**You are what you eat and where you live:**

**how territory size and diet relate to mercury concentration in riparian songbirds**

by

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# Abstract

Mercury (Hg) is a pollutant that has become a large threat to many ecosystems and the organisms that live within them. Mercury is a known neurotoxin that can cause extensive central nervous system damage, which can impact the behavior and survival of many species. Inorganic mercury is often deposited into the atmosphere by anthropocentric sources and can easily enter water sources through precipitation. These inorganic forms of mercury are then methylated by bacteria in anoxic environments, creating an organic form of mercury called methylmercury (MeHg). Methylmercury accumulates in individuals and easily moves up through the trophic levels. As a result, the bioavailability of methylmercury in anoxic environments becomes a pressing issue within aquatic food chains. Riparian songbirds, due to their mixed diet and the fact that they occupy both aquatic and terrestrial habitats, are often used to monitor methylmercury availability within these environments, but very little is known about how territory location can influence Hg exposure. In June and July 2018, we studied riparian songbirds at two sites in Acadia National Park: Gilmore Marsh near Aunt Betty Pond on Mount Desert Island and an unnamed beaver pond on the Schoodic Peninsula. The goal of this study was to relate habitat usage and diet to blood mercury concentrations in four riparian songbirds, the Common Yellowthroat Warbler, White-throated Sparrow, Song Sparrow, and Swamp Sparrow. We found that different species vary in their mercury levels and individuals of the same species vary in their mercury levels between sites, although these differences were not statistically significant. We also found that territory sizes differed for individuals at each site, which may be due to individual preferences, food availability, and competition. Diet analyses also showed that riparian songbirds are eating emergent aquatic insects, which may aid to the biomagnification of methylmercury within the food web. However, the results demonstrate that further research is necessary to determine relationships between habitat usage, diet, and mercury in riparian songbirds.

# Introduction

Mercury (Hg) is a pervasive pollutant that has become a large threat to many ecosystems and the organisms that live within them (Scheuhammer et al. 2007; Wolfe et al. 1998). Emissions of elemental and inorganic mercury into the air can occur naturally, through the weathering of Hg-bearing minerals, volcanic eruptions, and geological deposits, or anthropogenically, through mining, waste incineration, forest fires, and coal combustion (Driscoll et al., 2013; Rasmussen 1994). The atmosphere is a major pathway of mercury transportation across local, regional, and global scales. The long-range transport of elemental mercury, due to its long atmospheric residence time (up to a year), can allow for the transfer of mercury into locations that are not associated with local point sources (Driscoll et al., 2013; Wilson et al., 2006). While in the atmosphere, elemental mercury undergoes processes that transform it into inorganic mercury. These inorganic forms of mercury are then deposited into terrestrial and aquatic environments through the process of precipitation (Driscoll et al., 2013). Mercury, which doesn't break down over time, accumulates in these environments.

Mercury becomes methylated by bacteria in anoxic, aquatic environments (Gochfeld 2003; Morel et al., 1998). Methylated mercury, known as methylmercury (MeHg), is a bioavailable form of Hg and can be absorbed into an organism's body through ingestion. The bioavailability of methylmercury becomes a pressing issue within aquatic food chains because, like many contaminants, methylmercury increases in individuals over time. Mercury also readily biomagnifies within the food web as it travels from lower tier organisms to top-tier predators that feed on primary and secondary prey items in both aquatic and terrestrial environments. This poses a threat to all organisms within the food web because mercury is a known neurotoxin that is able to penetrate the blood-brain barrier, causing extensive central nervous system damage, which can impact the behavior and survival of many species (Hartman et al., 2013; Morel et al., 1998; Wolfe et al., 2009).

The negative effects of mercury have been well-documented in wildlife (Scheuhammer et al. 2007; Wolfe et al. 1998), but there has been a heavy concentration on utilizing piscivorous birds as indicators of mercury in aquatic environments (Jackson et al., 2017). Piscivorous birds are considered top-predators in many aquatic ecosystems, which puts them at high-risk for mercury exposure (Burgess and Meyer, 2007). Methylmercury, because it crosses the blood-brain barrier, can produce brain lesions, spinal cord degradation, and central nervous system dysfunctions in birds (Wolfe et al., 1998). Even acute mercury exposure can result in reduced food intake, difficulty flying, reduced hatchability, eggshell thinning, reduced clutch size, and atypical behavior of juveniles. Although research has focused on piscivorous birds, recent research has suggested that songbirds (order Passeriformes), may be as susceptible to the negative effects of methylmercury as piscivorous birds (Evers et al., 2005).

