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First documentation of malaria parasites (genus *Plasmodium*) in the Saltmarsh Sparrow (*Ammodramus caudacutus*) and patterns of infection with mercury exposure

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Honors Thesis

Defended April 29th, 2020

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Abstract

Avian malaria parasites, protist parasites of the genus *Plasmodium*, are extremely common in birds worldwide and have the ability to dramatically impact sensitive populations and species. Mercury, a heavy metal that accumulates in the tissues of birds, is a common environmental pollutant that may reduce immune system function and increase susceptibility to malaria parasite infection. I surveyed the endangered Saltmarsh Sparrow (*Ammodramus caudacutus*), a species with high exposure to mercury, for malaria parasites. Specifically, I investigated the presence, diversity, and distribution of malaria parasites in the species, the degree of sharing of parasite lineages with other bird species, and the relationship between blood mercury levels and infection status. Through collaboration with researchers at the Biodiversity Research Institute (BRI) and the University of New Hampshire (UNH), blood samples were collected from 290 individuals across the entire breeding range of the species from Maine to Maryland, U.S. By sensitive nested PCR methods, I screened each sample for malaria parasite infection and each positive infection was sequenced at the mitochondrial cytochrome *b* gene to identify parasite lineages, the standard gene used in defining *Plasmodium* lineages. Overall, 16.5% of Saltmarsh Sparrows were found to be infected with a total of six *Plasmodium* lineages. Prevalence and diversity of parasite lineages varied across the breeding range of the Saltmarsh Sparrow, with greater prevalence and diversity in northern states. The skewed distribution of prevalence and parasite diversity may be due to mosquito control measures, habitat type, or possibly climate change. All six parasite lineages were found to be shared with other passerine species including those also found to nest in or near saltmarshes. Blood mercury levels varied in the Saltmarsh Sparrow, ranging from 0.18 ppm to 2.52 ppm with a mean of 0.86 ppm. Although a positive correlation was expected between blood mercury levels and infection status because mercury negatively impacts an individual's ability to fight infection, I found a negative correlation. These results suggest that individuals with low blood mercury levels are more likely to be infected with malaria parasites. The discovery of a relatively high prevalence and diversity of malaria parasites in the Saltmarsh Sparrow, a species in rapid decline across its range, and the negative correlation relationship between mercury exposure and malaria parasite infection status, suggest the need for continued study of malaria parasite dynamics in the species. Further research will help elucidate the link between environmental pollution and infectious disease risk, which is a pressing topic as environmental contamination combined with climate change may heavily impact host, vector, and parasite distributions and host-parasite dynamics.

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CHAPTER ONE

Introduction

Tidal saltmarshes are home to an extremely diverse fauna and consist of dense saltmeadow cordgrass, saltgrass, and needlerushes. There are numerous vertebrate species that breed on saltmarsh coastlines around the world, approximately half of them birds, and numerous factors have resulted in a disproportionate number of endangered or threatened species in this coastal fauna group (Greenberg et al. 2006). Among the factors contributing to the endangerment of saltmarsh species are rises in sea level, human development, and environmental pollutants (Doody 2008). Sea level rise poses a substantial threat to birds as many species nest in vegetation only inches above the water, so nests are extremely vulnerable to getting flooded during extreme storms. The effects of sea level rise and extreme weather events are predicted to increase as temperatures warm with climate change. Unfortunately, the high density and increase in human development along coasts makes it difficult, if not impossible, for saltmarsh habitat and its inhabitants to move inland as sea levels rise.

The Saltmarsh Sparrow (*Ammodramus caudacutus)* is one such species found in tidal coastal marshes along the eastern United States, with its breeding range stretching from Maine to New York and its wintering range spanning from North Carolina to Florida. This species is of high conservation concern because across its breeding range much of its coastal habitat has been lost. Population declines of about 9% per year have resulted in this species being listed as a conservation priority (Shriver et al. 2015). Federally, the Saltmarsh Sparrow is considered to be vulnerable, and the IUCN lists this species as endangered due to their steady population declines and the lack of monitoring or management programs. Previous studies suggest that mercury exposure is an additional stressor for this sensitive wetland species, and central to this study, no

information exists on the presence or prevalence of malaria parasites in Saltmarsh Sparrows (Lane et al. 2011).

Malaria is caused by infection with protist parasites of the genus *Plasmodium,* which are transmitted by blood-feeding insects and reproduce within the red blood cells and internal organs of vertebrate hosts. Infection in avian hosts is characterized by a wide range of symptoms, spanning from no measurable effect to reduced reproductive success and premature death (Valkiunas 2005). *Plasmodium* has been known to cause mortality in naïve host species, as exhibited by bird populations in Hawaii, New Zealand, and the Galapagos Archipelago (van Riper et al. 1986, Wikelski et al, 2004, Shoener et al. 2014). The malaria parasites of birds are diverse and widely distributed, with hundreds of lineages representative of species recorded from avian hosts worldwide (Bensch et al. 2009, Clark et al. 2014). Given changes in host and parasite distributions due to host and vector introductions and range expansions, *Plasmodium* lineages are constantly affecting additional bird species, oftentimes with negative effects. The virulence of different lineages varies and affects hosts differently; a relatively common *Plasmodium* lineage affecting songbirds was recently discovered to cause mortality in the Common Loon (Martinsen et al. 2017). While most hosts are able to live with a malarial infection, individuals that are naïve or immunosuppressed may suffer more severe consequences. For example, previous studies have found a significant relationship between mercury exposure and malaria parasite infection in lab mice, with mercury impairing host resistance to malaria infection (Silberged et al. 2000). However, a relationship between mercury exposure and malaria parasite infection has not been demonstrated in a wild animal host.

Mercury contamination is well documented across the wetlands of the U.S. and reaches high concentrations near urbanized areas. Once atmospheric mercury is deposited via

precipitation, bacteria in marsh sediments transform the inorganic mercury into its bioavailable toxic form of methylmercury, which accumulates in organisms and biomagnifies through the food chain (Kopec et al. 2018). Thus, birds that occupy wetland habitats such as salt marshes may be especially vulnerable to mercury exposure. Indeed, a survey of 102 songbird species in eastern North America found mercury concentrations to be highest in wetland breeding birds when compared to birds that breed in other types of habitats (Jackson et al. 2015). Mercury causes adverse health effects when ingested due to its extreme toxicity and ability to bioaccumulate. Studies on the toxicology of mercury in wildlife have found that exposure caused profound damage to the reproductive, immune, and central nervous systems (Wolfe et al. 1998, Clarkson and Magos 2006). Saltmarsh Sparrows are subjected to environmental mercury and blood samples from this study indicated that their blood mercury levels ranged from 0.18 ppm to 2.52 ppm, with a mean of 0.85 ppm. Given the effects on the immune system, it is likely that mercury exposure reduces an individual's ability to combat other contaminants and pathogens that exist in the environment.

Warming temperatures also have direct impacts on the transmission rates of infectious diseases. The effects of climate change have been observed in the Hawaiian Islands as the prevalence of avian malaria has increased at higher altitudes as temperatures warm (Atkinson and Lapointe 2009). The emergence and spread of avian malaria parasites in Alaska and New Zealand also highlight how changes in the environment may improve conditions for insect vectors and pathogens (Loiseau et al. 2012, Schoener et al. 2014). The response of avian *Plasmodium* parasites to changing environmental conditions may give insight to the dynamics of other infectious diseases affected by climate change.

This thesis focuses on the *Plasmodium* parasites of the endangered Saltmarsh Sparrow. The first chapter investigates the prevalence, diversity, and distribution of *Plasmodium* parasites across the breeding range of the species as well as the degree of sharing of *Plasmodium* parasites with other bird species. The second chapter examines the relationship between blood mercury levels and malaria infection status of Saltmarsh Sparrows from Long Island, New York. The results from this study will help draw attention to the many factors driving the decline of the Saltmarsh Sparrow, including the possible role of malaria parasites, and hopefully contribute to their conservation as well as the conservation of other songbird species known to be exposed to mercury and malaria parasites. A diversity of bird and other wildlife species that breed in salt marshes are also exposed to various heavy metals, so this study will also serve as a benchmark for future studies focusing on the interactions between heavy metals and infectious disease risk.

Literature Cited

Atkinson, C. T., and LaPointe, D. A. (2009). Introduced Avian Diseases, Climate Change, and the Future of Hawaiian Honeycreepers. *Journal of Avian Medicine and Surgery*, *23*(1), 53–63.

Clarkson, T. W., and Magos, L. (2006). The Toxicology of Mercury and Its Chemical Compounds. *Critical Reviews in Toxicology*, *36*(8), 609–662.

Doody, J. P. (2008). *Saltmarsh Conservation, Management and Restoration*. Springer Science & Business Media.

Greenberg, R. (Ed.). (2006). *Terrestrial vertebrates of tidal marshes: Evolution, ecology, and conservation*. Cooper Ornithological Society.

Jackson, A. K., Evers, D. C., Adams, E. M., Cristol, D. A., Eagles-Smith, C., Edmonds, S. T., Gray, C. E., Hoskins, B., Lane, O. P., Sauer, A., and Tear, T. (2015). Songbirds as sentinels of mercury in terrestrial habitats of eastern North America. *Ecotoxicology (London, England)*, *24*(2), 453–467.

Kopec, A. D., Bodaly, R. A., Lane, O. P., Evers, D. C., Leppold, A. J., and Mittelhauser, G. H. (2018). Elevated mercury in blood and feathers of breeding marsh birds along the contaminated lower Penobscot River, Maine, USA. *The Science of the Total Environment*, *634*, 1563–1579.

Loiseau, C., Harrigan, R. J., Cornel, A. J., Guers, S. L., Dodge, M., Marzec, T., Carlson, J. S., Seppi, B., and Sehgal, R. N. M. (2012). First Evidence and Predictions of Plasmodium Transmission in Alaskan Bird Populations. *PLoS ONE*, *7*(9).

