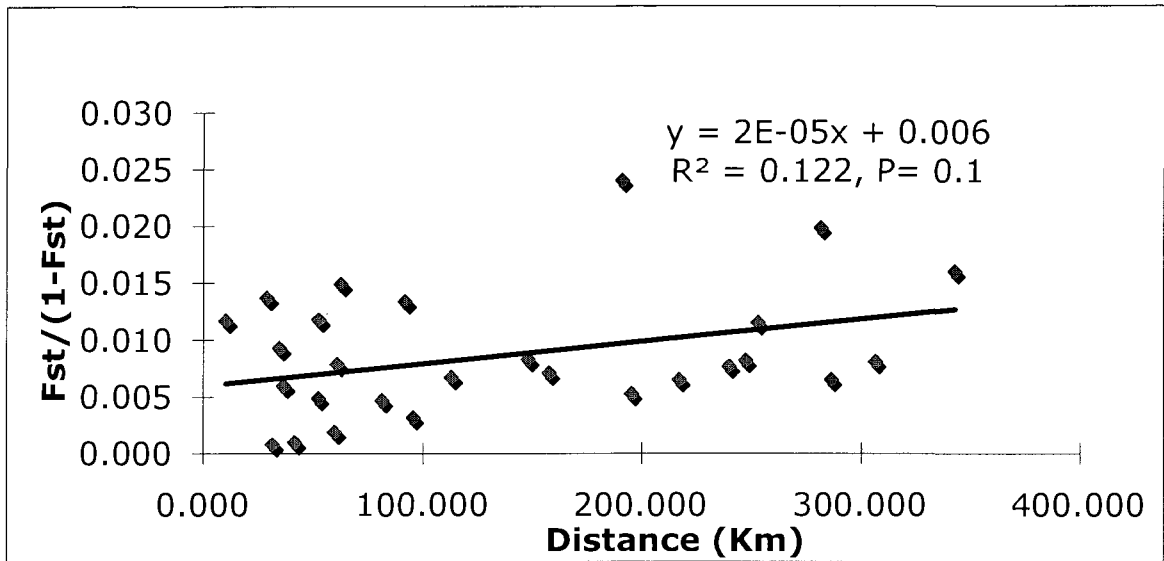
Results from SAMOVA and BARRIER were used to identify clusters of genetically similar populations. Results from SAMOVA yielded small but significant  $F_{CT}$  values ( $P < 0.001$ ), with  $K=6$  and  $K=5$  equally yielding the highest  $F_{CT}$  values (0.00797 and 0.00792, respectively). For  $K=5$ , the clusters were as follows: Chapman's Landing, Fairhill/Furbish/Hampton/Parker River, Spurwink, Rhode Island, and the 2 Long Island sites. For  $K=6$ , the Fairhill/Furbish/Hampton/Parker River cluster was split into two separate clusters comprised of Fairhill/Furbish and Hampton/Parker River. We chose 5 clusters (Figure 3) due to the high connectivity observed between Parker River, Fairhill, and Furbish. For all values of  $K$ , the CL population was consistently selected first as the most differentiated population and WNWR and MNC were invariably clustered together. Results from BARRIER coincided with the clusters identified in SAMOVA. BARRIER identified first, second and third order barriers separating CL, the Long Island cluster and JHC respectively (Figure 3). A first order barrier fully separating CL from all three adjacent populations was supported by 4 out of 10 loci, while at least a partial barrier, separating CL from one or more adjacent population, was supported by the remaining loci. A second order barrier fully separating the Long Island complex was supported by 7 out of 10 loci and a third order barrier fully separating RI from northern marsh complexes was supported by 3 out of 10 loci. A PCA provided further support for the population clusters (Figure 4); results correspond with those of SAMOVA and BARRIER.

**Table 2: Pairwise  $F_{ST}$  values for 9 Saltmarsh Sparrow populations. P values < 0.0014 are significant (after a Bonferroni correction) and are indicated in bold.**

	CL	FH	RCF	HB	WNWR	MNC	PR	JHC	SCAR
CL	***								
FH	<b>0.0117</b>	***							
RCF	<b>0.0137</b>	<b>0.0008</b>	***						
HB	<b>0.0118</b>	<b>0.0049</b>	<b>0.0047</b>	***					
WNWR	<b>0.0230</b>	<b>0.0097</b>	<b>0.0082</b>	<b>0.0071</b>	***				
MNC	<b>0.0150</b>	<b>0.0028</b>	<b>0.0082</b>	<b>0.0095</b>	<b>0.0015</b>	***			
PR	<b>0.0093</b>	<b>0.0010</b>	<b>0.0019</b>	-0.0001	<b>0.0075</b>	<b>0.0094</b>	***		
JHC	<b>0.0240</b>	<b>0.0053</b>	<b>0.0065</b>	<b>0.0083</b>	<b>0.0160</b>	<b>0.0096</b>	<b>0.0071</b>	***	
SCAR	<b>0.0149</b>	<b>0.0079</b>	<b>0.0060</b>	<b>0.0067</b>	<b>0.0154</b>	<b>0.0165</b>	<b>0.0032</b>	<b>0.0115</b>	***



a.



b.

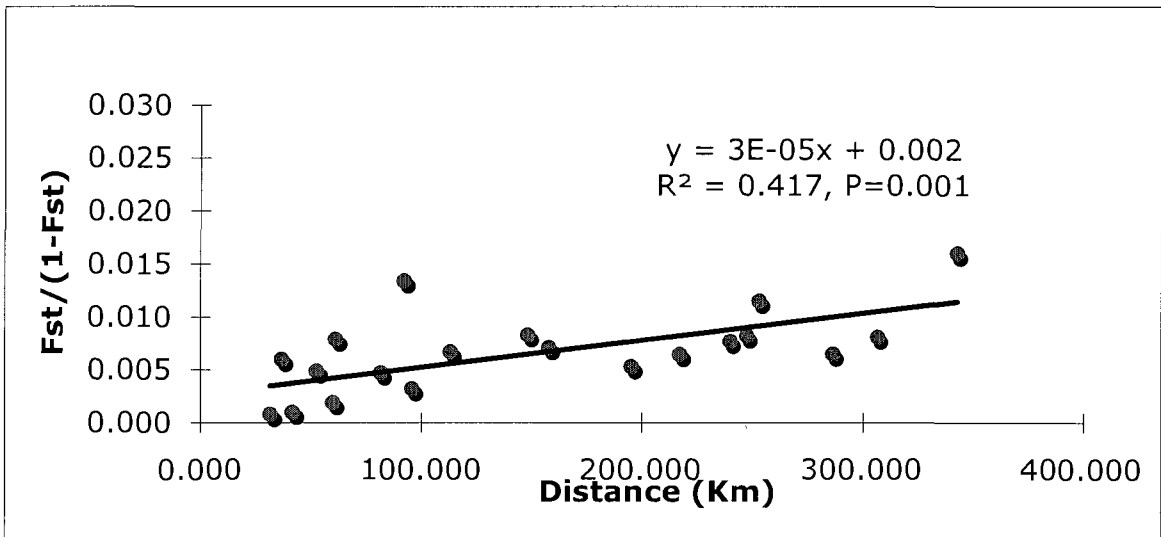


Figure 2: A Mantel test plotting Isolation by distance in Saltmarsh Sparrows: a. With all populations, the results are not significant. b. With Chapman's Landing removed.

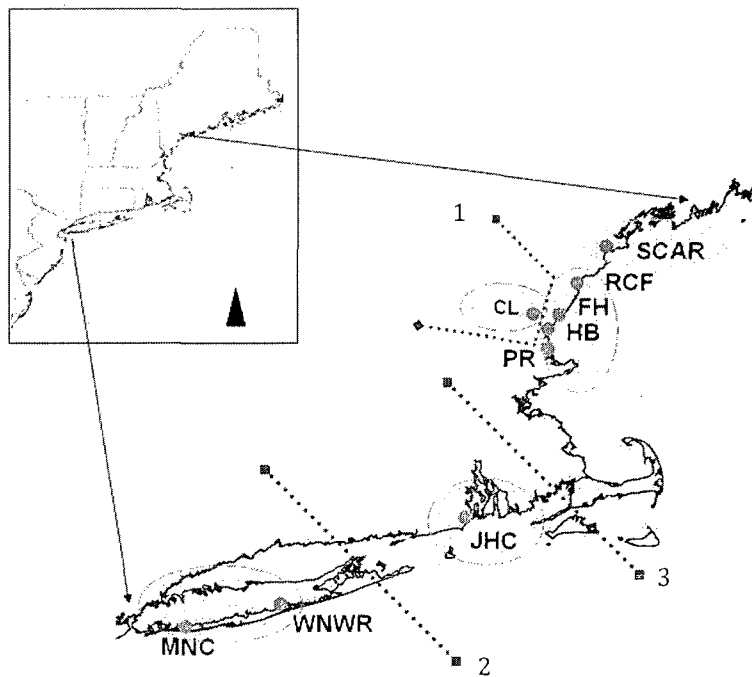


Figure 3: Genetically similar population groupings and dispersal barriers in 9 Saltmarsh Sparrow populations. Five clusters identified by SAMOVA are circled ( $F_{ct}=0.007$ ,  $P < 0.0001$ ) and the first, second and third order barriers identified by BARRIER are indicated by dotted lines.