Riparian songbirds living in areas adjacent to aquatic ecosystems are introduced to mercury through emergent aquatic prey and predatory invertebrates, such as spiders (Iwata et al., 2003; Cristol et al., 2008). Emergent aquatic insects live in the water for a majority of their lives and emerge onto land as winged adults. These insects are important in the transfer of aquatic contaminants, from aquatic to terrestrial systems (Walters et al., 2008). Predatory invertebrates, such as spiders, can also eat emergent aquatic prey, resulting in the biomagnification of mercury in the food web. Prior research has focused on top aquatic invertebrate predators, i.e., dragonflies (Eagles-Smith et al., 2018). However, little is known about the extent to which these insects contribute to songbird diet, especially because the diet of each bird species can vary greatly due to species-specific foraging behavior, site selection, and time of year.

Relatively little is known about riparian songbird territories, but we do know that site selection and habitat usage varies among birds, and even between individuals within the same species. This may be due to individual preferences in vegetation, food availability, and interspecific and intraspecific competition (Cody, 1981; Tinbergen, 1957). There is also the added factor of time of year. For example, territories only used for mating are expected to be smaller than territories utilized for breeding (Schoener, 1968). This difference in territory size can be explained in differences in activities performed during this period. For breeding, bird pairs would need a larger area to supply food for their young. These assumptions come from foundation research conducted in the mid-to-late 1900s, therefore, it is important that further research is conducted to determine how exactly individual preferences, food availability, and competition can influence site selection, habitat usage, and the diet of riparian songbirds.

This study aims to relate habitat usage and diet to blood mercury concentrations in riparian songbirds. The objectives of this study are to 1) relate average blood mercury in riparian birds to prior research conducted on aquatic predator invertebrates, 2) compare blood mercury concentrations between species, 3) determine if the territories of individuals of species vary in their distance to the water, 4) relate bird mass and territory size to blood mercury concentrations, and 5) analyze the diet of select riparian songbirds to determine if they are eating emergent aquatic insects.

# Materials and Methods

### Study Sites

Areas with no local sources of mercury contamination, such as Acadia National Park, Maine, may be exposed to mercury as a result of long-range transport in the atmosphere. Although National Parks are protected areas, recent research conducted in Acadia National Park has concluded there to be elevated levels of mercury in sediment, streams, fish, amphibians, and other wildlife (Bank et al. 2007a, b; Longcore et al. 2007a, b).