Martinsen, E. S., Sidor, I. F., Flint, S., Cooley, J., and Pokras, M. A. (2017). Documentation of Malaria Parasite (Plasmodium spp.) Infection and Associated Mortality in a Common Loon (Gavia immer). *Journal of Wildlife Diseases*, *53*(4), 859–863.

Riper, C. van, Riper, S. G. van, Goff, M. L., and Laird, M. (1986). The Epizootiology and Ecological Significance of Malaria in Hawaiian Land Birds. *Ecological Monographs*, *56*(4), 327–344.

Schoener, E. R., Banda, M., Howe, L., Castro, I. C., and Alley, M. R. (2014). Avian malaria in New Zealand. *New Zealand Veterinary Journal*, *62*(4), 189–198.

Shriver, W. G., O'Brien, K. M., Ducey, M. J., and Hodgman, T. P. (2016). Population abundance and trends of Saltmarsh (*Ammodramus caudacutus*) and Nelson's (A. nelsoni) Sparrows: Influence of sea levels and precipitation. *Journal of Ornithology*, *157*(1), 189–200.

Silbergeld, E. K., Nash, D., Trevant, C., Strickland, G. T., Souza, J. M. de, and Silva, R. S. U. da. (2002). Mercury exposure and malaria prevalence among gold miners in Pará, Brazil. *Revista Da Sociedade Brasileira de Medicina Tropical*, *35*(5), 421–429.

Wikelski, M., Foufopoulos, J., Vargas, H., and Snell, H. (2004). Galápagos Birds and Diseases: Invasive Pathogens as Threats for Island Species. *Ecology and Society*, *9*(1).

Wolfe, M. F., Schwarzbach, S., and Sulaiman, R. A. (1998). Effects of mercury on wildlife: A comprehensive review. *Environmental Toxicology and Chemistry*, *17*(2), 146–160.

CHAPTER TWO

**Diversity and Distribution of Malaria Parasites (genus *Plasmodium*) in Saltmarsh
Sparrows (*Ammodramus caudacutus)***

Introduction

The avian malaria parasites (genus *Plasmodium)* are extremely diverse, abundant, and
geographically widespread (Valkiunas 2005). With the exception of Antarctica, they are found
on every continent. The advent of molecular methods has enhanced our understanding of the
host and geographic distribution of avian malaria parasites as they may go unseen by traditional
methods of light microscopy of blood smears (Clark et al. 2014). Gene sequence data have also
revealed a greater diversity of these parasites than previously known by visual classification via
microscope (Martinsen et al. 2006). The malaria parasites of birds have been so well studied that
they have their own online database- MalAvi (http://mbio-serv2.mbioekol.lu.se/Malavi/, Bensch
et al. 2009). In this database, hundreds of *Plasmodium* lineages representative of species have
been documented. It is believed that the intermixing of occurrences of host switching, local
host-parasite coevolution, and long-distance dispersal has driven the diversification of malaria
parasites of birds worldwide with an even greater diversity still yet to be discovered (Ricklefs et
al. 2014).

Malaria parasites have a complex life cycle in both avian and mosquito vector hosts.
Once malaria parasites are injected into an avian host, they undergo a phase of asexual
reproduction in the liver and then move out to invade the red blood cells where they can multiply
via asexual reproduction. Gametocytes are then formed, which circulate in the peripheral blood
so that they can be taken up by a mosquito when it feeds (Valkiunas 2005). Once a bird becomes

infected with malaria, it typically maintains infection for many years or even for life, thus acting as the source of infection for vectors. Infection in avian hosts is characterized by a wide range of symptoms, spanning from no measurable effect to reduced reproductive success and premature death (Valkiunas 2005). The consequences of *Plasmodium* infection on avian fitness are usually relatively mild, but the severity depends on the parasite lineage and the sensitivity of the host (Böhme et al. 2018). *Plasmodium* lineages differ in their ability to develop in different hosts, and one lineage that might cause severe disease in one species of bird may not be able to develop in and thus infect another species of bird (Valkiunas et al. 2008). In general, novel host parasite interactions usually result in increased virulence and can cause acute disease and high mortality when introduced to naïve birds (Egizi et al. 2018). For instance, the introduction of avian malaria parasites to the Hawaiian Islands played a major role in the endangerment and extinction of native honeycreeper species (Riper et al. 1986).

The avian *Plasmodium* parasites are considered to be host generalists as many are able to infect and develop in bird taxa belonging to different species and genera, and sometimes even families and orders (Valkiunas 2005). For example, the most common avian malaria parasite worldwide, *Plasmodium relictum,* has an extensive host range covering many bird orders (Schoener et al. 2014, Valkiunas et al. 2018). With a warming climate, the geographic range of mosquito vectors is moving northward (Egizi et al. 2018). The reproduction of malaria parasites in their mosquito hosts increases with increasing temperatures, and a 2-3-fold increase in the prevalence of malaria parasites in birds has been predicted for every 1-degree Celsius rise of global temperatures (Garamszegi 2011). With vector host range expansion and increased prevalence due to global warming, malaria parasites are expected to increase in incidence and prevalence at more northern latitudes. Although previously not known to infect birds in Alaska,

malaria parasites were recently discovered in resident Alaskan birds (Loiseau et al. 2012). This is especially troubling as the *Plasmodium* parasites of birds are capable of expanding into new host taxa and are likely to have devastating impacts on immunologically naïve bird populations and species. Recently, disease and mortality from malaria parasite infection was found in the Common Loon (*Gavia immer*), a species with a northern distribution thought previously to be malaria parasite free (Martinsen et al. 2017). In light of global warming, the screening of malaria parasites in naïve host taxa including those of conservation concern is imperative.

Avian malaria parasites are very common in birds in the Northeastern U.S., with light microscopy revealing approximately 40% of songbirds sampled in Vermont found to be infected (Barnard and Bair 1986). No studies to date have documented the presence and distribution of avian malaria parasites in other areas of the Northeast, so we know very little about the parasites that currently inhabit birds in this region. In addition, although a positive correlation exists between habitats that may harbor more vectors and the prevalence of malaria parasites among avian host species, very little is known about the malaria parasites of wetland breeding bird species (Mederios et al. 2015). Here, I studied the blood parasites of a threatened and declining bird species that inhabits wetlands: The Saltmarsh Sparrow (*Ammodramus caudacutus*). This species has experienced a steep population decline of 9% per year due to human development and rises in sea level (Shriver et al. 2015). In addition, this species is heavily exposed to environmental mercury, which may serve as an additional stressor for this sensitive wetland species. To date, this species has not been screened for malaria parasites.

Through collaboration with researchers at the Biological Research Institute in Portland, ME and the University of New Hampshire, I obtained blood samples from breeding Saltmarsh Sparrows from sites across their breeding range. Using sensitive molecular methods, I screened

these samples for malaria parasites. I hypothesized that the Saltmarsh Sparrow would be infected with similar malaria parasite lineages to other bird species that breed in similar habitat as they may be exposed to the same mosquito vectors. In addition, I hypothesized that parasite lineages will vary between sites because the distribution of mosquitos may differ throughout the breeding range of the Saltmarsh Sparrow.

Materials and Methods

Field Sampling

Adult Saltmarsh Sparrows were sampled during the breeding season from sites across their breeding range by researchers at the Biodiversity Research Institute and the University of New Hampshire. Birds were caught in the field in mist nets, banded with USF&WS leg bands, measured, and blood sampled. Approximately 50-70 micrograms of blood were collected from each individual from the ulnar vein. Blood was stored in microcapillary tubes at -20 degrees Celsius until molecular analysis. For a small number of birds, blood smears were also prepared in the field. These smears were stained with Giemsa stain in the lab and examined for the presence of malaria parasites under a light microscope at 40X and 100X. For this project, I examined 290 blood samples collected between 2007 and 2017 from 17 sampling sites. This included 63 samples from Maine, 55 samples from New Hampshire, 16 samples from Massachusetts, 35 samples from Rhode Island, 100 samples from New York, 12 samples from Delaware, and 9 samples from Maryland (See Map, Figure 1). All sites were marshlands located either near or on the coastline. The sites in Rhode Island and Massachusetts were both wildlife refuges. All sites were located along the coastline with the exception of Scarborough, ME, John H. Chafee National Wildlife Refuge, RI, Chapman's Landing, NH, and Sawmill Creek, NY.

These sites were located inland along saltmarshes of bodies of water that were connected to the ocean.

Figure 1. Map of the field sites from which blood samples were collected from Saltmarsh Sparrows (*Ammodramus caudacutus*). Each dot represents a sampling site; sites were located in Maryland, Delaware, New York, Rhode Island, Massachusetts, New Hampshire, and Maine.