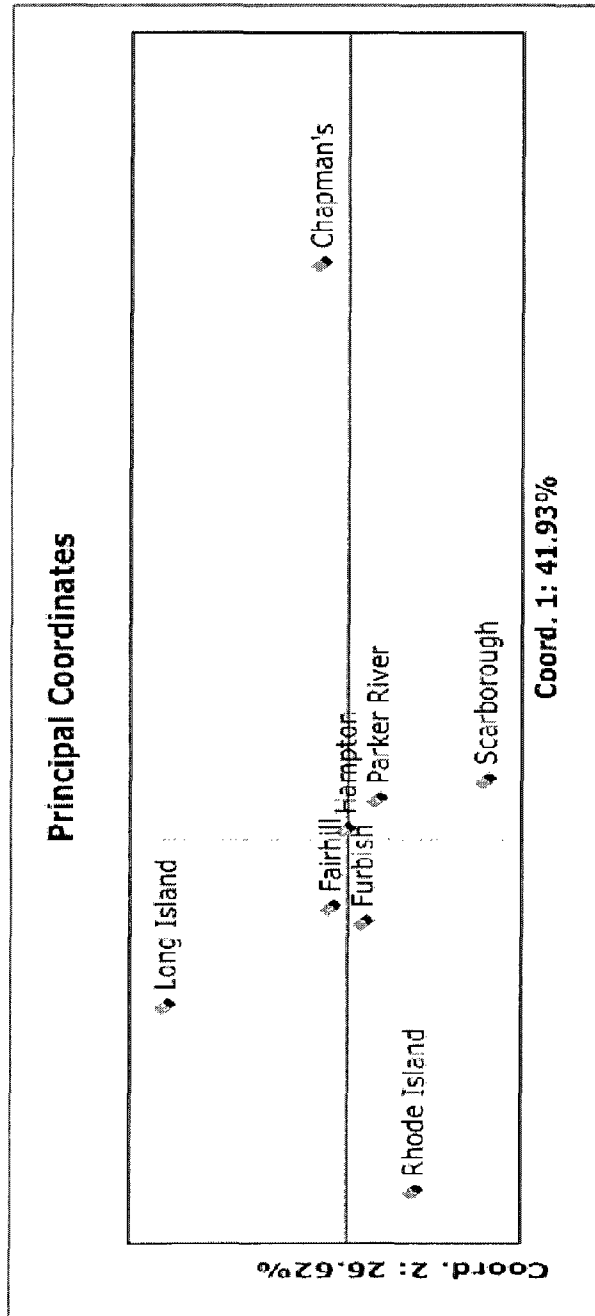


Figure 4: Principle component analysis of pairwise  $F_{ST}$  values for 8 populations of Saltmarsh Sparrows (2 Long Island sites combined). Cumulatively, axis one and two account for 68.5% of the variation detected in the data set.

## **Patterns of Dispersal and Source Sink Dynamics**

We ran assignment tests on the 5 and 6 population clusters identified by SAMOVA and BARRIER. The number of individuals correctly assigned ranged from 40.6-43.26% for 6 and 5 populations, respectively. Although the Fairhill/Furbish cluster was differentiated enough from the Hampton/Parker River cluster to detect migrant individuals, our assignment results indicated that the only other population of origin for Fairhill/Furbish individuals was the Hampton/Parker River cluster. Based on these results, we chose 5 clusters as the most appropriate grouping for the sampled populations. Using these 5 populations, 31% of the total individuals were assigned, with the number of individuals assigned back to the population from which they were sampled ranging from 4-28% and the number of individuals immigrating into a population ranging from 1.8-30% (Table 3). The PR/HB/RCF/FH cluster had the highest percentage of resident individuals and the lowest percent of immigrants in comparison to the other populations. The number of individuals dispersing from a given population ranged from 3-27, with the PR/HB/RC/FH cluster as the origin of the highest number of dispersers. The results from the 'detect migrants' function in GENECLASS were consistent with the results of the assignment tests 86 times out of 105 (82%) and detected 13 additional migrants.

We found no evidence of sex-biased dispersal, using the randomization test in FSTAT for comparisons of  $F_{ST}$  ( $P=0.1461$ ),  $F_{IS}$  ( $P=0.6015$ ) and relatedness ( $P=0.1801$ ). Similarly, the sex ratio of migrants detected by the assignment tests showed no clear patterns of a dispersal bias (Table 3). In CL and the PR/HB/FH/RCF cluster a higher percentage of females were identified as resident and a higher percentage of males as

migrants, however, assignment results from the remaining population clusters did not support this. Corrected assignment indices also did not show a consistent pattern, with *Aic* values negative for males in some populations and for females in others; overall no *Aic* values were strongly negative in any population, suggesting no sex-bias in dispersal. Relatedness values were consistently close to 0 (indicating unrelated) for all populations, averaging -0.02 for MM pairs, -0.017 for FF pairs, and -0.019 for MF pairs.

**Table 3: Dispersal patterns and ratio of female:male migrants in five Saltmarsh Sparrow population clusters.**

<b>Population Cluster</b>	<b>Ratio of migrants by population</b>		
	<b>% Resident</b>	<b>% Immigrants</b>	<b># of dispersers</b>
CL	10%	30%	5
PR/HB/RCF/FH	28%	1.8%	28
MNC/WNWR	4%	21.8%	2
JHC	7.5%	22.6%	3
SCAR	7.5%	22.5%	6

**Females:Males**  
 1:4  
 7:21  
 2:0  
 0:3  
 2:4

## Discussion

### Population Structure and Connectivity

We observed small yet significant differences (0.0001 to 0.0240 and an overall  $F_{ST}$  value of 0.008) in the genetic structuring of Saltmarsh Sparrow populations. Five clusters were identified based on groups of genetically similar populations. The five clusters we identified in BARRIER and SAMOVA were comprised of a Long Island complex (WNWR and MNC), Rhode Island, Chapman's Landing, Spurwink and Parker River/Hampton Beach/Fairhill/Furbish. Although the clusters we identified appear to follow an overall isolation by distance trend, where distance contributes to genetic differentiation, population outliers indicate that factors other than geography are impacting population structure. This raises the question of the role of geographic scale in forming management units, as our results indicate that location and the proximity of a marsh to other marshes is not necessarily indicative of high connectivity.

Chapman's Landing was the most genetically differentiated from all other sampled marshes. This degree of genetic differentiation observed in Chapman's Landing is unexpected, as it is located within 16.1 Km of Fairhill and Hampton and within 38.5 Km of Parker River and Furbish. One interesting feature of Chapman's Landing is that it is the most inland of any of our sampled marshes, located approximately 15 Km from the coast. Similarly to Chapman's Landing, Spurwink was significantly genetically distinct from all other sampled marshes, and in comparison is further inland (approximately 3.5 Km from the coast). The location of these sites (Figure 1) combined with their relatively small size may influence the ability of dispersing individuals to detect the habitat patch;

as the spatial scale of a patch and inter-patch distances effect the movement and the perceptual range of a species (Moilanen and Hanski, 2006). Because Saltmarsh Sparrows are restricted to coastal wetlands, the degree of fragmentation creates “islands” of suitable habitat patches. Thus, this is similar to the theory of island biogeography, in which smaller islands or patches support fewer individuals simply because they provide smaller “targets” for potential colonists (MacArthur and Wilson, 1963; Lomolino, 1990). Saltmarsh Sparrows are also likely following a typical coastal migration pattern characteristic of birds breeding in tidal marshes, and are thus less likely to detect the inland marshes. Furthermore, field observations and banding recapture data indicate that Saltmarsh Sparrows display some level of site fidelity (O. Lane, Biodiversity Research Institute, personal communication). Therefore, the small percentage of individuals that detect Chapman’s Landing and Spurwink as suitable breeding habitat may return for subsequent breeding seasons, contributing to the level of genetic differentiation observed in these populations.

Long Island and Rhode Island were also identified as genetically distinct by all of our methods. This is likely due to geographic isolation relative to the other sampled populations (Figure 1). The Long Island populations (WNWR and MNC) and the Rhode Island population (JHC) are relatively distant from both each other (approximately 140 Km) and from the northern Massachusetts/New Hampshire/Maine cluster (approximately 312 Km from WNWR to Hampton and 172 Km from Rhode Island to Hampton). This finding is consistent with the results from the Mantel test, which detected an increase in genetic differentiation in correlation with geographic distance.