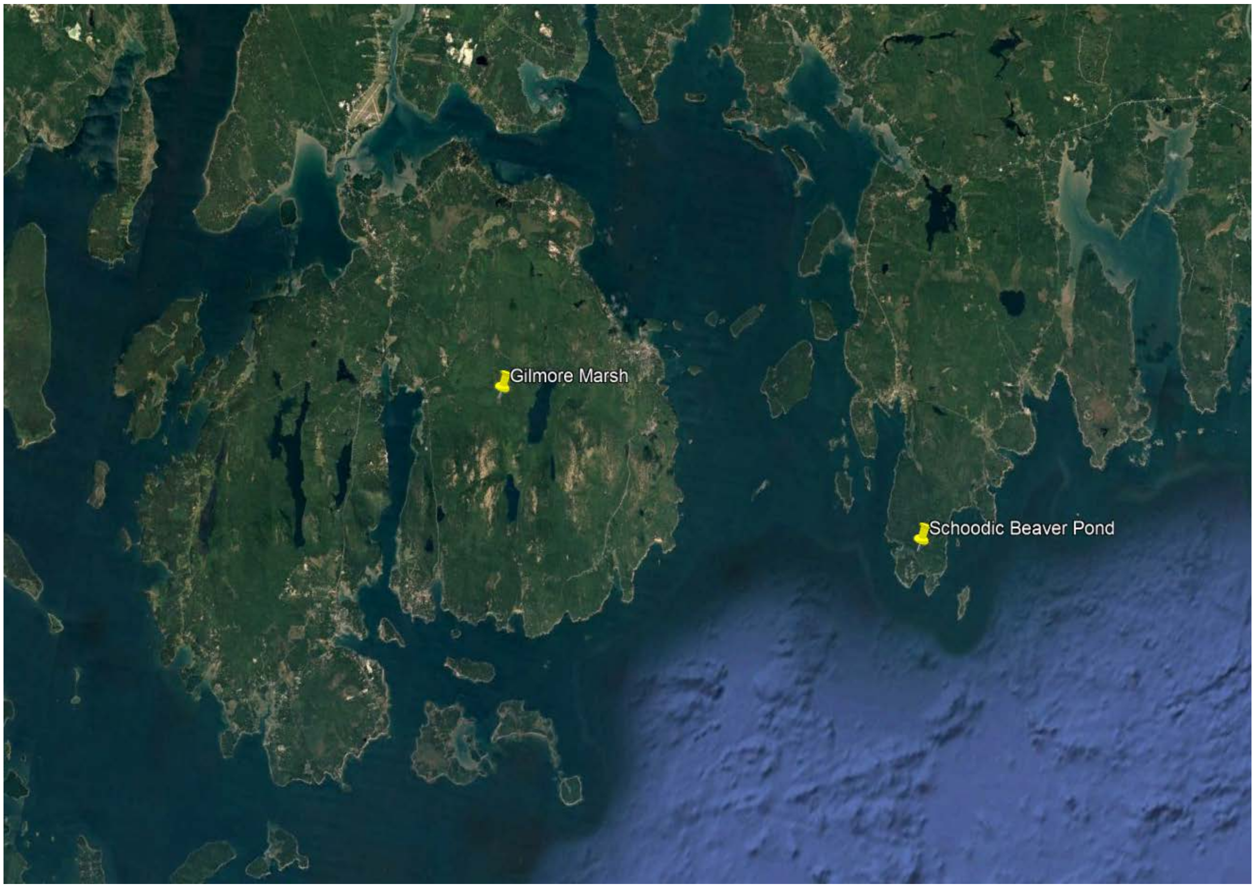


Figure 2. Map of two study sites in relation to each other in Acadia National Park, Maine.

Samples were collected at two sites in Acadia National Park, Maine (Figure 1). Schoodic Beaver Pond (44.344452, -68.058775), located on the Schoodic Peninsula, is a beaver pond complex known for high levels of mercury in dragonfly larvae (Eagle-Smith et al., 2018). The concentrations of mercury found in dragonfly larvae were based on a nationwide initiative, called the Dragonfly Mercury Project, that utilizes the help of citizen scientists to collect dragonfly larvae for mercury analysis (Figure 2). Schoodic Beaver Pond is also located near the site of a former Navy Base, the Naval Security Group Activity, Winter Harbor, that closed down in 2002 ("The Navy at Schoodic Point", n.d.). However, no references could be found that indicate that this site contributed to the levels of mercury seen throughout Acadia National Park. Gilmore Marsh (44.364539, -68.272303), located on Mount Desert Island, is a large wetland complex that flows into a nearby pond, called Aunt Betty's Pond.