Molecular Methods

To detect avian malaria parasite infection, all blood samples were screened by sensitive nested PCR methods. DNA was first extracted from each blood sample using the Qiagen DNeasy Blood and Tissue Kit following manufacturer's instructions. The nested PCR amplifies ~500 base pairs of the parasite's mitochondrial cytochrome *b* gene and allows detection of low-level infections not visible by light microscopy. The outer PCR utilized primers HaemNF and HaemNR2 and the inner PCR utilized primers HaemF and HaemR2. The thermocycler

conditions were 10 minutes of denaturation at 94 degrees Celsius, followed by 35 cycles at 94 degrees Celsius for 30 seconds, 52 degrees Celsius for 30 seconds, 72 degrees Celsius for 45 seconds, and a final elongation step at 72 degrees Celsius for 10 minutes (Bensch et al. 2000, Waldenstrom et al. 2004). By gel electrophoresis, PCR products were visualized (Figure 2). A band of approximately 500 base pairs indicated a positive infection. Positive and negative controls were included with each round of nested PCRs to control for contamination and to ensure successful parasite amplification. To test for the presence of false negatives, 20 samples found to be negative by one round of nested PCR were retested by nested PCR.
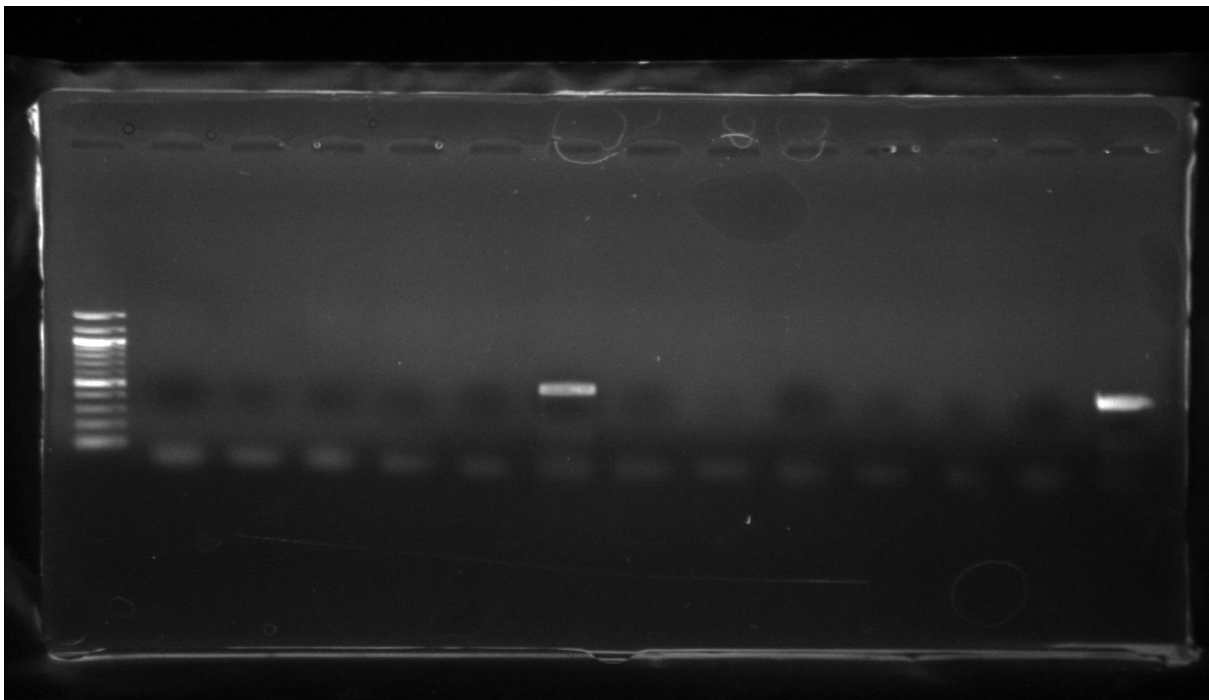

Figure 2. Image of an agarose gel showing the base pair ladder in the far left and the PCR products (lanes 7 and 14). The bright bands at ~500 base pairs indicate the presence of malaria parasites in a sample. All other samples without bands present were scored negative for malaria parasite infection.

Samples that scored positive for malaria parasite infection by gel electrophoresis were purified enzymatically using ExoSAP-IT. Samples were then sequenced using the forward

primer on an ABI Prism 3130xl Genetic Analyzer at the University of Vermont's Integrative

Genomics Resource Center. Sequences were visualized and edited with review of each base pair

using the program 4Peaks. To identify parasites to lineage, each sequence was compared to all

other avian malaria parasite lineages in the online databases MalAvi (http://mbio-

serv2.mbioekol.lu.se/Malavi/, Bensch et al. 2009) and Genbank by BLAST analysis (Basic Local

Alignment Search Tool). Parasite sequences were assigned or designated with a previously

documented lineage name only if they were identical (100% genetic similarity) to previously

documented parasite lineages in these databases.

Results

Of the 290 blood samples screened for *Plasmodium* infection by nested PCR, 48 (16.5%)

tested positive. The gametocytes of *Plasmodium* blood parasites were visualized by light

microscopy in blood smears from a few of the infected individuals indicating that the parasites

are able to complete their life cycle in the Saltmarsh Sparrow and that the species is a host for the

parasites. Rhode Island had the highest prevalence or percentage of infected individuals, with 12

out of 35 samples testing positive (34%). Massachusetts, Maine, and Maryland also had

relatively high prevalence rates of 31%, 25%, and 22% respectively. All samples from Delaware

were negative (N=12). New York and New Hampshire had the lowest prevalence of 5% and

14.5% (Table 1).

Table 1. The number of Saltmarsh Sparrows (*Ammodramus caudacutus)* sampled per state including the number of individuals deemed positive for malaria parasite (genus *Plasmodium)* infection by nested PCR.

State	# of Individuals Sampled	# of Infected Individuals	Prevalence of Infected Individuals
Maine	63	16	25%
New Hampshire	55	8	14.5%
Massachusetts	16	5	31%
Rhode Island	35	12	34%
New York	100	5	5.00%
Delaware	12	0	0%
Maryland	9	2	22%

Malaria parasite prevalence also varied widely by individual sampling site. The highest infection rates were found in John H. Chafee National Wildlife Refuge, RI (34%), Scarborough, ME (31%), and Parker River National Wildlife Refuge, MA (31%) (Table 2). There were several sites that had no infected individuals at all. The site in Delaware had zero infected individuals. In New York, five out of the eight sites did not have infected individuals (Figure 3). The New York sites that had infected individuals were Accabonac Harbor, Four Sparrow Marsh, and Idlewild.

Table 2. The number of Saltmarsh Sparrows (*Ammodramus caudacutus)* sampled per site including the number of individuals deemed positive for malaria parasite (genus *Plasmodium)* infection by nested PCR.

| Site	# of Individuals Sampled	# of Individuals Infected	Prevalence of Infected Individuals
Popham Beach, ME	18	4	22%
Furbish Marsh, ME	10	1	10%
Scarborough, ME	35	11	31%
Chapman's Landing, NH	35	7	20%
Hampton Beach, NH	20	2	10%
Parker River National Wildlife Refuge, MA	16	5	31%
John H. Chafee National Wildlife Refuge, RI	35	12	34%
East Quogue, NY	5	0	0%
Four Sparrow Marsh, NY	10	2	20%
Accabonac Harbor, NY	14	1	7%
Idlewild, NY	10	2	20%
Marine Nature Center, NY	23	0	0%
North Cinder Island, NY	17	0	0%
North Greensedge Island, NY	7	0	0%
Sawmill Creek, NY	14	0	0%
Delaware	12	0	0%
Maryland	9	2	22%
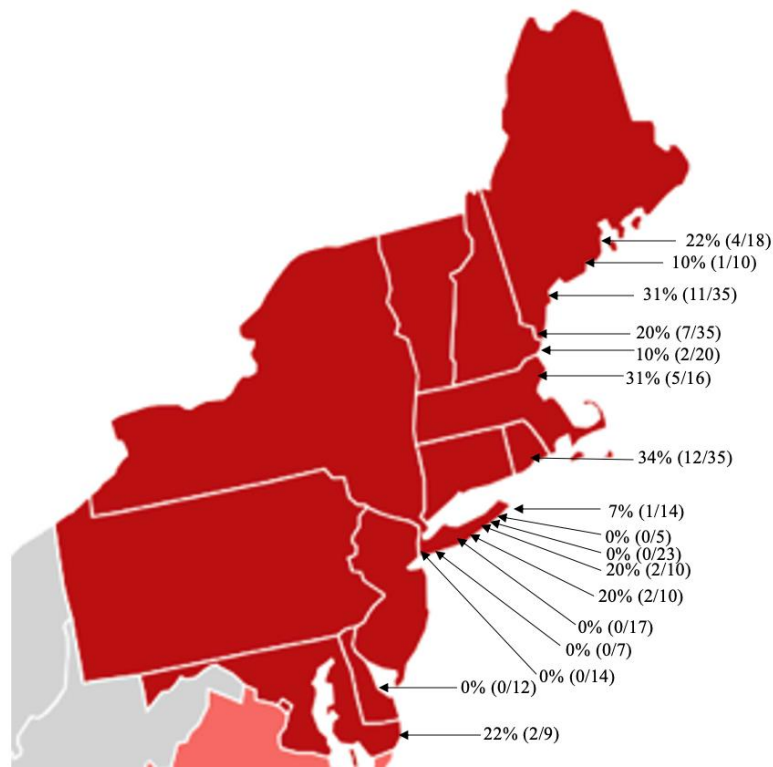
Figure 3. Map indicating the prevalence of Saltmarsh Sparrows (*Ammodramus caudacutus*) infected with *Plasmodium* parasites at each sampling site.

Sequencing and BLAST analysis of samples that tested positive for malaria parasites revealed the presence of six different parasite lineages. All sequences matched known lineages in the MalAvi database. These lineages included GEOTRI09, MELMEL01, MELMEL02, RWB01, SEIAUR01, and MELMEL05. The Scarborough and Chapman's Landing sites had the greatest number of parasite lineages (Figure 4). The site in Scarborough, Maine had four lineages: GEOTRI09, RWB01, MELMEL02, and SEIAUR01. The other two sites in Maine only had the GEOTRI09 lineage present in the infected individuals. The site in Chapman's Landing also had four lineages present: GEOTRI09, SEIAUR01, MELMEL02, and MELMEL05. All other sites with infected individuals had less than three lineages present.