The detection of genetic structure over the geographic scale in which we sampled is further consistent with findings from previous studies on population structure in saltmarsh breeding passerines. A study by Chan (2006) found detectable patterns of genetic structure in populations of seaside sparrows (*Ammodramus maritimus*) over geographic distances of 100 Km. The small pairwise  $F_{ST}$  values observed in Saltmarsh Sparrow populations fall within the range detected in previous studies on avian species in fragmented habitats (Barnett, 2008; Lehtonen, 2009), which suggests that disrupted or patchily distributed habitat may be less of a barrier to movement in vagile avian species. Despite this commonality of low  $F_{ST}$  with previous studies on avian populations, however, the above studies failed to detect genetic differentiation over distances of thousands of kilometers in some instances and found no trends of isolation by distance. This indicates that despite the migratory nature of Saltmarsh Sparrows, there are factors that are responsible for detectable patterns of genetic structuring.

### **Patterns of Dispersal and Source Sink Dynamics**

The assignment tests in GENECLASS suggest that the Parker River cluster is sending out the largest number of dispersers and is receiving the lowest percentage of immigrants, indicating that this cluster may be acting as source populations. Furthermore, we detected migrants from the Parker River cluster in all of the populations that we sampled, including populations separated by large geographic distances (Long Island and Rhode Island), which is inconsistent with our findings of isolation by distance. Although our analyses were conducted on the five population clusters, marshes from the Parker River/Hampton Beach/Fairhill/Furbish cluster were individually analyzed in separate

assignment tests to identify individual population effects. Our results indicated that when Parker River was removed from the cluster, the remaining marshes are comparable to that of the four other clusters, in that they display higher immigration rates and low numbers of dispersers. This indicates that Parker River is the true source population as it was representative of the only sampled population characterized by high numbers of dispersers.

Results from the assignment tests are consistent with pairwise  $F_{ST}$  values, as Parker River displayed the most genetic similarity in comparison to other populations. An important feature of Parker River is that it is the largest stretches of continuous marsh habitat in our study (approximately 9,000 hectares). The size of the marsh complexes may contribute to the level of connectivity observed. Previous research has found that the density of breeding Saltmarsh Sparrow adults is positively correlated to marsh size (Benoit and Askins, 2002; Shriver, 2004). Furthermore, field data from breeding bird surveys (see chapter three) indicate that Parker River supports the highest abundance of breeding adults in comparison to four of the other sampled marshes (Furbish, Chapman's Landing, Fairhill and Hampton). Thus, large quantity of continuous marshland habitat characteristic of Parker River may be responsible for the degree of connectivity we are observing. This would seem likely in terms of source-sink dynamics, as areas with better quality habitat support higher reproductive success in individuals, making these habitats sources due to their relatively high population productivity (Brown and Kodric-Brown, 1977). This is often seen in cases of patchy populations, where high dispersal rates between sites that are spatially separated create homogenous populations (Scheiman et al., 2007).

We found no evidence of sex-biased dispersal in Saltmarsh Sparrows. Sex-biased dispersal is a common occurrence in higher vertebrates, with one sex dispersing and one sex remaining in their natal territory for subsequent breeding seasons (Möller, 2004). Patterns of sex-biased dispersal are often shaped by mating behavior, seasonal cues, and resource availability (Mossman and Waser, 1999). In cases where the female is responsible for rearing young and defending territories, there is a stronger selective pressure for philopatry in females than in males (Favre, 1997). The breeding behavior of Saltmarsh Sparrows is such that females provide all of the parental care (Greenlaw and Rising, 1994), and thus sex-biased dispersal may be expected, as it would be beneficial for the females to return to an area where they were successful in previous years. We did not find distinct evidence of sex-biased dispersal, although this may be due to a limitation of our data set, as the ratio of males to females sampled was almost 2:1, which, along with uneven sample sizes, may bias our estimates. Furthermore, Saltmarsh Sparrows are highly promiscuous and do not defend territories. High rates of infidelity can complicate the interpretation of biased dispersal, as promiscuity can reduce patterns of genetic structure by mediating gene movement (Double et al., 2005).

### **Conservation Implications**

Our study contributes to the larger understanding of population structure and dispersal patterns among populations of Saltmarsh Sparrows. We identified five population clusters based on genetic similarity and connectivity between breeding populations. Our results indicate that some degree of substructuring is occurring despite the vagility of this species. Overall, populations follow an isolation by distance model but the smaller more inland marshes appear to be less connected, indicating that geographic

distance is not the only factor contributing to genetic structure. Our results provide new insight for future management initiatives. Further thought may be required to assess whether Saltmarsh Sparrow populations should be managed as one contiguous population, or if smaller management units are more appropriate. It would be beneficial to fill in geographic gaps in the sampling locations (Connecticut and extend data collection to the most southern point of the species' range) to further understand the scale of genetic structuring in Saltmarsh Sparrow populations. Our results show that creating management units based on a pre-chosen geographic distance is not appropriate for maintaining diversity, as some marshes display comparatively high levels of genetic differentiation despite close geographic proximity to other wetlands.

Our findings also lend support to the idea that larger stretches of continuous marshes are important. Our largest and most continuous stretch of wetland habitat displayed the highest amount of connectivity and was identified as a source of dispersers to populations as far as 300 Km away. Although smaller marshes, such as Chapman's Landing, appear to sustain healthy, viable populations (see chapter three), these populations are characterized by low numbers of dispersers. Such considerations should be taken into account when implementing management systems. Large marshes may not be essential for healthy populations but may be important in that they are large enough to sustain smaller, less suitable patches of habitat. We highlight the need for further research concerning the scale at which Saltmarsh Sparrows should be managed. Large populations such as Parker River are a conservation priority as they are sending out large numbers of dispersers to surrounding marshes. Efforts should be made to determine whether similarly large stretches of continuous wetland habitat in other portions of the species' range

display comparable levels of genetic connectivity. However, we also show that smaller, inland populations are the most genetically distinct. Thus, in terms of preserving genetic diversity, considerations should also be made for these divergent populations in terms of their adaptive potential.

## CHAPTER 3

### NESTING SUCCESS AND DENSITY OF BREEDING SALTMARSH SPARROWS (*AMMODRAMUS CAUDACUTUS*) IN FIVE NEW ENGLAND SALT MARSHES

#### Abstract

To assess reproductive success and overall density of breeding adult Saltmarsh Sparrows (*Ammodramus caudacutus*), we monitored nests and conducted point count surveys in five salt marshes in Maine, New Hampshire, and Massachusetts. Breeding bird surveys were used to obtain a relative index of adult Saltmarsh Sparrows that ranged from 3 to 27 individuals. Furthermore, we found a positive correlation between Saltmarsh Sparrow density and size of continuous marsh ( $R^2=0.8954$ ,  $P= 0.0148$ ). Models of daily survival were used to estimate the probability of a Saltmarsh Sparrow chick surviving a 26-day nesting cycle. Nesting success varied from 0.50-0.05. In three of the five marshes, the primary cause of nest failure was flooding, while in two of the marshes predation was the primary cause.

#### Introduction

The Saltmarsh Sparrow (*Ammodramus caudacutus*) breeds in coastal wetlands ranging from Maine to Virginia, USA (Greenlaw and Rising, 1994) and is one of only two passerines exclusively restricted to salt marshes (Greenberg, 2006). Due to their limited range and specialized habitat requirements, the Saltmarsh Sparrow is classified as

a species of conservation priority (U.S. Fish and Wildlife Service, 2002) and is considered globally vulnerable to extinction (Birdlife International, 2004). The conservation status of the Saltmarsh Sparrow coupled with increased impacts of anthropogenic stressors on nesting habitat (habitat loss and the spread of invasive vegetation; Greenberg, 2006) raise the need for baseline information on nesting success and overall population viability.

As ground nesting birds, Saltmarsh Sparrows have adapted to nest in a habitat characterized by daily tidal inundation. The females choose areas where the vegetation is taller and denser for nest construction (Gjerdrum, 2005). Saltmarsh Sparrows may rely on vegetation cues to indicate both substrate elevation and tidal flow when selecting nesting sites (DiQuinzio, 2002). Furthermore, female Saltmarsh Sparrows display synchrony with tidal cycles, as they, on average, build nests within three days after a tidal flood (Shriver, 2007). The initiation of nest construction immediately after a high tide increases the probability that nesting will be completed before the next flood (Shriver, 2007). The period between egg laying and fledging is about 26 days long (Greenlaw and Rising, 1994), with spring flood tides occurring every 28 days. The incubation period averages 11 days; and although Saltmarsh Sparrows are altricial upon hatching, chicks are ready to fledge 8-11 days after hatching (Greenlaw and Rising, 1994).