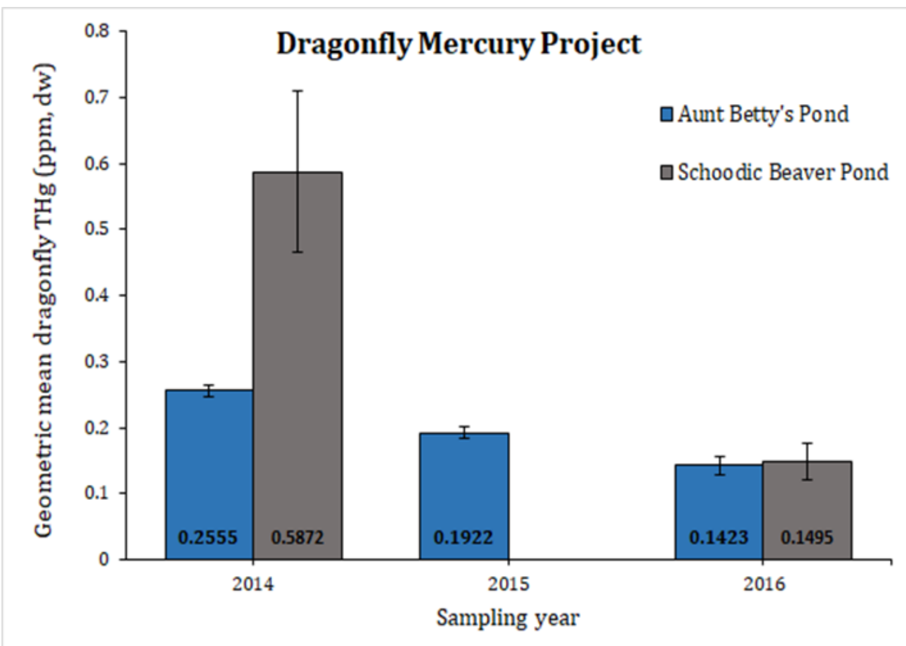


Figure 2. The geometric mean of dragonfly total mercury (ppm, dw) at Aunt Betty's Pond and Schoodic Beaver Pond between 2014 and 2016.

**Blood and Fecal Sampling Regime**

During the months of June and July 2018, riparian songbirds were captured using mist nets by utilizing playback recordings for conspecific songs. When captured, these birds were placed in sterilized cloth bags and kept in the bags until they defecated. Fecal samples were collected, stored in 95% ethanol, and kept cold in the field until it could be placed in the freezer at -20°F for long term storage. Blood samples of each bird were taken from the brachial ulnar vein, using 27 gauge needles (BD PrecisionGlide, Fisher Scientific), stored in heparinized microhematocrit capillary tubes (Fisherbrand, Fisher Scientific), capped with Critocaps ™ (Leica Microsystems), and kept cold in the field until it could be placed in the freezer for long term storage. Both blood and fecal samples were stored in a freezer within six hours of sampling. Morphometric measurements of unflattened wing length, tail length, tarsus length, bill (nares to tip), and bird mass were recorded as well. All samples were collected under authority of appropriate scientific collection permits, including USGS master bander permit to Allyson Jackson #24142, National Park Service Scientific Research and Collecting Permit #ACAD-2018-SCI-0018, State of Maine Department of Inland Fisheries and Wildlife, Wildlife Scientific Collection Permit 2018-536, and Purchase College Institutional Animal Care and Use Permit #2018-AJ01. Blood samples were run on a Nippon MA-3000 Hg Analyzer (Nippon Instruments, College Station, Texas, USA) at Weber State University.

Although multiple bird species were captured, this study focuses on four species of birds: Common Yellowthroat Warbler (*Geothlypis trichas*), Song Sparrow (*Melospiza melodia*), White-throated Sparrow (*Zonotrichia albicollis*), and Swamp Sparrow (*Melospiza georgiana*).

**GIS Data Analysis: Territory Map**

Two territory maps were produced for these two sites in 2019 using ArcMap 10.4.1. The Esri Imagery Basemap, with aerial photography from Esri, DigitalGlobe, Geoeye, Earthstar Geographics, CNE/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community, was used to delineate the outline of both Schoodic Beaver Pond and Gilmore Marsh field sites and the water bodies at each site. GPS coordinates of the four target species were collected in the months of June and July 2018. Coordinate points were taken anytime a male of one of these species was seen foraging or heard singing. The GPS coordinates were imported into ArcMap and projected into the WGS 1984 World Mercator Auxiliary Sphere. The recorded points served as the bounds of each territory and were used to create polygons depicting the observed territories of these species. The geoprocessing workflow was conducted as follows:

- Buffer functions were used to create 20 meter buffers around the waterbody polygons of each site. Buffers went up to 120 meters at Schoodic Beaver Pond and up to 140 meters at Gilmore Marsh.
- Union functions were used to combine all of the buffers at each site.
- Territory polygons were then intersected with the union buffer layers.
- The territory polygon was then unioned with the buffer layer to determine the proportion of each habitat within each 10 meter buffered distance from the waterbody.
- These proportions were recorded as percentages of the field site polygon and used in the analysis.