Twenty individuals were found infected with the GEOTRI09 lineage, making this lineage the most common within the sites sampled. The only state that did not have the GEOTRI09 lineage present was New Hampshire. The next most common lineage was SEIAUR01 with 14 individuals infected across Maine, New Hampshire, Massachusetts, Rhode Island, and Maryland. MELMEL01, RWB01, and MELMEL05 lineages were each only isolated from a single infected individual. MELMEL01 was found in an individual from Parker River NWR, MA, RWB01 was found in an individual from Scarborough, ME, and MELMEL05 was found in an individual from Chapman's Landing, NH (Figure 4).
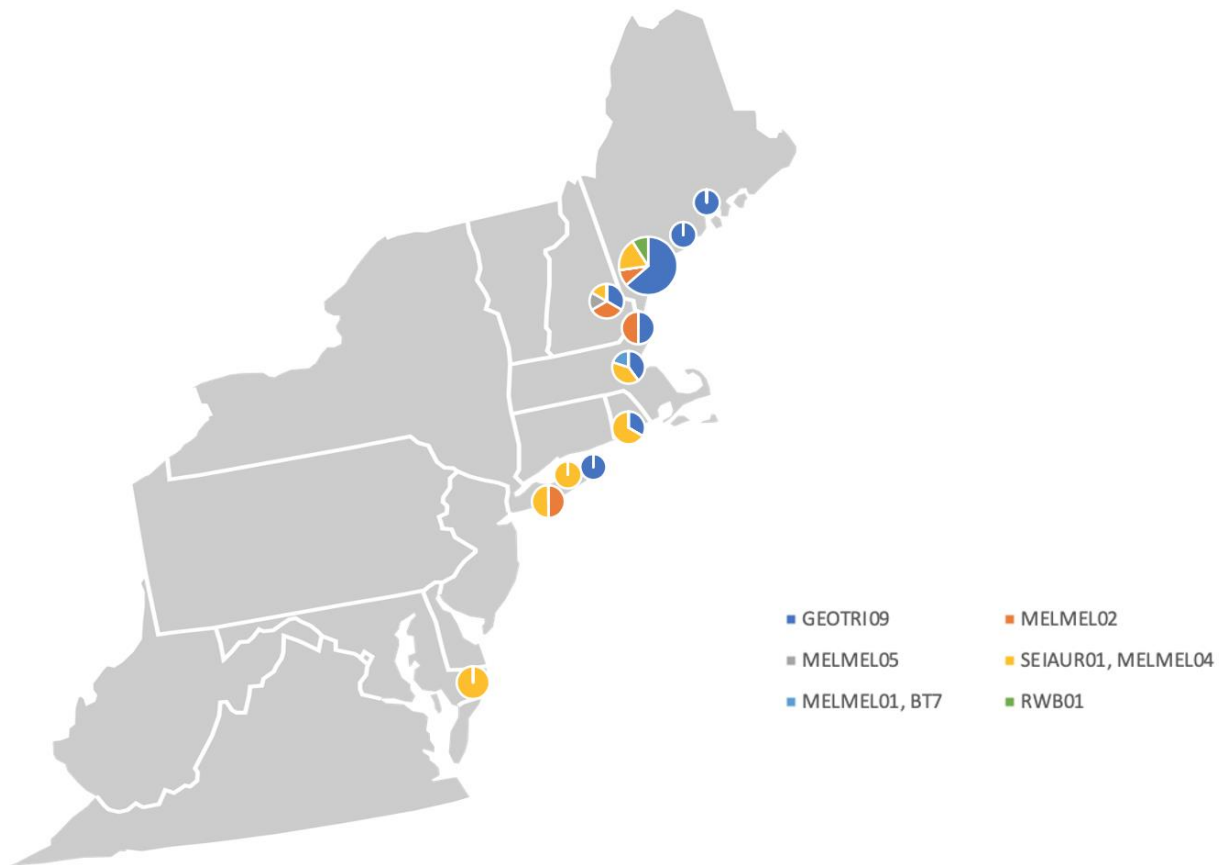

Figure 4. Map of the geographic distribution of six different *Plasmodium* lineages across the breading range of Saltmarsh Sparrows (*Ammodramus caudacutus*)

The GEOTRI09 lineage has previously been found in nine other host species in the United States, Canada, and Brazil (MalAvi database). MELMEL01, MELMEL02, MELMEL04 and MELMEL05 have only been found in Song Sparrows (*Melospiza melodia*) in Ontario, Canada (Sarquis-Adamson and MacDougall-Shackleton 2016). RWB01 has been found in six hosts across four families from New York, Wisconsin, Vermont, Michigan, and Ontario (MalAvi database). The SEIAUR01 lineage morphologically is a *Plasmodium cathemerium* species and is present in 29 hosts across 10 families in the United States, Canada, Bermuda, Mexico, and Colombia (MalAvi database).

Discussion

Overall, I found a 16.5% infection rate, or prevalence of malaria parasites, in the Saltmarsh Sparrows sampled in this study. These results are significant given that these are the first records of malaria parasites in the species and the Saltmarsh Sparrow is a species of conservation concern. Malaria parasites were found across the breeding range, with the greatest prevalence and diversity of parasites concentrated in the northern portion of the breeding range of the species, including Rhode Island, Massachusetts, New Hampshire, and Maine. New York and Delaware had substantially lower rates of infected individuals than the other states. Differences in prevalence were also discovered among sampling sites within states. In Maine, Scarborough had the highest prevalence of malaria infection (31%), but the Popham Beach and Furbish Marsh sites had much lower infection rates of 22% and 10%, respectively. The prevalence of infection also varied in New York, as only three of the eight sites had infected individuals and they were located on the southeastern coast of Long Island.

Differences in prevalence of malaria parasites across the breeding range of the Saltmarsh Sparrow may be due to different land use practices and mosquito control efforts. For some of the counties in which the sample sites were located, extreme mosquito control measures have been taken. For example, in the 1900's, Long Island used mosquito ditches in attempt to drain pooled waters in marshes where mosquito larvae developed. Many mosquito species on Long Island are now uncommon due to ditching and control methods including species which bite humans (Penny 2010). Accabonac Harbor, NY had the greatest ditching efforts, and there are still various source reduction techniques presently being used to stop the production of mosquitos by eliminating breeding sites. Suffolk County, which encompasses the majority of Long Island, regularly uses ultra-low volume sprays that dispense aerosol droplets of insecticides that stay aloft and kill mosquitos on contact (suffolkcountyny.gov). Delaware also takes similar measures to reduce the number of breeding mosquitos in saltmarshes. Small, shallow ponds are selectively installed to eliminate alternately wet and dry potholes where saltmarsh mosquitos lay eggs. Many counties in Delaware also routinely spray insecticides to kill adult mosquitos in the area (dnrec.delaware.gov). Only 5% of the Saltmarsh Sparrows sampled from Long Island were infected with malaria, and zero individuals from Delaware were infected. As these two states had the lowest prevalence of infections, it suggests that the mosquito control programs were successful in reducing the number of mosquito vectors in the area including those species that transmit avian *Plasmodium* parasites.

A greater diversity of *Plasmodium* lineages was found in states in the northern part of the Saltmarsh Sparrow's breeding range, which could be a result of the greater number of infected individuals in the northern states. The sites in Scarborough, Maine, and Chapman's Landing, New Hampshire had the greatest number of infected individuals, and they also had the most

lineages present. In addition, these sites in Maine and New Hampshire also had novel lineages

that were not present at any other sampled sites. The RWB01 lineage was only seen in a single

bird from Scarborough, and the MELMEL05 lineage was only found in a single bird from

Chapman's Landing. These results may be explained by the presence of genetically distinct

populations of Saltmarsh Sparrows at different sites across their breeding range. Indeed, a study

on the fine-scale population structure of Saltmarsh Sparrows revealed significant differences in

genetic variation across populations from nine different marshes along the breeding range, with

the discovery of five genetically distinct breeding populations of the Saltmarsh Sparrow (Walsh

et al. 2012, Figure 5). As many of the samples in this study were obtained from the Kovach lab

at the University of New Hampshire, which was involved in this population genetics study,

including samples from all five of these distinct populations, our results may indicate local host-

parasite associations. Genetic variation in the host species may influence the survival and

reproduction of different lineages of *Plasmodium* species and explain differences in individual

parasite lineage distribution (Valkiunas et al. 2008). Interestingly, the three lineages documented

to only infect a single bird were isolated from different populations of the Saltmarsh Sparrow.
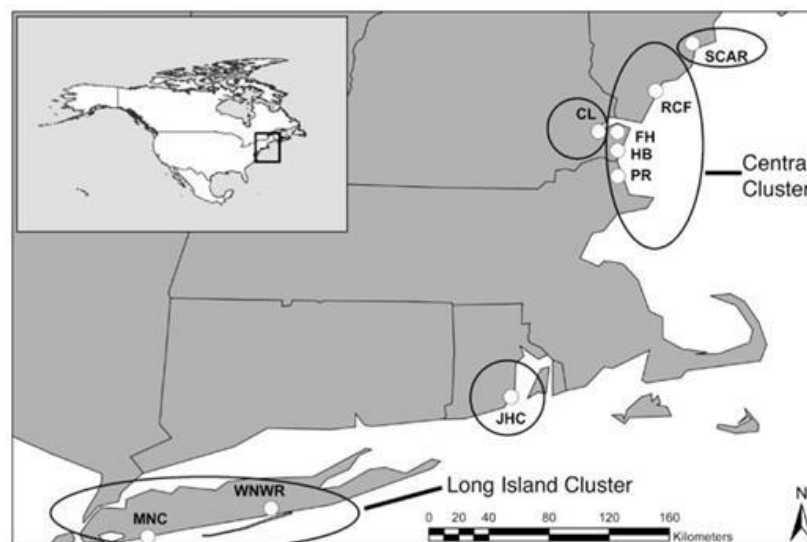
The two *Plasmodium* lineages distributed across the entire breeding range of the Saltmarsh Sparrow were GEOTRI09 and SEIAUR01. According to the MalAvi database, the GEOTRI09 lineage appears in nine species across five families in North America while the SEIAUR01 has been found in 29 avian hosts across 10 families in North America. All of the lineages documented in Saltmarsh Sparrows are restricted to hosts of the order Passeriformes across North America. The six *Plasmodium* lineages observed in Saltmarsh Sparrows were also all found in Song Sparrows (*Melospiza melodia*), suggesting a high degree of *Plasmodium* lineage sharing between these two species. The MELMEL01, MELMEL02, and MELMEL05 lineages were only found in Song Sparrows. The geographic distribution of the Saltmarsh Sparrow overlaps with the northeast portion of the Song Sparrow's range, which spans from Alaska to the eastern United States. Song Sparrows are found in a large variety of open habitats, including tidal marshes, grasslands, desert scrub, and woodlands. This overlap in ranges and habitat would allow the same vectors to come into contact with both species as supported by these findings. It would be beneficial to determine the mosquito vector species that transmit the specific lineages of *Plasmodium* found in the Saltmarsh Sparrows to elucidate the relationship between habitat location and exposure to mosquito vectors and *Plasmodium* parasites.