Flooding is generally documented as the leading cause of nest failure in Saltmarsh Sparrow nests (Greenlaw and Rising, 1994; Shriver, 2007), and has been documented to cause up to 60% of nest failures in past studies (Greenlaw and Rising, 1994). Additional causes of nest failure include predation and failure of the eggs to hatch due to either embryo mortality or infertility (Greenlaw and Rising, 1994). Although nesting success is

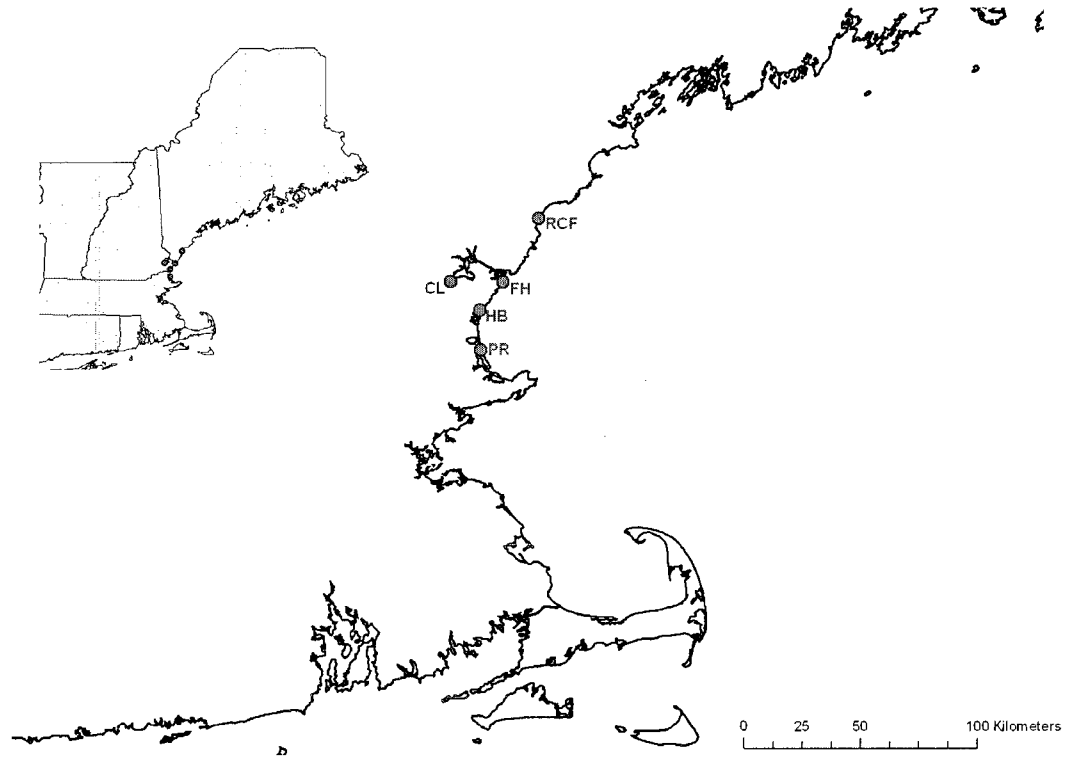
highly variable among sites and years (Post and Greenlaw, 1982; DiQuinzio et al., 2002), previous studies focused in Rhode Island and New York have documented nesting success averaging 52% (Greenlaw and Rising, 1994). To identify causes of nesting mortality, we monitored nesting success in Saltmarsh Sparrows within five marshes located in Maine, New Hampshire and Massachusetts and discuss possible causes for variability among sites. We also conducted breeding bird surveys at our five sites to assess the density of breeding adults. Our objectives were to evaluate the relative productivity of these sites based on the reproductive success and abundance of Saltmarsh Sparrows and to lend support to the identification of source/sink populations. We predicted: i) flooding would be the leading cause of nest failure in the five sites; ii) larger marshes would support a larger density of breeding adults.

## **Methods**

### **Study System**

Surveys were conducted during the 2008 summer breeding season at multiple subsites within five marshes along the New England coast: Wells, ME (Rachel Carson NWR), Hampton, NH, Rye, NH, Stratham, NH and Parker River, MA (Plum Island NWR; Figure 5). We defined the area of the salt marsh as any wetland habitat connected by tidal flow and where patches were separated by less than 500 m of open water or 50 m of upland habitat (Benoit and Askins, 2002). When possible, the entire marsh was surveyed for the collection of both point count and nest data. Some marshes were only partially sampled due to large size or lack of accessibility; the size of the marshes and the number of points varied broadly between the five sample sites (Table 4).





**Figure 5: Location of Saltmarsh Sparrow point count surveys and nest monitoring efforts. Marsh location code names: Furbish marsh (RCF), Fairhill marsh (FH), Chapman's Landing (CL), Hampton marsh (HB), and Parker River (PR).**

## **Adult Abundance and Point Counts**

To estimate population abundance of Saltmarsh Sparrows, we conducted bird surveys during the months of June through August of 2008. Population density was estimated using the point count method (counting the number of birds seen or heard within a specified range over ten minutes). The number of points we surveyed was dependent on the size of the marsh. Once chosen, the points were flagged and their locations recorded with a GPS unit (Conway, 2006). To avoid counting the same individuals twice, all survey points were a minimum of 250 meters apart. To ensure that the individuals surveyed were within the marsh being sampled, all points were located a minimum of 75 meters from upland habitats (Benoit and Askins, 2002). At each point, we surveyed birds for 10 minutes by passive observation and recorded all individuals seen or heard within 100 meters of the point (Shriver et al. 2004). We conducted point counts between dawn and 11:00 am. Surveys were not conducted in the rain or when wind speed exceeded 20km/hr, as this would have affected the probability of detection (Conway, 2006). We conducted three replicate surveys, increasing the probability of conducting a survey during the period of peak seasonal response for individuals within the focal marsh (Conway, 2006). Points were re-surveyed in random order, with a minimum of two weeks separating surveys at the same site (Benoit and Askins, 1999).

All five of the marshes surveyed were located in a region where Saltmarsh Sparrows occur sympatrically with a congener, the Nelson's Sparrow (*Ammodramus nelsoni*). Although visual and auditory cues can be used to differentiate between Nelson's and Saltmarsh Sparrows in the field, accurate visual and auditory species identification decreases with distance. Therefore, for adult abundance estimates, point count data from

Nelson's Sparrows and Saltmarsh Sparrows were combined to include data on individuals that could not be visually identified as one species or the other. An index of abundance for each marsh was calculated as the maximum number of individuals counted at a single point (Hodgman et al., 2002). Relative abundance of sparrows was quantified as the mean number of individuals surveyed across all points at a given marsh. We used linear regression to assess the relationship between marsh area and the relative abundance of Sharp-tailed species.

### **Nest Monitoring and Survival**

To determine nesting success of Saltmarsh Sparrows, we monitored nests over three nesting cycles (June, July and August). Nests were located through extensive searches of the salt marsh sites. Once a nest was found, we marked the area 5 meters to the North with a flag and checked the nest every 3-5 days. If at least one chick fledged from the nest, we considered it a success (Mayfield, 1975). Nests were defined as failed based on one of the following categories: flooding, defined by a minimum of one egg found outside of the nest cup and with the female no longer attending the nest, and depredation, when nests showed signs of predation (torn nests, broken egg shells, or chicks too young to fledge disappearing from nests; Gjerdrum, 2005; DiQuinzio, 2002). We classified the cause of nest failure as unknown if neither of the above scenarios could be used to explain nest failure

We modeled daily survival rates (DSR) using the software program MARK (White and Burnham, 1999). Data were grouped by sample site; we additionally included an individual covariate for distance of nest to edge to model edge effect on nest survival. The simplest model (constant) used a single constant parameter to estimate daily survival

and is comparable to the approach implemented by the Mayfield estimator (Mayfield, 1975). We also modeled linear and quadratic time trends to account for temporal variation in nest survival (T and TT). To obtain an average survival rate per site, we combined nest success data from the first and second nesting cycle. Due to small sample sizes (n= 11), data from the third nesting cycle were not included in the analysis.

## **Results**

### **Adult Abundance and Point Counts**

The maximum number of Saltmarsh and Nelson's Sparrows surveyed at a single point ranged from 0-27, with the average number of sparrows at a site ranging from 1.7-10 (Table 4). We observed birds at 88%-100% of all of the points we surveyed. We found a positive correlation between marsh size (ha) and the relative abundance of breeding Saltmarsh Sparrows ( $R^2=0.8954$ ,  $P= 0.0148$ ; Figure 6).

### **Nest Monitoring and Survival**

We located and monitored a total of 112 nests over two cycles in our five sample sites (Table 4). The percentage of failed nests ranged from 33-74%. Flooding was the leading cause of failure in Chapman's Landing, Fairhill, and Hampton. Predation was the leading cause of nest failure in Furbish and Parker River, with 85% and 91% of the nest failures caused by predation, respectively. The best model for nest survival (Table 5) included site and a linear time trend (site\*T). Overall nesting success for Saltmarsh Sparrows varied from 0.5 to 0.05 (Table 6). Survival rate estimates decreased daily over the duration of the study (Figure 7).