**DNA Extraction and PCR**

Ethanol was removed from fecal samples using an Eppendorf vacufuge on V-AL mode. DNA was extracted from a single fecal pellet weighing 0.0092-0.1118 g (average weight = 0.0412 g) with the DNA Stool Mini Kit (Qiagen #51604), following the manufacturer's instructions (with the modifications for ethanol preservation proposed by Zeale et al. 2011). DNA yields were consistently low, so we performed remaining extractions using the Quick DNA Fecal/Soil Microbe Microprep Kit (Zymo #D6012), following the manufacturers protocol. DNA was quantified using a Qubit 4 Fluorometer, with the 1X dsDNA HS Assay Kit (Thermo Fisher Scientific), and stored in a freezer at -20°C until ready for PCR.

We performed a 5x dilution on extracted DNA samples to remove inhibitors and then pooled samples by site. The pooled samples were amplified by PCR with AmpliTaq GOLD 360 Master Mix (Thermo Fisher Scientific). The primers mlCOIintF (GGWACWGGWTGAACWGTWTAYCCYCC, Leray et al., 2013) and jgHCO2198 (TAIACYTCIGGRTGICCRAARAAYCA, Geller et al., 2013) were used to amplify a 333 bp section of the COI mitochondrial DNA barcoding region. Each 25 µl total volume PCR reaction contained 12.5 µl of master mix, 1 µl of forward primer, 1 µl of reverse primer, and a combined total of 10.5 µl of DNA template and distilled $H_2O$. PCR cycling was performed by initial denaturation at 95°C for 20 s, 38 cycles of 95°C for 30 s, 50°C for 30 s, and 70°C for one minute, followed by a final extension of 72°C for 7 minutes. PCR efficiency was visualized using gel electrophoresis on 2% agarose gel.

**Metagenomic Sequencing**

Sequencing was performed on April 1st, 2019 using a MinION (Oxford Nanopore Technologies) and R9.4 Chemistry. Pooled PCR products from both Qiagen and Zymo kits were chosen for sequencing because individual PCR products yielded no visible bands. Amplified DNA from each site was barcoded using the PCR Barcoding Kit (SQK-PBK004), following the manufacturers protocol. The two samples were combined in equimolar amounts and run on one flow cell (Flow cell #106). The flow cell had 865 active pores.

**Bioinformatics**

After MinION sequencing, basecalling and demultiplexing were performed using Guppy v2.3.7. Barcodes and adapters were trimmed using default parameters in Porechop v0.2.3_seqan2.1.1 (https://github.com/rrwick/Porechop/) and reads with a quality less than 6 and minimum sequence length less than 100 were filtered with Nanofilt v2.2.0 (https://github.com/wdecoster/nanofilt). A BLAST database was created from COI nucleotide sequences downloaded from the National Center for Biotechnology Information (NCBI). We used BLAST to align sequences to the COI database and imported the BLAST results into MEGAN6 (MEtaGenome ANalyzer). We filtered results in MEGAN using the weighted LCA algorithm in order to assign sequence reads to specific taxon levels. The following MEGAN LCA parameters were used: Min Score = 50.0, Max Expected = 1.0E-20, Min Percent Identity = 85.0, Top Percent = 10, Min Support Percent = 0 (off), Min Support = 1000, Min Complexity = 0.0, Percent to cover = 80.0, and Read Assignment Mode = readCount. Functional analyses were completed using eggNOG and InterPro2GO. The sequences assigned to species level taxa were aligned in MEGAN and the consensuses were exported. These consensus sequences were compared to reference sequences in the NCBI nucleotide Web BLAST database in order to exclude false positives. Reads in MEGAN were extracted for taxa that was lower than species level and blasted in Geneious Prime v2019.1.1 against the NCBI NT database. A Geneious Multiple Sequence Alignment was performed if there were four or more reads blasted to the same species. A consensus sequence was built and reblasted against the NCBI NT database in Geneious. In MEGAN and Geneious, the species were considered present if the pairwise identity was equal to or greater than 97%, the EVALUE was equal to or greater E-20, and the sequence length was equal to or greater than 300 bp. Diet taxa were considered either absent or present in the dataset based on these parameters. Taxonomic IDs in MEGAN with more than 1000 reads were considered high confidence hits and taxonomic IDs in Geneious with less than 1000 reads were considered mid-confidence hits.