Only one of the lineages found in Saltmarsh Sparrows has been previously isolated by molecular methods from mosquitos. Lineage SEIAUR01 has been isolated from *Culex pipens*, *Culex restuans*, and *Aedes canadensis* mosquitoes in upstate New York (Kimura et al. 2010). These different mosquito species utilize different habitats for larval development and are all

widespread in the continental U.S. My results indicate that lineage SEIAUR01 is widely

distributed in Saltmarsh Sparrows across their breeding range. Its broad mosquito host range for

this parasite lineage may explain why it is so widespread and found in every state sampled and

also why it infects a diversity of songbird species including the Saltmarsh Sparrow.

     The Saltmarsh Sparrow shares its *Plasmodium* parasite lineages with some but not all

other saltmarsh-nesting songbird species screened for blood parasites. Marsh Wrens

(*Cistothorus palustris*) have only been found to harbor *Leucocytozoon* parasites, which are in a

different haemosporidian parasite genus. Red-winged Blackbirds (*Agelaius phoeniceus*) are also

a marsh-nesting species and have been found to be infected with four *Plasmodium* lineages, two

of which are SEIAUR01 and RWB01 (Smith et al. 2018). Their geographic range, which spans

the United States, overlaps with that of the Saltmarsh Sparrow so it is likely that the same

mosquito vectors bite both species. *Plasmodium* parasites have also been found in other

Ammodramus species including the Yellow-browed Sparrow (*Ammodramus aurifrons*),

Henslow's Sparrow (*Ammodramus henslowii*), and the Grassland Sparrow (*Ammodramus*

humeralis). Henslow's Sparrows that were sampled from Platte River, Nebraska were infected

with the SEIAUR01 lineage, which was very common among the Saltmarsh Sparrows sampled

in this study (Levin et al. 2016). Further research is needed to determine the degree to which

lineages are shared between the Saltmarsh Sparrow and other avian species including those that

also nest in marshlands.

As temperatures warm with climate change, the geographic distribution of vectors and

parasites will shift, impacting host-parasite dynamics. It is predicted that malaria parasites will

spread to any area that presents suitable environmental conditions, including those at higher

elevations and more northernly latitudes (Loiseau et al. 2012). Some habitats that were too cool

to sustain vector populations may now be more favorable for vectors and parasites, whereas

other habitats at lower latitudes and elevations may become less conducive to vector and parasite

reproduction. The high rates of malaria infection in individuals found in Rhode Island and other

more northern states could potentially be explained by the recent warming in temperatures. A

temporal study on the distribution of malaria parasite lineages found in the Northeastern U.S.

would be helpful in determining exactly how *Plasmodium* parasites are being affected by climate

change and their effects on Saltmarsh Sparrows. The increase in avian malaria prevalence has

accelerated in recent years, and a surge in avian malarial infection is predicted to take place in

the near future (Garamszegi 2011). It is important to track the changes in malaria distribution as

increases in infection rates may endanger naïve bird populations, and avian malaria may serve as

a model for how vector-mediated effects due to climate change are manifested in human malaria

parasites.

Literature Cited

About the Refuge—Parker River—U.S. Fish and Wildlife Service. (n.d.). Retrieved April 20,
        2020, from https://www.fws.gov/refuge/Parker_River
Barnard, W. H., and Bair, R. D. (1986). Prevalence of avian hematozoa in central Vermont.
        Journal of Wildlife Diseases, *22*(3), 365–374.
Bensch, Staffan, Hellgren, O., and Pérez-Tris, J. (2009). MalAvi: A public database of malaria
        parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome
        b lineages. *Molecular Ecology Resources*, *9*(5), 1353–1358.
Bensch, Stffan, Stjernman, M., Hasselquist, D., Örjan, Ö., Hannson, B., Westerdahl, H., and
        Pinheiro, R. T. (2000). Host specificity in avian blood parasites: A study of Plasmodium
        and Haemoproteus mitochondrial DNA amplified from birds. *Proceedings of the Royal
        Society of London. Series B: Biological Sciences*, *267*(1452), 1583–1589.

Böhme, U., Otto, T. D., Cotton, J. A., Steinbiss, S., Sanders, M., Oyola, S. O., Nicot, A., Gandon, S., Patra, K. P., Herd, C., Bushell, E., Modrzynska, K. K., Billker, O., Vinetz, J. M., Rivero, A., Newbold, C. I., and Berriman, M. (2018). Complete avian malaria parasite genomes reveal features associated with lineage-specific evolution in birds and mammals. *Genome Research*, *28*(4), 547–560.

Clark, N. J., Clegg, S. M., and Lima, M. R. (2014). A review of global diversity in avian haemosporidians (Plasmodium and Haemoproteus: Haemosporida): new insights from molecular data. *International Journal for Parasitology*, *44*(5), 329–338.

Egizi, A., Martinsen, E. S., Vuong, H., Zimmerman, K. I., Faraji, A., and Fonseca, D. M. (2018). Using Bloodmeal Analysis to Assess Disease Risk to Wildlife at the New Northern Limit of a Mosquito Species. *EcoHealth*, *15*(3), 543–554.

Garamszegi, L. Z. (2011). Climate change increases the risk of malaria in birds. *Global Change Biology*, *17*(5), 1751–1759. https://doi.org/10.1111/j.1365-2486.2010.02346.x

Kimura, M., Darbro, J. M., and Harrington, L. C. (2010). Avian Malaria Parasites Share Congeneric Mosquito Vectors. *Journal of Parasitology*, *96*(1), 144–151.

Levin, I. I., Colborn, R. E., Kim, D., Perlut, N. G., Renfrew, R. B., and Parker, P. G. (2016). Local parasite lineage sharing in temperate grassland birds provides clues about potential origins of Galapagos avian Plasmodium. *Ecology and Evolution*, *6*(3), 716–726.

Loiseau, C., Harrigan, R. J., Cornel, A. J., Guers, S. L., Dodge, M., Marzec, T., Carlson, J. S., Seppi, B., and Sehgal, R. N. M. (2012). First Evidence and Predictions of Plasmodium Transmission in Alaskan Bird Populations. *PLoS ONE*, *7*(9).

MalAvi: Avian Malaria Initiative. (n.d.). Retrieved April 21, 2020, from http://130.235.244.92/Malavi/

Martinsen, E. S., Paperna, I., and Schall, J. J. (2006). Morphological versus molecular identification of avian Haemosporidia: an exploration of three species concepts. *Parasitology*, *133*, 279-288.

Martinsen, Ellen S., Sidor, I. F., Flint, S., Cooley, J., and Pokras, M. A. (2017). Documentation of Malaria Parasite (Plasmodium spp.) Infection and Associated Mortality in a Common Loon (*Gavia immer*). *Journal of Wildlife Diseases*, *53*(4), 859–863.

Medeiros, M. C. I., Ricklefs, R. E., Brawn, J. D., and Hamer, G. L. (2015). *Plasmodium* prevalence across avian host species is positively associated with exposure to mosquito vectors. *Parasitology*, *142*(13), 1612–1620.

Mosquito Control. (n.d.). Retrieved April 21, 2020, from http://www.dnrec.delaware.gov/fw/mosquito/Pages/default.aspx

Penny, L. (2010). Healing Salt Marshes from the Scars of Mosquito Ditches. *Memoirs of the Torrey Botanical Society*, *26*, 154–161. JSTOR.

Riper, C. van, Riper, S. G. van, Goff, M. L., and Laird, M. (1986). The Epizootiology and Ecological Significance of Malaria in Hawaiian Land Birds. *Ecological Monographs*, *56*(4), 327–344.

Schoener, E. R., Banda, M., Howe, L., Castro, I. C., and Alley, M. R. (2014). Avian malaria in New Zealand. *New Zealand Veterinary Journal*, *62*(4), 189–198.

Shriver, W. G., O'Brien, K. M., Ducey, M. J., and Hodgman, T. P. (2016). Population abundance and trends of Saltmarsh (Ammodramus caudacutus) and Nelson's (A. nelsoni) Sparrows: Influence of sea levels and precipitation. *Journal of Ornithology*, *157*(1), 189–200.

Smith, J. D., Gill, S. A., Baker, K. M., and Vonhof, M. J. (2018). Prevalence and diversity of avian Haemosporida infecting songbirds in southwest Michigan. *Parasitology Research*, *117*(2), 471–489.

Suffolk County Vector Control 2020 Plan of Work.pdf. (n.d.). Retrieved April 17, 2020, from https://www.suffolkcountyny.gov/portals/0/formsdocs/publicworks/pdf/Suffolk%20County%20Vector%20Control%202020%20Plan%20of%20Work.pdf

Valkiunas, G. (2004). *Avian Malaria Parasites and other Haemosporidia*. CRC Press.