**Table 4: Maximum numbers of Nelson's and Saltmarsh Sparrows observed at a single point. Columns include number of points at each site surveyed, the maximum number of Saltmarsh and Nelson's Sparrows observed, the number of individuals that could not be identified as Nelson's or Saltmarsh sparrows (Sharp-tailed species), and the range, median, and mean number of individuals (Nelson's or Saltmarsh) identified at a single point.**

Site	# of Points	Marsh Size (ha)	Maximum # of birds observed at a point					All points combined by site			% of points with either species present
			Saltmarsh	Nelson's	Sharp-tailed species	Range	Median	Mean			
Furbish- Wells, ME	4	100	4	5	4	0-4	1	1.7	100%		
Chapman's Landing- Stratham, NH	5	19.47	5	2	9	0-13	3	3.9	100%		
Fairhill- Rye, NH	9	87.38	3	1	8	0-8	1	1.6	88%		
Hampton Beach- Hampton, NH	11	1,700	8	0	15	0-18	5	5.1	90%		
Parker River- Newburyport, MA	5	9,000	27	2	0	2-27	8	10	100%		

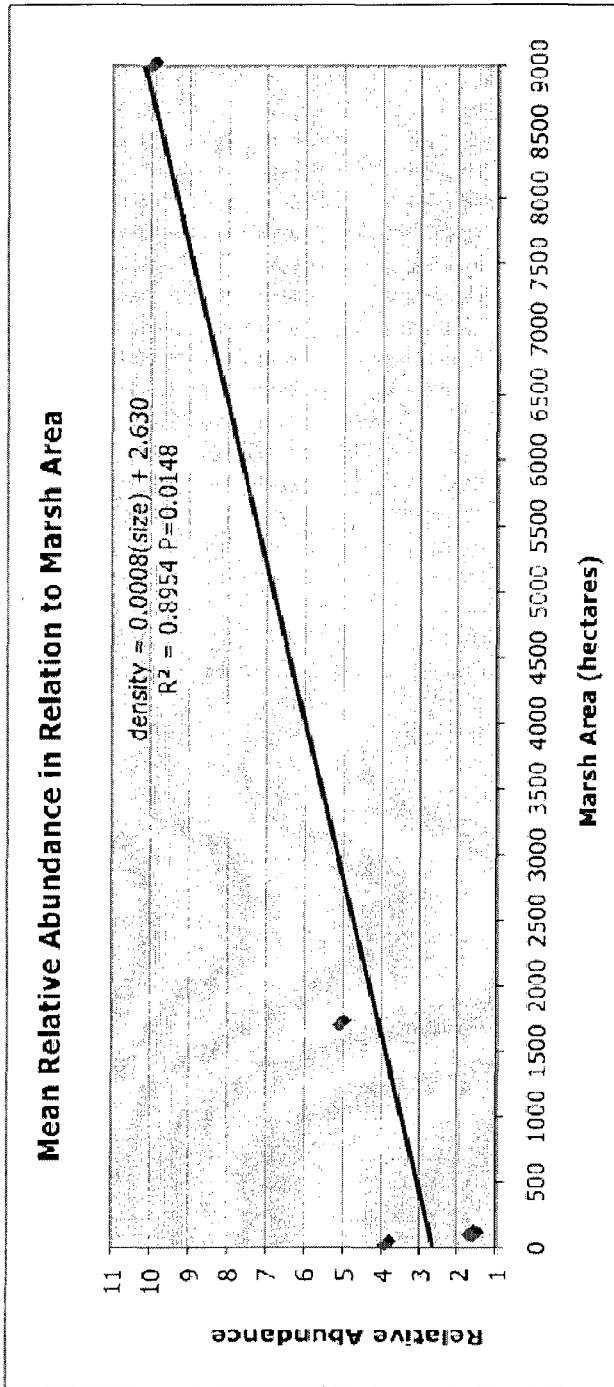


Figure 6: Linear regression shows a significant correlation between continuous marsh area and the average density of birds in a marsh.

**Table 5: Number of Saltmarsh Sparrow nests monitored, % failed, and outcome (predated or flooded) on five marshes.**

Site	Total nests	Failed	Predated	Flooded	Unknown
Chapman's Landing	12	33%	0	75%	25%
Fairhill	7	71%	40%	60%	0
Hampton	27	63%	12%	82%	6%
Parker river	39	61%	91%	9%	0
Furbish	27	74%	85%	15%	0
<b>Total</b>	<b>112</b>				

Table 6: Nest success models for Saltmarsh Sparrows in five marshes in New England.

Model	AICc	Delta AICc	AICc Weight	Model Likelihood	# of parameters	Deviance
Site*T	354.348	0.00	0.3214	1.000	6	342.262
Site*TT	354.348	0.00	0.3214	1.000	6	342.262
Site*TT*distance	354.856	0.51	0.24925	0.775	7	340.742
Constant	357.043	2.69	0.08354	0.259	1	355.038
Site	359.503	5.16	0.02441	0.076	5	349.442



**Table 7: Probability of Saltmarsh Sparrow chicks surviving the duration of a 26-day nesting cycle. Survival estimates were calculated in MARK using the Site\*T model.**

<b>Study Site</b>	<b>Survival</b>	<b>Standard Error</b>	<b>Lower CI</b>	<b>Upper CI</b>
Chapman's Landing	0.508111	0.19914	0.17811	0.83120
Fairhill	0.142154	0.13572	0.01836	0.59480
Hampton	0.193783	0.06807	0.09283	0.36085
Parker River	0.120608	0.04630	0.05508	0.24396
Furbish	0.058071	0.03448	0.01761	0.17497

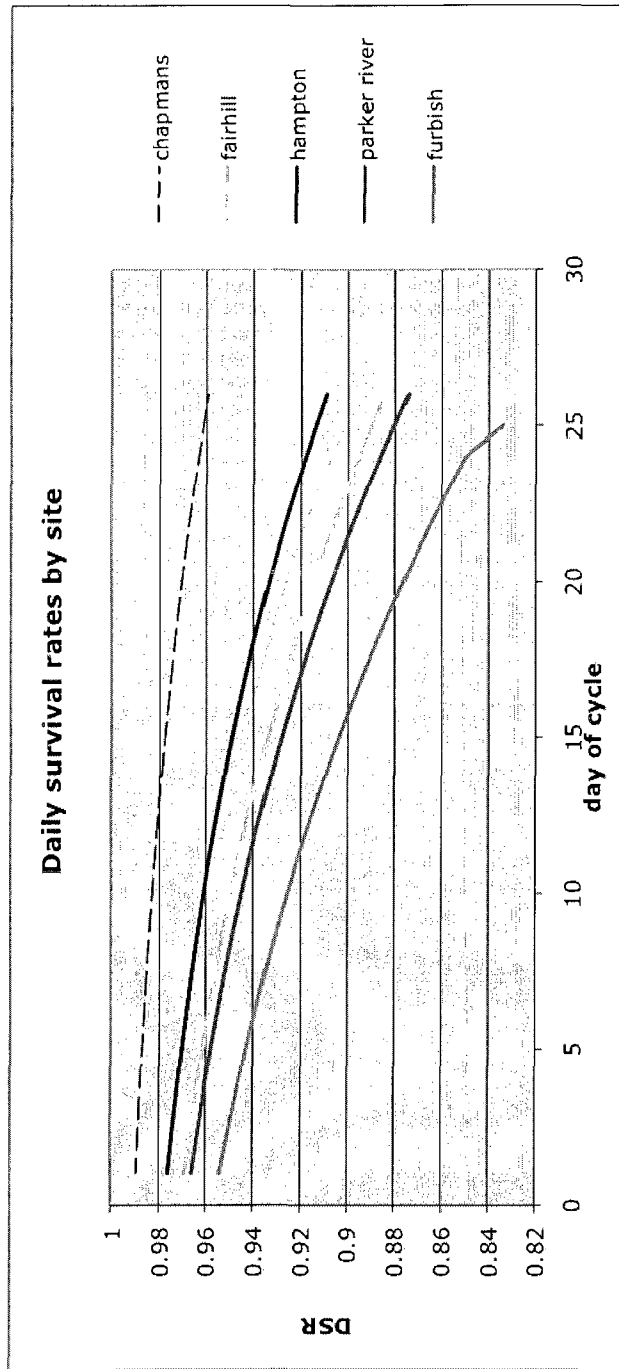


Figure 7: Daily survival rates for Saltmarsh Sparrow chicks plotted over a 26-day nesting cycle.

## Discussion

### Adult Abundance and Point Counts

Point count surveys provided an estimate of the density of breeding adults within our five sample sites. The highest number of Saltmarsh Sparrows surveyed at a single point was documented at Parker River and Hampton Beach, which were the two largest marshes surveyed. These results are consistent with genetic findings (see chapter two), which indicate that Parker River is highly connected to surrounding marshes through the large number of dispersers originating from the Parker River marsh complex. We additionally found a significant, positive correlation between the density of breeding adult sparrows and the size of continuous marsh habitat ( $P=0.0148$ ). Although our study only included five sampling locations, our results are consistent with previous research on sparrow abundance and marsh size (Benoit and Askins, 2002; Shriver, 2004). To further test for a correlation between marsh size and sparrow density, it would be beneficial to sample marshes that range from 2,000-8,000 hectares to obtain more data on the middle range of marsh area not covered in this study.