**Statistical Analyses**

All statistical analyses were conducted using Excel (v16.0.4738.1000, 64-bit) and R statistical software (version 3.5.2). Area in $m^2$ of each territory was calculated using ArcGIS ArcMap 10.4.1. To convert area ($m^2$) to acres, each value was divided by 4046.856 and to convert acres to hectares (ha), each value was divided by 2.471 (Table 1 and Table 2). To calculate portion of area within the riparian zone, the portion of each individual territory within the water buffer and 20 m buffer was combined and compared with the total area for that respective polygon. The average area of observed territories were calculated by combining the individuals of the same species at each site (Figure 4). Similarly, the average blood mercury concentrations per species was calculated by combining all individuals for that species at each site (Figure 7). An unpaired t-test was used to determine site differences in mercury concentrations. A linear regression was used to determine the relationship between mercury concentrations of individual songbirds by species and the percent of territory in the riparian zone (Figure 14). Mercury concentrations for the following comparisons were $\log_{10}$-transformed: mercury concentration and bird mass, within-species site differences in mercury concentrations for Song Sparrows and Common Yellowthroat Warblers, and mercury concentration and territory size ($m^2$). We then used linear regression to determine the relationships between mercury concentration and bird mass, number of observations and territory size, and mercury concentration and territory size ($m^2$). Within-species site differences for Song Sparrows and Common Yellowthroat Warblers were tested using an unpaired *t*-test. We compared among-species differences in mercury concentrations using ANOVA and Tukey's post hoc tests. The significance level for all statistical analyses was set at alpha = 0.05.