Valkiūnas, G., Iezhova, T. A., Križanauskienė, A., Palinauskas, V., Sehgal, R. N. M., and Bensch, S. (2008). A Comparative Analysis of Microscopy and PCR-Based Detection Methods for Blood Parasites. *Journal of Parasitology*, *94*(6), 1395–1401.

Valkiūnas, G., Ilgūnas, M., Bukauskaitė, D., Fragner, K., Weissenböck, H., Atkinson, C. T., and Iezhova, T. A. (2018). Characterization of Plasmodium relictum, a cosmopolitan agent of avian malaria. *Malaria Journal*, *17*(1), 184.

Waldenström, J., Bensch, S., Hasselquist, D., and Ostman, O. (2004). A new nested polymerase chain reaction method very efficient in detecting Plasmodium and Haemoproteus infections from avian blood. *The Journal of Parasitology*, *90*(1), 191–194.

Walsh, J., Kovach, A. I., Babbitt, K. J., and O'Brien, K. M. (2012). Fine-Scale Population Structure and Asymmetrical Dispersal in an Obligate Salt-Marsh Passerine, the Saltmarsh Sparrow (Ammodramus caudacutus)Estructura Poblacional a Escala Fina y Dispersión Asimétrica en Ammodramus caudacutus, un Paserino Habitante de Marismas. *The Auk*, *129*(2), 247–258.

CHAPTER THREE

Patterns of *Plasmodium* Infection with Blood Mercury Levels in the Saltmarsh Sparrow

(*Ammodramus caudacutus*)

Introduction

Anthropogenic activities produce environmental pollutants, including trace metals, as byproducts that can produce harmful effects in high quantities. Trace metals increase in concentration in industrialized, urbanized, and intensive agricultural areas and contaminate organisms through air, water, and food (Bichet et al. 2013). Mercury is one such trace metal commonly found in the environment due to the use of mercury in the pulp and paper industry and emissions from coal-fired power plants, of which there are many in the United States (Scheuhammer 1987, US EPA 2015). Mercury contaminants pose a particular threat in wetland habitats, as atmospheric mercury is deposited via precipitation. Once mercury precipitates into wetlands, bacteria in marsh sediments transform the inorganic mercury into its bioavailable toxic form of methylmercury, which then accumulates in organisms (Kopec et al. 2018).

Methylmercury is a potent neurotoxin that affects the central nervous, endocrine, reproductive, and immune systems and can cause permanent changes in organisms, especially vertebrates. Once ingested, it is absorbed by the gastrointestinal tract and distributed to red blood cells and plasma, which allows it to cross the blood-brain barrier (Clarkson et al. 2003). The brain is the primary target tissue, as methylmercury is preferably taken up by glial cells and can result in regional destruction of neurons in the visual cortex and cerebellar granule cells (Clarkson et al. 2003). Methylmercury is also known to suppress the immune system by inhibiting lymphocyte functions (Moszczyński 1996, Lane et al. 2011, Perkins et al. 2019). The lymphatic system is the body's first line of defense against disease, so disruption of the

lymphocytes reduces the body's ability to produce antibodies and lymphocytes that fight off infection (Swartz 2001). Lymphoid tissue depletion as well as atrophy of the thymus, spleen, and bursa were related to chronic exposure to methylmercury in juvenile great egrets (*Ardea alba*) (Spalding et al. 2000).

As exposure to heavy metals including mercury is known to impair immunological function, heavy metals have shown to impact host-pathogen dynamics (Bichet, et al. 2013). A study on the effects of mercury on the immune system of bottlenose dolphins found that high exposures to mercury led to reduced antibody concentration to common marine micro-organisms, suggesting that dolphins with high mercury exposures may be more susceptible to infectious diseases (Reif 2015). Mercury is also known to cause immune system effects in humans. Correlations between exposure to mercury and a history of malaria infections were reported in Amazonian gold mining communities exposed through fish consumption and occupational exposure (Silberged et al. 2012). Occupational exposures to mercury were associated with alterations in B lymphocytes, T-helper cells, T-suppressor cells, and T-cell proliferation (National Research Council (US) Committee on the Toxicological Effects of Methylmercury 2014). These findings led researchers to conclude that exposure to mercury increases risk for contracting infectious diseases in humans and impacts host-pathogen dynamics.

Due to biomagnification, exposure to methylmercury is especially pronounced in organisms higher on the food chain that live in wetlands. Indeed, a survey of 102 songbird species in eastern North America found mercury concentrations to be greatest in wetland breeding birds compared to birds that breed in other habitats (Jackson et al. 2015). The Saltmarsh Sparrow (*Ammodramus caudacutus*), which spends its entire lifetime in wetlands and marshlands on the Atlantic Coast of the U.S., is one such species known to have high blood

mercury concentrations. Studies have shown body mercury loads averaged 0.7 ppm in the summer and 0.4 ppm in the winter, presumably because of the dietary shift from invertebrates to greater seed consumption in the winter (Cristol et al. 2011). Long term studies by the Biodiversity Research Institute (BRI) have also found that blood mercury concentrations in the Saltmarsh Sparrow were extremely high, with the majority of birds sampled found to have blood mercury levels well above the 0.7 ppm threshold for risk of decreased reproductive success as mercury crosses the fetal membrane and concentrates selectively in the fetal brain (Wolfe et al. 1998, Lane et al. 2011, Hong et al. 2012). These results are concerning because the species has shown annual declines of 9% per year (Cristol et al. 2011). Although the Saltmarsh Sparrow has been a model system for understanding the effects of methylmercury on immune functioning and reproductive success, no studies have focused on the pathogens of this species or the effect of methylmercury exposure on host pathogen dynamics in the species.

In this study, I explored the relationship between mercury exposure and infection by a common and widespread pathogen group, the malaria parasites (genus *Plasmodium*) in the Saltmarsh Sparrow. Conservation biologists at the Biodiversity Research Institute in Portland, ME, provided 100 blood samples and blood mercury data from breeding Saltmarsh Sparrows in Long Island, New York and I screened them for malaria parasites using molecular methods. As methylmercury has immunocompromising capabilities and impairs defense against pathogens, I hypothesized that Saltmarsh Sparrows with higher blood mercury levels are more prone to infection with malaria parasites.

Methods

Field sampling and blood mercury analysis

To examine the relationship between environmental mercury exposure and malaria parasite infection, blood samples were collected from Saltmarsh Sparrows during the breeding season at eight sites along Long Island, New York by the Biological Research Institute (BRI) in 2013 and 2017. The sites included Accabonac Harbor, East Quogue, Four Sparrow Marsh, Idlewild, Marine Nature Center, North Cinder Island, North Greensedge Island, and Sawmill Creek (See Map, Figure 1). BRI conducted in-kind laboratory services for blood mercury analyses. The samples were analyzed at Texas A&M Trace Element Research Lab using direct combustion/trapping atomic absorption method on a Milestone DMA 80. The instrument detection limit was 0.05 nanograms. The total blood mercury level was recorded, 95% of which is considered to be methylmercury.

1. Accabonac Harbor
2. East Quogue
3. Marine Nature Center
4. Idlewild Park
5. North Cinder Island
6. Four Sparrow Marsh
7. North Greensedge Island
8. Sawmill Creek

Figure 1. Map of the field sites from which blood samples were collected from Saltmarsh Sparrows (*Ammodramus caudacutus*) in Long Island, New York. Each number represents a sampling site; the corresponding sites are listed on the right-hand side of the map.

Molecular screening for malaria parasite infection

The Saltmarsh Sparrow blood samples were screened for avian malaria parasite infection using sensitive nested PCR methods. DNA was extracted from each blood sample using the Qiagen DNeasy Blood and Tissue Kit following manufacturer's instructions. The outer PCR utilized primers HaemNF and HaemNR2 and the inner PCR utilized primers HaemF and HaemR2. The thermocycler conditions were 10 minutes of denaturation at 94 degrees Celsius, followed by 35 cycles at 94 degrees Celsius for 30 seconds, 52 degrees Celsius for 30 seconds, 72 degrees Celsius for 45 seconds, and then ended with an elongation step at 72 degrees Celsius for 10 minutes (Bensch et al. 2000, Waldenstrom et al. 2004). PCR products were visualized by gel electrophoresis, and a band of approximately 500 base pairs indicated a positive infection. Positive and negative controls were included with each round of nested PCRs to control for contamination and to ensure successful parasite amplification. All samples were sequenced on an ABI Prism 3130xl Genetic Analyzer at the University of Vermont's Integrative Genomics Resource Center. Sequences were then visualized and carefully edited with review of each base pair using the program 4Peaks.

Using R platform (R version 3.6.2), a logistic regression plot determined the correlation between malaria infection status and blood mercury levels. These data were fitted using a generalized linear model.

Results

The mean blood mercury level among the 100 Saltmarsh Sparrow samples from New York was 0.8645 ppm (range 0.18 ppm to 2.521 ppm). The highest blood mercury level was

found in an individual from North Cinder Island, which also had the highest mean blood mercury

level of 1.475 ppm. Idlewild had the lowest mean blood mercury level of 0.25 ppm (Table 1).

Table 1. The number of Saltmarsh Sparrows (*Ammodramus caudacutus)* sampled per state including the number of individuals deemed positive for malaria parasite (genus *Plasmodium)* infection by nested PCR and the mean blood mercury level at each site (measured in ppm).