The percentage of points where sparrows were observed ranged from 88%-100% indicating that the areas we surveyed were representative of appropriate breeding habitat for Saltmarsh and Nelson's Sparrows. For Parker River, Furbish, and Hampton Beach, we were unable to survey the entire marsh due to the large size and overall lack of accessibility. However, the percentage of points with individuals again indicates that the areas of marsh we chose to survey were appropriate and are representative of the overall abundance within the marsh, specifically in areas that are composed of similar habitat

features to the areas in which surveys were conducted. For Fairhill and Chapman's Landing, the size of the marsh was small enough that we could survey a high proportion of the area, and thus are confident that the results from the point count surveys are representative of the relative abundance of individuals inhabiting those sites.

The point count surveys provide a baseline index of the number of adults occupying the marshes surveyed. This may be particularly important for some of the sites in New Hampshire, which are not managed by U.S Fish and Wildlife Services. Thus, information on the relative abundance of breeding adults in these areas may be useful to local management agencies. A benefit of the radial point count method implemented in this study is that it is standard protocol that is also used in many of the National Wildlife Refuges (Conway, 2006), thus this format of data collection provides flexibility in that data is easily transferable to that of similar research efforts, and as a result data from multiple marsh monitoring projects can be pooled together to gain insight on local and regional trends (Conway, 2006). Because we chose this standardized survey method, our results can be easily translated to fit the formats of local and regional management agencies, which may prove to be beneficial for future endeavors to monitor local and regional population trends of Saltmarsh Sparrows in the area.

### **Nesting Success**

We found nesting success to be highly variable among marshes, with the percentage of failed nests ranging from 33% to 74%. Previous research has documented nesting success in Saltmarsh Sparrows as 58.7% (Rhode Island), 46.9% (New York), and 41% (Connecticut), with flooding generally identified as the leading cause of failure

(Greenlaw and Rising, 1994; Gjerdrum et al., 2005). With the exception of Chapman's Landing, nesting success within our sampled marshes was slightly below the average (26-39%) in comparison to previous nesting studies. It should be noted that in some cases, such as Fairhill, low nesting success might be a product of small sample size (n=7), and thus may not accurately reflect nest success.

Although previous studies have documented flooding as the leading cause of failure for Saltmarsh Sparrow nests, this was only observed in our three New Hampshire sites. In Parker River and Furbish, predation was the leading cause of failure, accounting for 91% and 85% of the total nests failed, respectively. High predation rates in Furbish and Parker River may be attributed to a number of factors. Furbish is surrounded by roads and neighborhoods, which may provide easy corridors and access points for predators. In Parker River, the surrounding woodland habitat is additionally under the protection of U.S Fish and Wildlife Services and thus it is possible that the number of predators in these areas is higher. There was a higher occurrence of research activity surrounding nests in Furbish and Parker River, which may also have contributed to the predation rates observed. In some instances, visiting a subject can temporarily decrease its chance of survival, particularly in bird studies as researchers may lead predators to the nest (Bart and Robson, 1982). A temporal factor may also be contributing to the low survival estimates, as previous research has documented extreme annual fluctuations in Saltmarsh Sparrow productivity (Post and Greenlaw, 1982; DiQuinzio et al., 2002). Despite the high predation rates observed in Parker River and Furbish, however, it should be noted that the overall nesting success of Saltmarsh Sparrows was comparable to that of Fairhill and Hampton Beach. So although predation was higher in these sites in comparison to

flooding, the resulting survival rates from these sites are similar. Survival in Chapman's Landing was comparably high in relation to the other marshes sampled, with low flooding and predation rates. The relatively inland location of Chapman's Landing may contribute to the low flooding rates observed, although future research may be warranted to determine if tidal patterns vary enough to have less of an impact on Saltmarsh Sparrow nesting success.

### **Conservation Implications**

Our results indicate higher than expected predation rates in two of our study sites (Furbish and Parker River), with only a 5% and 12% probability of chicks surviving the duration of the study, respectively. However, the percentage of nests that failed in Parker River and Furbish is comparable to that observed in Fairhill and Hampton Beach (Table 5), indicating that the higher observation of predation rates in these areas may not have a significant impact on the overall productivity of these marshes. If high predation rates can be contributed to an edge effect or a larger number of predators in these managed areas it may be difficult to control these factors. However, if predation rates are indicative of an observer effect, it may be worth considering the impacts of nesting studies on Saltmarsh Sparrows when planning future field experiments, as this is something that wildlife managers can control. We recommend designing a field study that can control for observer effects to assess whether frequent nest visits or increased activity surrounding nesting sparrows increases predation.

Nest studies and breeding bird surveys can provide valuable insight on the reproductive success and abundance of a species. Our study resulted in general trends of

survival and estimates of relative abundance that can act as a basis for future research initiatives. It is recommended that data from multiple years be collected to provide future insight on long-term population trends. Our results provide baseline data for future studies on nesting success and point count surveys, information from which will be an important contribution to future management initiatives for a species of conservation priority.

## CHAPTER 4

### CONCLUSION

The objectives of this study were to collect data on dispersal rates and population connectivity in Saltmarsh Sparrow populations and to correlate findings with field data on relative abundance and nesting success to provide insight on source-sink dynamics. Results from the genetic analyses suggest that there are significant levels of genetic differentiation observed in the sampled populations. Overall, populations follow an isolation by distance trend ( $R^2 = 0.41$ ,  $P = 0.001$ ), but this is not applicable to all of the populations we sampled, indicating that other factors in addition to geography are shaping patterns of genetic substructuring in Saltmarsh Sparrows. Furthermore, results from assignment tests highlight Parker River as a possible source population, with the highest numbers of dispersers originating from this population. We found no evidence of sex-biased dispersal in the populations we sampled, however it is possible that the male bias in our sampled individuals is responsible, and thus closer inspection is warranted.

We were additionally able to define five population clusters based on genetic similarity among sampled marshes. The five clusters we defined consist of a Long Island complex (WNWR and MNC), Rhode Island, Spurwink, Chapman's Landing, and Parker River/Hampton Beach/Fairhill/Furbish. Although  $F_{ST}$  values were small, genetic structuring was detected in the sampled populations providing insight on the formation of appropriate management units for this species. Results from the genetic analyses also



provide insight into the movement and connectivity between populations of a migratory avian species. Although genetic structuring is unexpected in organisms characterized by high vagility, we have shown that there are mechanisms, whether behavioral or related to habitat features, which are resulting in patterns of substructuring in a mobile species.

Results from the field component of our study further are consistent with the genetics findings that Parker River is a source population. A linear regression of continuous marsh size and the density of breeding adults resulted in a significant, positive relationship ( $P=0.0148$ ), with the highest observations of breeding adults occurring in Parker River, representing approximately 9,000 hectares of continuous marshland. Although our nesting data suggest that Parker River displayed the highest predation rates in 2007, the overall nest failures observed during the season were not lower than that seen in marshes with low predation rates (Hampton Beach and Fairhill). This suggests that although predation rates were high, the number of chicks surviving to fledge in this site is as expected based on data from the other sites monitored during this year. Thus, results from our nesting study further support the finding that Parker River is a source population. The average nesting success in Parker River combined with its large size supports our findings of high connectivity and large numbers of dispersing individuals.

Our study provides valuable insight on the population trends, dispersal patterns and genetic connectivity among Saltmarsh Sparrow populations. Future research focused on filling in the geographic gaps between Massachusetts and Rhode Island and in marshes further south of Long Island, would be beneficial in determining the scale of management required to preserve the genetic diversity of these populations. Identification and preservation of source populations, including Parker River, should also be an

important consideration for future management initiatives. To assess whether predation rates observed in Parker River and Furbish are a product of annual fluctuations or of increased research activity within the marsh, we also suggest conducting a study on observer effects and modeling the survival data in MARK. Data from both the field and genetics component of this study can be used in conjunction with past and current research efforts as a source of baseline information on determining the most appropriate strategy for effective management plans for a species that is globally vulnerable to extinction.

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**Appendix A.** Implementing a DNA-barcoding approach to identify Saltmarsh Sparrow (*Ammodramus caudacutus*) and Nelson's Sparrow (*Ammodramus nelsoni*) hybrids.