# Results

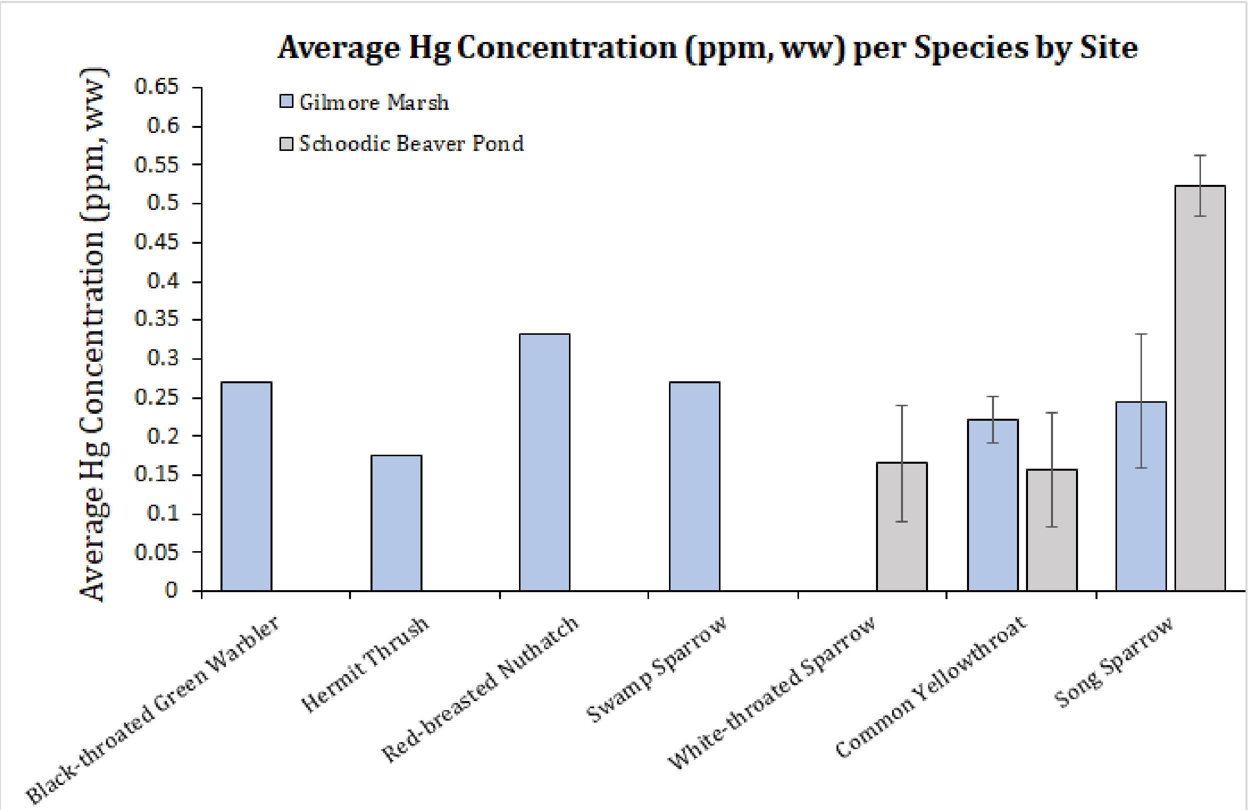


Figure 3. Average blood mercury (Hg) concentration for each individual captured using mist-nets at both Schoodic Beaver Pond and Gilmore Marsh. Standard error bars are also given.

Although Song Sparrows (*Melospiza melodia*), Common Yellowthroats (*Geothlypis trichas*), Swamp Sparrows (*Melospiza georgiana*), and White-throated Sparrows (*Zonotrichia albicollis*) were the target species for this study, other species were also captured in mist-nets and had their blood samples analyzed. From Gilmore Marsh, a total of 8 individuals were sampled for blood mercury: 3 Common Yellowthroat Warblers, 1 Black-throated Green Warbler (*Dendroica virens*), 1 Hermit Thrush (*Catharus guttatus*), 1 Red-breasted Nuthatch (*Sitta canadensis*), 1 Swamp Sparrow, and 2 Song Sparrows (Figure 3). From Schoodic Beaver Pond, a total of 7 individuals were sampled for blood mercury: 3 Common Yellowthroat Warblers, 2 Song Sparrows, and 3 White-throated Sparrows (Figure 3).

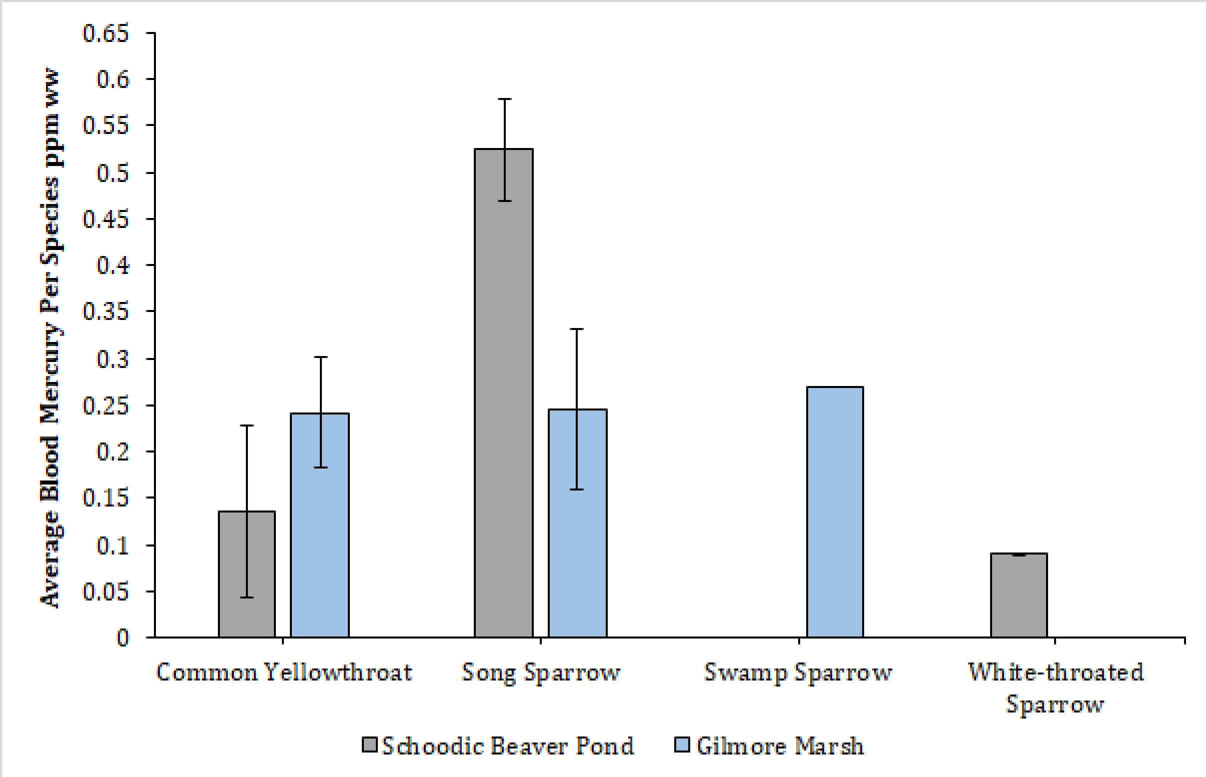


Figure 4. Average blood mercury concentration (ppm ww) per species separated by site. Standard errors are also given.