Site	# of Individuals Sampled	# of Individuals Infected	Prevalence of Infected Individuals	Average Blood Hg (ppm)
Accabonac Harbor	14	1	7%	0.57
East Quogue	5	0	0%	0.77
Four Sparrow Marsh	10	2	20%	0.34
Idlewild	10	2	20%	0.25
Marine Nature Center	23	0	0%	0.99
North Cinder Island	17	0	0%	1.47
North Greensedge Island	7	0	0%	0.95
Sawmill Creek	14	0	0%	0.95

Five of the 100 blood samples tested positive for malaria parasites, and the blood

mercury levels of the infected individuals ranged from 0.183 ppm to 0.590 ppm. The infected

individuals were found in Accabonac Harbor, Four Sparrow Marsh, and Idlewild. The mean

blood mercury level of the infected individuals was 0.363 ppm, while the mean blood mercury

level for the uninfected individuals was 0.884 ppm. When plotted in a logistic regression the

estimated slope was -5.413, indicating a negative correlation between blood mercury levels and
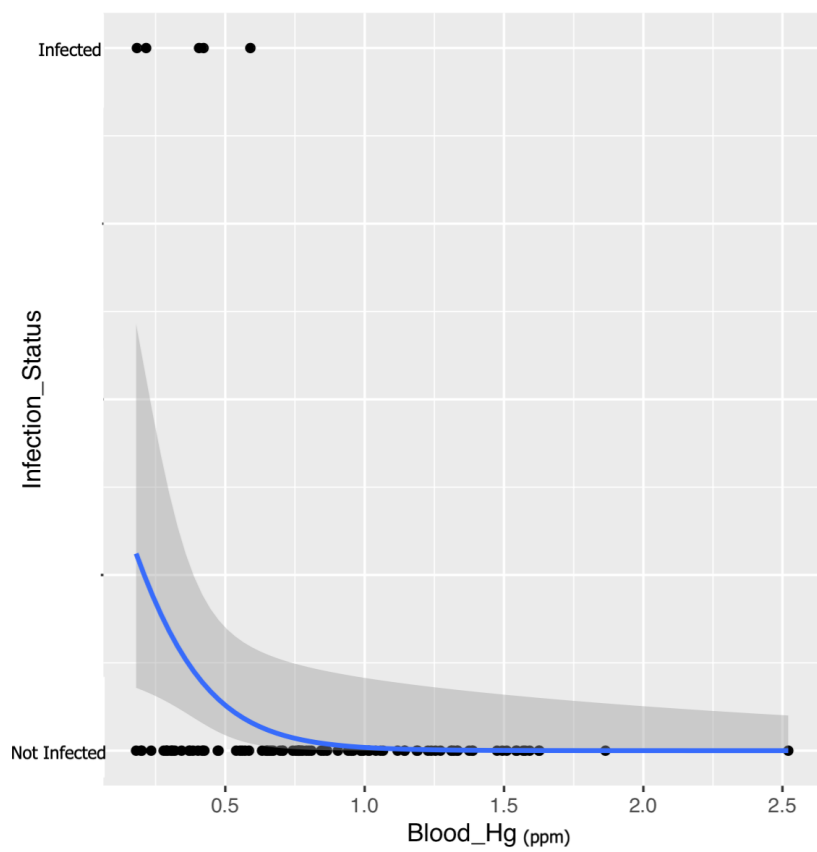
infection status ($p= 0.0325$, Figure 2).

Figure 2. Logistic regression plot in which the malaria parasite (genus *Plasmodium)* infection status (infected or not infected) of Saltmarsh Sparrows (*Ammodramus caudacutus)* is shown on the Y-axis, and the blood mercury level (measured in ppm) is shown on the X-axis.

Discussion

This study is the first to look into the relationship between infection by malaria parasites and mercury exposure in a songbird species. The Saltmarsh Sparrow makes an ideal study species as it is exposed to environmental mercury due to its insectivore foraging habits during the breeding season when it feeds on marshland invertebrates exposed to methylmercury. As a potent neurotoxin and an immunosuppressant, it is likely that mercury impacts host-pathogen dynamics as exposure negatively impacts the host's immunity to infectious diseases. The purpose of this study was to examine the immunocompromising effects of mercury exposure in a breeding bird exposed to avian malaria parasites. My previous study (Neuhaus et al.

unpublished, thesis Chapter 2) demonstrated that avian malaria parasites are present and diverse in Saltmarsh Sparrows across their breeding range.

Results from this study demonstrate a significant association between blood mercury levels and malaria parasite infection status in Saltmarsh Sparrows. Originally, I had predicted that high Saltmarsh Sparrows with high levels of blood mercury would be more prone to infection by malaria parasites because mercury is known to have immunocompromising effects on exposed individuals. However, the hypothesis that individuals with higher blood mercury levels were more likely to be infected with *Plasmodium* was not supported. The observed correlation was negative, indicating that individuals with low blood mercury levels were actually more likely to be infected with malaria parasites than those with high levels. The infected individuals had blood mercury levels ranging from 0.183 ppm to 0.590 ppm, while individuals that were not infected had much higher blood mercury levels of up to 1.475 ppm. These results were surprising and may indicate that other factors are at play.

A correlation between blood mercury levels and the presence of *Plasmodium* has not been previously documented in a wild vertebrate host, although studies in humans and lab mice have suggested that chronic exposure to low doses of mercury may increase host susceptibility to malaria infection and block acquisition of immunity (Silberged et al. 2012). A study on the effects of trace metals (zinc, lead, and cadmium) on malaria parasite infection status in the House Sparrow (*Passer domesticus)* found that chronic exposures at low concentrations affect T-cell activity, which could increase the host's susceptibility to infection (Bichet et al. 2013). Thus, it is possible that the relationship between blood mercury levels and malaria infection status varies paradoxically; the toxicity of mercury increases at low dose levels but decreases at high dose levels.

My findings are in contrast with the immunological effects of other studied trace metals, including lead, which is known to suppress the immune system at high levels and is positively correlated with *Plasmodium* prevalence in House Sparrows (Bichet et al. 2013). Mercury is known to impair immunologic function, so it is unexpected that a negative correlation was observed in this study. While high concentrations of mercury are positively correlated with neurologic effects, our results suggest low concentrations may be negatively correlated with immunologic effects (Lane et al. 2011). The threshold for decreased immune system response has not yet been determined, so further research is needed to determine the exact levels of mercury at which immune system function decreases in avian species.

The presence of mercury in the blood could have unforeseen effects on host-pathogen dynamics. While this study is purely correlational, it leaves open the possibility of additional unmeasured variables that may be involved with both infection status and mercury levels in a host. Instead of simply directly suppressing the immune system of the host, it is possible that mercury interacts with the pathogen. No research has been done on the effects of mercury directly on malaria parasites, but an interactive effect may exist directly between malaria parasites and mercury such that the parasites can only tolerate low levels of mercury in the blood of their host? Further studies are needed to examine the effects of mercury on the acquisition of malaria parasite infection, and whether high blood mercury levels actually decrease the chance of infection.

Numerous other factors, such as habitat use and type, may also affect mercury levels and parasite transmission (Bryan et al. 2015). For example, *Plasmodium* prevalence across avian host species has been documented to be positively associated with exposure to mosquito vectors (Medeiros et al. 2015). Saltmarsh sparrows live in wetland habitats that are ideal for breeding

saltmarsh mosquitos, so it would be beneficial to determine which mosquito species serve as vectors for the *Plasmodium* parasites of Saltmarsh Sparrows and if they are more or less prevalent in marshes with varying degrees of mercury contamination. In this way, the level of exposure to malaria parasite infection could be estimated. A separation of mosquito habitat and mercury contamination may help explain why lower levels of blood mercury were found in infected individuals.

Human land management practices are also likely at play in determining a host's exposure to malaria parasite infection and mercury. As mercury and other trace metals typically persist where there is more human development, there may be an increased need for mosquito control programs in areas with high levels of mercury contamination (Bichet et al. 2013). Saltmarsh areas on the south shore of Long Island are likely breeding sites for mosquitoes as high tides and heavy rains cause areas of wetlands to flood. Historically, Long Island took extreme measures to reduce the number mosquitoes in the region to prevent the spread of vector-borne infectious diseases to humans. Mosquito ditching, which allows mosquito breeding pools to drain, was a commonly used technique until negative effects on the landscape were observed in the late 1900's. Currently, Long Island regularly manually eliminates breeding sites to kill mosquito larvae and dispenses ultra-low volume insecticide sprays to kill adult mosquitoes (nassaucountyny.gov, suffolkcountyny.gov). The long-term mosquito control programs used in Suffolk County and Nassau County, which encompass all the sites used in this study, may have impacted the prevalence of malaria parasites as there are fewer mosquito vectors for transmission. This would explain the relatively low 5% prevalence rate of malaria infection found in the sampled Saltmarsh Sparrows throughout Long Island. With fewer vectors due to

mosquito control programs, malaria parasites may not be able to transmit to avian hosts to complete their life cycle.

Since the 2000's, the Environmental Protection Agency (EPA) has put multiple regulations in place to reduce mercury levels in the environment. In 2011, the standards were set to reduce mercury and other toxic air-pollution from coal- and oil-fired power plants, as powerplants are currently the dominant emitters of mercury in the United States (US EPA 2015). In addition, the Clean Air Act and the Clean Water Act were put in place to reduce the emissions of mercury from factories and the amount of mercury pollution that enters waterways (US EPA 2015). Despite these attempts to reduce the impacts of mercury on the environment, mercury still persists in the atmosphere for up to two years and in the environment as methylmercury in soils and wetlands for decades (Heinz 1996). Mercury will therefore continue to be a threat to the health of the Saltmarsh Sparrow as well as other wildlife species due to its persistence in the environment.

Literature Cited

Bensch, S., Stjernman, M., Hasselquist, D., Örjan, Ö., Hannson, B., Westerdahl, H., and Pinheiro, R. T. (2000). Host specificity in avian blood parasites: A study of Plasmodium and Haemoproteus mitochondrial DNA amplified from birds. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *267*(1452), 1583–1589.