***ABSTRACT***

We developed a DNA bar-coding approach to discriminate between Nelson's Sparrows (*Ammodramus nelsoni*) and Saltmarsh Sparrows (*Ammodramus caudacutus*) and applied it to 441 putative Saltmarsh Sparrows sampled from Maine to Long Island. Although all individuals were identified in the field as Saltmarsh Sparrows based on plumage characteristics, 34 (7.7%) were found to have Nelson's specific mitochondrial DNA, indicating that they were of hybrid origin. This discrepancy in morphological and genetic data highlights the difficulties associated with accurate field identification and may hinder conservation efforts by confounding attempts to identify and monitor "pure" populations. We found evidence of an expansion of the hybrid zone, with Nelson's mitochondrial DNA prevalent in the most southern point of the previously documented overlap zone and as far south as Rhode Island. Our findings raise additional questions concerning the fitness of hybrids and the extent of introgression into both the nuclear and mitochondrial genomes of each species and highlight the need for further investigation into the consequences of hybridization on Saltmarsh Sparrows. The latter is especially important in light of other stressors potentially affecting the persistence of this threatened species.



## INTRODUCTION

The distribution and taxonomic classification of the Nelson's Sparrow (*Ammodramus nelsoni*) and Saltmarsh Sparrow (*Ammodramus caudacutus*) has been a topic of ornithological debate for over a century (Rising and Avise 1993, Greenlaw 1993, Shriver et al. 2005). Both species breed in coastal marshes, with a subspecies of Nelson's Sparrow (*A. n. subvirgatus*) breeding in marshes from coastal Québec to northeastern Massachusetts and the two subspecies of Saltmarsh Sparrow (*A. c. caudacutus* and *A. c. diversus*) breeding from Maine to New Jersey and New Jersey to Virginia, respectively (Greenlaw and Woolfenden 2007). Both Species are a high conservation priority in the northeastern United States (U.S Fish and Wildlife Service 2008) because of the high proportion of the global populations breeding in these areas (Hodgman et al. 2002). Hybridization between Nelson's and Saltmarsh Sparrows has been previously documented, as the two species are sympatric within an overlap zone spanning from central Maine to the northeast shore of Massachusetts (Hodgman et al 2002, Shriver et al. 2005).

In light of the potential negative consequences of hybridization in wildlife populations (Rhymer and Simberloff 1996), it is important to monitor and consider the impacts of hybridization on these threatened species. The possible expansion of the overlap zone and the potential for increased hybridization may present another threat to the long-term persistence of the Saltmarsh Sparrow, which as a salt marsh obligate, is limited to a heavily fragmented range of coastal habitat. Thus the identification and monitoring of genetically "pure" populations of this species may warrant consideration as a conservation priority.

In this paper, we present the results of a DNA-barcoding approach to identify hybrids in a large sample of Saltmarsh Sparrow individuals studied in multiple salt marshes across the northeastern U.S. We report evidence that hybridization between Nelson's and Saltmarsh Sparrows is occurring at a greater rate than previously thought and that the hybrid zone may be expanding.

## **METHODS**

### **STUDY AREA AND SAMPLE COLLECTION**

Genetic samples used in this study were collected from Nelson's and Saltmarsh Sparrows during ongoing toxicological (Lane and Evers 2007; Lane et al. 2008) and population genetics (Walsh et al. in prep; see chapter 2) research. To capture adult sparrows, we deployed two to six 12-m mist nets with size 36 mm mesh. Blood samples (30-50  $\mu$ l) were drawn from the cutaneous ulnar vein using a non-heparinized capillary tube. In a few cases, one or two tail feathers were obtained instead of blood. Individuals were identified in the field as either Nelson's or Saltmarsh Sparrows by plumage characteristics; measurements of bill size (culmen, depth and width), wing chord and weight were also recorded. All birds were released within 10-20 minutes of capture. Blood samples were stored at room temperature on Whatman filter cards for later genetic analysis.

To develop a genetic assay for species identification, blood or feather samples were obtained from known individuals of each species outside of the overlap zone ( $n= 4$  Nelson's Sparrows from Penobscot River, ME and  $n= 10$  Saltmarsh Sparrows from Shirley and Oceanside, NY). Individuals were also sampled within the overlap zone, for which species identification was based on morphological features and differences in

nesting behavior and spatial segregation (O. Lane, personal observation;  $n= 4$  Saltmarsh Sparrows and  $n= 2$  Nelson's Sparrows from Wells and Scarborough, ME). For morphological comparisons, we also collected field measurements of culmen length, bill width, bill depth and weight for 34 Nelson's Sparrows sampled along the Penobscot River and 29 Saltmarsh Sparrows sampled from Shirley, NY.

We applied our genetic test (see below) to 441 samples collected from putative Saltmarsh Sparrows (based on morphology) during 2006-2008 on nine marshes along the northeastern coast. Study marshes were located in Wells, ME (Rachel Carson National Wildlife Refuge; NWR), Scarborough, ME (Rachel Carson NWR), Hampton, NH, Rye, NH, Stratham, NH, Newburyport, MA (Parker River NWR), Narragansett, RI (John H. Chaffee NWR), Shirley (Wertheim NWR), NY and Oceanside, NY.

## GENETIC IDENTIFICATION

To develop our assay for species identification, we employed a DNA bar-coding approach (Hebert et al. 2003). DNA was extracted from the known Nelson's and Saltmarsh Sparrow samples using a DNeasy Blood Kit (Qiagen, Valencia, CA). Universal avian primers (BirdF1 and BirdR2) were used to amplify a 648 base pair region of the cytochrome *c* oxidase I (COI) gene in a 12.5 $\mu$ l polymerase chain reaction following the conditions described by Hebert et al. (2004). Samples were sequenced by Geneway Research, LLC (Hayward, CA) or by the Hubbard Center for Genome Studies at the University of New Hampshire.

To identify variation within and between species, Nelson's and Saltmarsh Sparrow sequences were edited to 600 base pairs and aligned in Geneious Pro 4.7.6 (Biomatters Ltd, Auckland, NZ). In addition, we included three Nelson's sequences from GenBank

(accession numbers DQ433298, DQ432709 and DQ432708) in our alignment; these specimens originated from the Midwest (Minnesota and Illinois) and were highly consistent with the sequences from our Nelson's reference individuals despite being representative of a different subspecies (*A. n. nelsoni*). We consistently found the same species-specific nucleotide variations at seven sites (1.2% interspecific variation) when comparing Nelson's and Saltmarsh Sparrow sequences. With the exception of one Saltmarsh individual at one site, there was no intra-specific variation within these seven sites when comparing our Nelson's samples with those on GenBank, nor when comparing our species-specific samples collected within and outside of the overlap zone; the latter confirms that our reference individuals for each species were correctly identified in the field. Outside of the seven sites, we observed 0.2% and 0.4% intraspecific variation in Saltmarsh and Nelson's Sparrow sequences, respectively. The higher intraspecific variation observed in the Nelson's Sparrow sequences might be reflective of the large geographic distance between sampling locations and genetic variation between two subspecies.

We identified a nucleotide difference in one of the seven sites that was located in a *Hinf*I specific restriction site, and used this as the basis for the development of a species-specific diagnostic assay. Amplified products were digested in a 10.5 $\mu$ l reaction (9 $\mu$ l template DNA, 0.5 $\mu$ l enzyme *Hinf*I and 1.0 $\mu$ l of buffer 2) and incubated overnight at 37°C. When resolved on a 3% agarose gel, the test yielded 2 fragments (approximately 100 and 550 in size) in Saltmarsh Sparrows, and 3 fragments (approximately 100, 150 and 400 in size) in Nelson's Sparrows.

We then applied this diagnostic assay to the 441 individuals identified morphologically as Saltmarsh Sparrows from Maine to Long Island. Individuals with Nelson's mitochondrial DNA were identified as potential hybrids.

#### MORPHOLOGICAL COMPARISONS

Field measurements of the putative hybrids were compared to those of known Nelson's and Saltmarsh individuals sampled outside of the overlap zone, from the Penobscot River in Maine ( $n=34$ ) and from Shirley, NY ( $n=29$ ). Averages and standard deviations were calculated for four morphological features (culmen length, bill width, bill depth and weight) for the three groups (hybrid, Nelson's and Saltmarsh). ANOVA and a Tukey's test were used to assess differences between morphological characteristics among the three groups.

#### RESULTS

Our genetic testing revealed 34 of the 441 putative Saltmarsh Sparrows had Nelson's mitochondrial DNA (Figure 1). The majority ( $n=18$ ) of the sparrows with Nelson's mtDNA were captured on Plum Island (Newburyport, MA). The most southern site at which we identified an individual with Nelson's mtDNA was in the John H Chafee Wildlife Refuge in Narragansett, Rhode Island.

On average, bill measurements and weights were smaller in pure Nelson's individuals in comparison to pure Saltmarsh individuals (Table 1) and hybrid measurements were more similar to that of pure Saltmarsh Sparrows. For all four morphological features, pure Nelson's measurements were significantly different ( $P<0.0001$ ) from those of the Saltmarsh and hybrid groups.

## **DISCUSSION**

We found the occurrence of Nelson's specific mtDNA in about 8% of the individuals identified morphologically as Saltmarsh Sparrows, suggesting that they were of hybrid origin. Our findings indicate an expansion of the hybrid zone, with a large proportion of hybrids identified in what was previously thought to be the most southern point of the overlap zone (Parker River) and one individual identified as a hybrid as far south as Rhode Island. In a previous study, Hodgman et al. (2002) conducted point count surveys within numerous marshes to evaluate the extent of the overlap zone. Hodgman et al. (2002) surveyed 40 points in Parker River and documented a maximum of one Nelson's Sparrow at 3% of their points in comparison to a maximum of 10 Saltmarsh Sparrows at 78% of their points. Of the 95 blood samples collected from Parker River in this study, 18 individuals (19%) were identified as having Nelson's mitochondrial DNA, indicating a higher proportion of Nelson's individuals present on Plum Island than previously recorded. Furthermore, previous research documenting hybridization between Nelson's and Saltmarsh Sparrows identified putative hybrids only as far south as Scarborough, ME (Shriver et al. 2005). We identified hybrids in Maine, New Hampshire, and Massachusetts indicating a southern expansion of Nelson's alleles.

Our study did not assess the expansion of Saltmarsh Sparrow genes into Nelson's populations, as we focused only on the genetic analysis of Saltmarsh Sparrows. However, more information on the direction of introgression will be critical in determining the role of hybridization in these species. The use of mitochondrial DNA to assess hybridization is a further limitation to our study, as mtDNA is maternally inherited. Thus we are unable to detect hybrid individuals that have inherited Nelson's DNA paternally. We may be

grossly underestimating the extent of hybridization by restricting our analysis to maternally inherited genes.

## CONSERVATION IMPLICATIONS

Due to the morphological similarities between the hybrids and the Saltmarsh Sparrows, there may be an underestimation of the size and range of the hybrid zone. Our results indicate that a higher proportion of Nelson's Sparrows inhabit the most southern point of the overlap zone than previously documented. Furthermore, results from our population genetic study (Walsh et al., in prep; see chapter 2) indicate that Parker River is genetically similar, in pairwise genetic comparisons, to other marshes along the northeastern coast. This is likely due to the large size of the marshes sampled on Plum Island. Due to the difficulty associated with accurate species identification in the field, we may be grossly underestimating the percentage of hybrids present in the Parker River population. This fact combined with the degree of connectivity observed between Parker River and other marshes may result in the spread of Nelson's alleles further south, and may impact pure Saltmarsh Sparrow populations originally believed to be outside of the overlap zone. It is likely that the range of overlap between these two species, and consequently the degree of introgression, is more dynamic than previously believed.

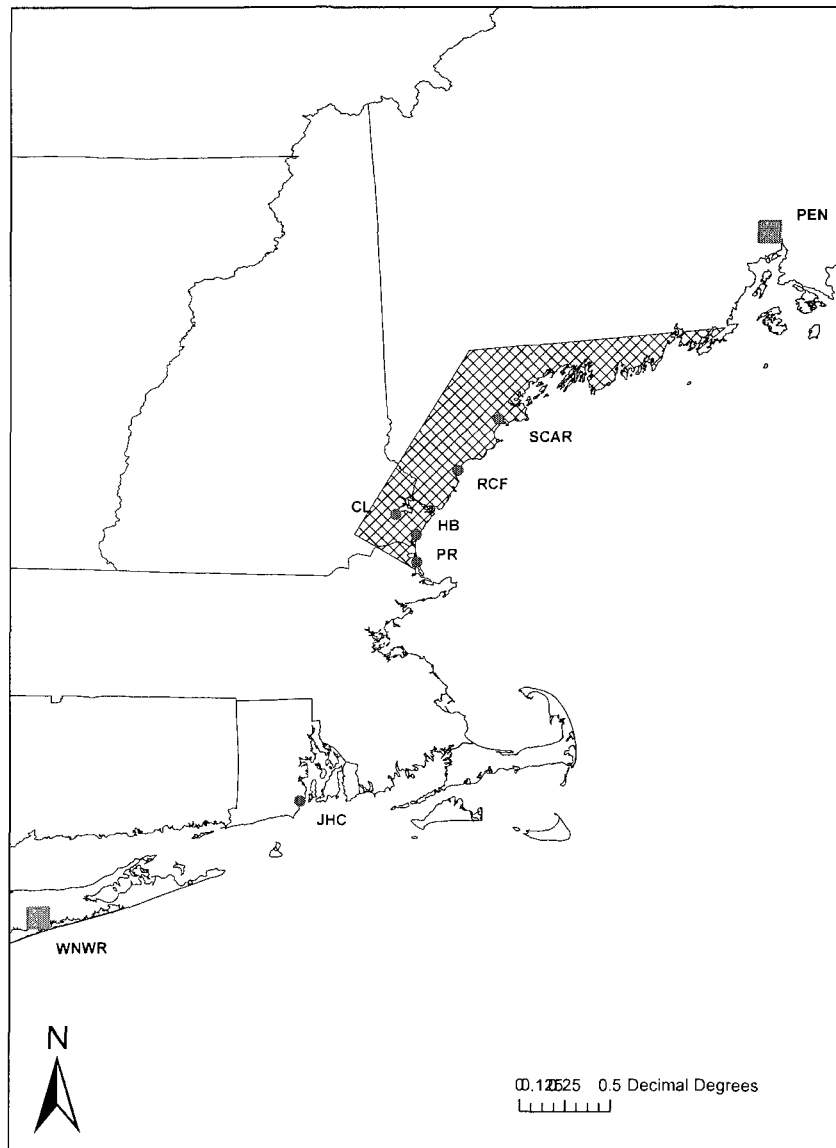
Because of the limited habitat range and conservation status of the Saltmarsh Sparrow, it is important that we understand the rate of hybridization between Saltmarsh and Nelson's Sparrows. More information is required to examine the occurrence of Nelson's alleles in Massachusetts and Connecticut, to fill in gaps between our sampling locations. If these putative hybrids are more prevalent than previously thought, research on hybrid fitness and behavior may also be important. Furthermore, future studies should

incorporate maternal and paternal DNA (microsatellites) to fully assess the direction and extent of hybridization. We recommend strict adherence to the plumage index created by Shriver et al. (2005) and the collection of field data including bill measurements and weight. This information will allow for more accurate field identification of hybrids, and should be used even if marshes are further south than the hypothesized overlap zone. Consideration of hybrid expansion will become increasingly important when implementing future management strategies for both species.



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**FIGURE 1:** Map of Saltmarsh and Nelson's Sparrow sampling locations. Red boxes represent marshes where pure Nelson's (PEN) and Saltmarsh (WNWR) samples were collected. Blue circles indicate marshes where putative hybrids were identified: SCAR(n=4), RCF(n=2), CL (n=5), HB (n=4), PR (N=18) and JHC (N=1). Shaded area represents the currently hypothesized Nelson's-Saltmarsh overlap zone.

**TABLE1: Average measurements and standard deviation for four morphological features compared between the three groups. Bold and \* represents a significant difference ( $P<0.0001$ ).**

Field Measurements	Nelson's Sparrow	saltmarsh sparrow	Hybrids
Culmen	<b>8.42<math>\pm</math> 0.30*</b>	9.22 $\pm$ 0.40	9.26 $\pm$ 0.53
Bill width	<b>4.02<math>\pm</math> 0.29*</b>	4.42 $\pm$ 0.21	4.31 $\pm$ 0.19
Bill depth	<b>5.01<math>\pm</math> 0.21*</b>	5.12 $\pm$ 0.12	5.26 $\pm$ 0.19
Weight	<b>17.32<math>\pm</math> 1.25*</b>	18.57 $\pm$ 1.24	18.96 $\pm$ 1.87

## APPENDIX B: IACUC APPROVAL

### University of New Hampshire

Research Integrity Services, Office of Sponsored Research  
Service Building, 51 College Road, Durham, NH 03824-3585  
Fax: 603-862-3564

18-Jun-2009

Babbitt, Kimberly J  
Natural Resources & The Environ  
Rudman Hall Rm G15C  
Durham, NH 03824

**IACUC #:** 070604

**Project:** Salt Marsh Sparrow Ecology, Population Genetics, and Assessment of Impacts of Mercury

**Category:** B

**Next Review Date:** 13-Jul-2010

The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved your request for a time extension for this protocol with the following comment(s):

1. *The Committee added the three-year number of animals approved to the annual review form.*
2. *As the current protocol does not cover the collection of dead chicks, the researcher needs to submit to the IACUC for the file a description of the procedures for collecting these animals, their use, and disposition.*

Approval is granted until the "Next Review Date" indicated above. You will be asked to submit a report with regard to the involvement of animals in this study before that date. If your study is still active, you may apply for extension of IACUC approval through this office.

The appropriate use and care of animals in your study is an ongoing process for which you hold primary responsibility. Changes in your protocol must be submitted to the IACUC for review and approval prior to their implementation.

**Please Note:**

1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,



Jessica A. Bolker, Ph.D.  
Chair

cc: File