Bichet, C., Scheifler, R., Cœurdassier, M., Julliard, R., Sorci, G., and Loiseau, C. (2013). Urbanization, Trace Metal Pollution, and Malaria Prevalence in the House Sparrow. *PLOS ONE*, *8*(1), e53866.

Bryan, A. L., Love, C. N., Mills, G. L., Borkhataria, R. R., and Lance, S. L. (2015). Testing for associations between hematozoa infection and mercury in wading bird nestlings. *Journal of Wildlife Diseases*, *51*(1), 222–226.

Clark, N. J., Clegg, S. M., and Lima, M. R. (2014). A review of global diversity in avian haemosporidians (Plasmodium and Haemoproteus: Haemosporida): new insights from molecular data. *International Journal for Parasitology*, *44*(5), 329–338.

Cristol, D. A., Smith, F. M., Varian-Ramos, C. W., and Watts, B. D. (2011). Mercury levels of Nelson's and saltmarsh sparrows at wintering grounds in Virginia, USA. *Ecotoxicology*, *20*(8), 1773–1779.

Heinz, G. H. (1996). *Mercury poisoning in wildlife*. 118–127.

Hong, Y.-S., Kim, Y.-M., and Lee, K.-E. (2012). Methylmercury Exposure and Health Effects. *Journal of Preventive Medicine and Public Health*, *45*(6), 353–363.

Jackson, A. K., Evers, D. C., Adams, E. M., Cristol, D. A., Eagles-Smith, C., Edmonds, S. T., Gray, C. E., Hoskins, B., Lane, O. P., Sauer, A., and Tear, T. (2015). Songbirds as sentinels of mercury in terrestrial habitats of eastern North America. *Ecotoxicology (London, England)*, *24*(2), 453–467.

Kopec, A. D., Bodaly, R. A., Lane, O. P., Evers, D. C., Leppold, A. J., and Mittelhauser, G. H. (2018). Elevated mercury in blood and feathers of breeding marsh birds along the contaminated lower Penobscot River, Maine, USA. *The Science of the Total Environment*, *634*, 1563–1579.

Lane, O. P., O'Brien, K. M., Evers, D. C., Hodgman, T. P., Major, A., Pau, N., Ducey, M. J., Taylor, R., and Perry, D. (2011). Mercury in breeding saltmarsh sparrows (Ammodramus caudacutus caudacutus). *Ecotoxicology*, *20*(8), 1984.

Medeiros, M. C. I., Ricklefs, R. E., Brawn, J. D., and Hamer, G. L. (2015). *Plasmodium* prevalence across avian host species is positively associated with exposure to mosquito vectors. *Parasitology*, *142*(13), 1612–1620.

Mosquito Control | Nassau County, NY - Official Website. (n.d.). Retrieved April 20, 2020, from https://www.nassaucountyny.gov/1872/Mosquito-Control

Moszczyński, P., and Moszczyński, P. (1995). Exposure to mercury and population health. I. Immunotoxicity of mercury. *Medycyna Pracy*, *46*(4), 385–393.

National Research Council (US) Committee on the Toxicological Effects of Methylmercury. (2014). *Toxicological Effects of Methylmercury*. National Academies Press (US).

Perkins, M., Lane, O. P., Evers, D. C., Sauer, A., Adams, E. M., O'Driscoll, N. J., Edmunds, S. T., Jackson, A. K., Hagelin, J. C., Trimble, J., and Sunderland, E. M. (2019). Historical patterns in mercury exposure for North American songbirds. *Ecotoxicology (London, England)*.

Prasad, A. S. (2008). Zinc in Human Health: Effect of Zinc on Immune Cells. *Molecular Medicine*, *14*(5–6), 353–357.

Reif, J. S., Schaefer, A. M., and Bossart, G. D. (2015). Atlantic Bottlenose Dolphins (Tursiops truncatus) as A Sentinel for Exposure to Mercury in Humans: Closing the Loop. *Veterinary Sciences*, *2*(4), 407–422.

Silbergeld, E. K., Nash, D., Trevant, C., Strickland, G. T., Souza, J. M. de, and Silva, R. S. U. da. (2002). Mercury exposure and malaria prevalence among gold miners in Pará, Brazil. *Revista Da Sociedade Brasileira de Medicina Tropical*, *35*(5), 421–429.

Spalding, M. G., Frederick, P. C., McGill, H. C., Bouton, S. N., Richey, L. J., Schumacher, I. M., Blackmore, C. G. M., and Harrison, J. (2000). Histologic, neurologic, and immunologic effects of methylmercury in captive great egrets. *Journal of Wildlife Diseases*, *36*(3), 423–435.

Suffolk County Vector Control 2020 Plan of Work.pdf. (n.d.). Retrieved April 17, 2020, from https://www.suffolkcountyny.gov/portals/0/formsdocs/publicworks/pdf/Suffolk%20County%20Vector%20Control%202020%20Plan%20of%20Work.pdf

Swartz, M. A. (2001). The physiology of the lymphatic system. *Advanced Drug Delivery Reviews*, *50*(1), 3–20.

US EPA, OAR. (2015, November 9). *Cleaner Power Plants* [Overviews and Factsheets]. US EPA. https://www.epa.gov/mats/cleaner-power-plants

US EPA, OCSPP. (2015, September 15). *Environmental Laws that Apply to Mercury* [Overviews and Factsheets]. US EPA. https://www.epa.gov/mercury/environmental-laws-apply-mercury

Waldenström, J., Bensch, S., Hasselquist, D., and Ostman, O. (2004). A new nested polymerase chain reaction method very efficient in detecting Plasmodium and Haemoproteus infections from avian blood. *The Journal of Parasitology*, *90*(1), 191–194.

Wolfe, M. F., Schwarzbach, S., and Sulaiman, R. A. (1998). Effects of mercury on wildlife: A comprehensive review. *Environmental Toxicology and Chemistry*, *17*(2), 146–160.

CHAPTER FOUR

Conclusions

Wildlife species face multiple challenges, including habitat destruction, environmental degradation, pathogens, and climate change. These factors are not independent and may interact to cause species declines. The Saltmarsh Sparrow is a species of conservation concern due to the recent declines in population size, so it is important to assess the factors that threaten the species in order to maintain the current populations. Rises in sea level and human development are known to have direct negative impacts on the breeding habitat of the species. Other factors, such as infectious disease and environmental contamination, are predicted to pose additional threats. Despite the increasing rate at which human activity impacts natural habitats, the role of environmental pollution in the dynamics of infectious diseases has not been thoroughly studied. The purpose of this study was to examine the prevalence, diversity, and distribution of malaria parasites in the Saltmarsh Sparrow and to elucidate the role of mercury on the susceptibility to malarial infection in hopes to contribute to the conservation of this species as well as other wildlife species that occupy similar wetland habitats.

For the first time, the malaria parasites of Saltmarsh Sparrows were surveyed to determine the presence and distribution of *Plasmodium* lineages throughout the breeding range of the species. According to the MalAvi database, there are currently 1,851 unique avian host species infected by malaria parasites. As there are many avian species affected by malaria parasites, it was likely that Saltmarsh Sparrows would also be infected since they occupy a wetland habitat conducive to the breeding of mosquito vectors. The distribution of infected individuals was somewhat unpredicted, as sites in the southern portion of the breeding range had fewer infected individuals and a fewer number of parasite lineages present. Mosquito control

programs may likely be a major limiting factor in the geographic distribution of malaria parasites. New York and Delaware regularly use insecticides and larvicides to kill mosquitoes, which may explain why such a small number of Saltmarsh Sparrows were found to be infected in these states. More lineages were also discovered in the northern states, which is likely due to the fact that there were more infected individuals. The distribution of lineages seems to align with the genetically distinct groups of the Saltmarsh Sparrow documented in a previous population genetic study (Walsh et al. 2012).

Songbirds serve as sentinels of mercury in terrestrial habitats of eastern North America as they are abundant and can be effectively sampled (Jackson et al. 2015). Saltmarsh Sparrows are an especially good indicator species of environmental contamination because they feed on invertebrates in wetlands and are vulnerable to bioaccumulation of pollutants. The species is continually monitored by BRI for the effects of methylmercury, but the link between mercury exposure and susceptibility to infection by blood parasites has not been previously studied. The results of this study demonstrated an unexpected negative correlation between the blood mercury levels of Saltmarsh Sparrows and malaria infection status. Mercury is generally reported to depress the immune response, as the effects on the immune system of wildlife species have been demonstrated both *in vitro* and *in vivo*. Exact mechanisms are unknown, but mercury exposure has been shown to disrupt lymphocyte function, which is important in producing antibodies to fight off infections (Spalding et al. 2000). The negative correlation found between mercury and infection status suggests that lymphocytes and other immune functions may be affected only when blood mercury concentrations are low. While it is tempting to conclude that mercury contamination alters the immune system and increases an organism's susceptibility to disease, there are other factors including habitat use and temperature that may be contributing to the

pattern. Thus, the paradoxical relationship warrants further research to examine the role of low concentrations of mercury on host-parasite dynamics.

With climate change, more infectious diseases are emerging, and host-pathogen dynamics will be greatly affected. The results from this study are useful for highlighting the effects of environmental conditions on the health of wildlife species. More specifically, the interactions between environmental contaminants and infectious diseases are important to monitor as climate change is likely to have a large impact in the near future. Avian malaria parasites serve as an ideal natural model system for studying the mechanisms of host-parasite interactions, including the immunological effects during infections, the ecology and evolution of host-parasite associations, and patterns of mosquito transmission with a warming climate (Valkiunas et al. 2018). The ultimate goal of this study is to draw attention to the many factors that threaten Saltmarsh Sparrows and other wildlife species in similar habitats. Given the negative impacts of mercury and malaria parasites on the health of avian species, I hope that additional conservation measures are taken in the future to protect wildlife species, including the Saltmarsh Sparrow, and their habitats.

Acknowledgements