There were no significant differences in mercury (Hg) concentration (ppm,ww) between Common Yellowthroat Warblers at Gilmore Marsh and Schoodic Beaver Pond (unpaired t-test, $t$= 1.1354, $p$= 0.3593). Similarly, although Song Sparrow blood mercury averages differed between Gilmore Marsh and Schoodic Beaver Pond, this difference was not significant (unpaired t-test, $t$= -2.20, $p$= 0.26). A one-way ANOVA was conducted to compare the average blood mercury concentrations between the four target species, but there was no significant difference found [$F_{(3, 9)} = 1.524$, $p = 0.274$].

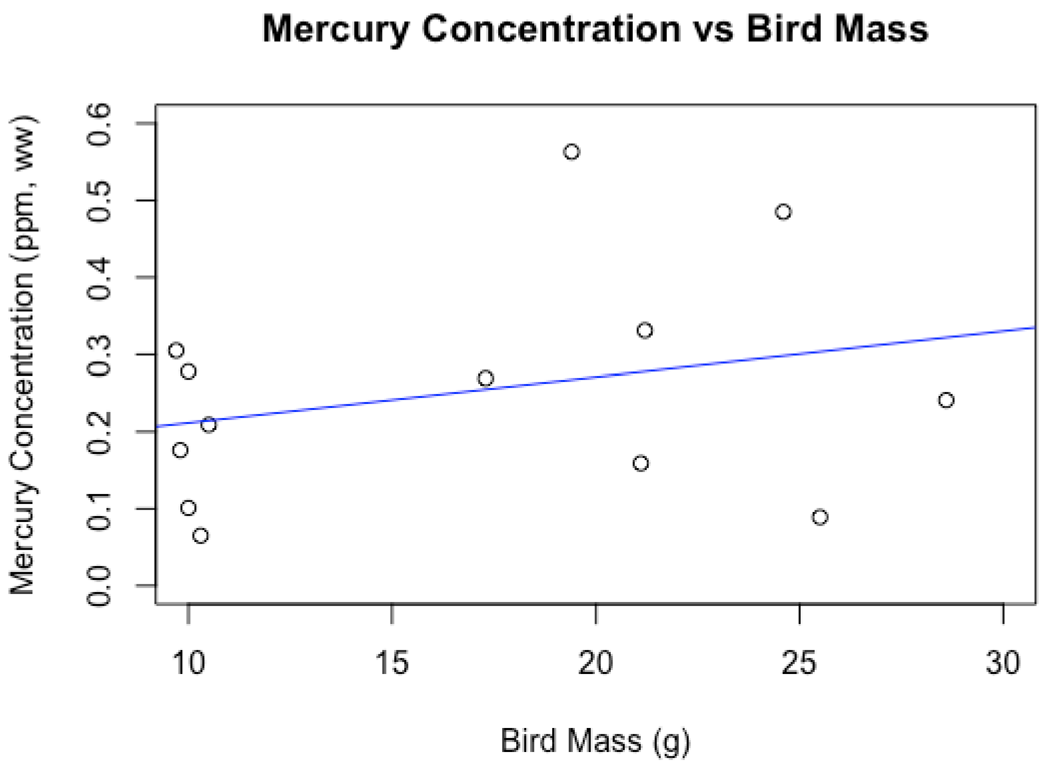


Figure 5. Blood mercury (Hg) concentration (ppm, ww) versus bird mass (g) of each individual at both sites. The line in blue shows a linear regression ($r^2$ = 0.062, $p$=0.41).

The linear regression of $\log_{10}$ blood mercury concentration on bird mass (g) was not significant ($r^2$ = 0.06, p=0.41).

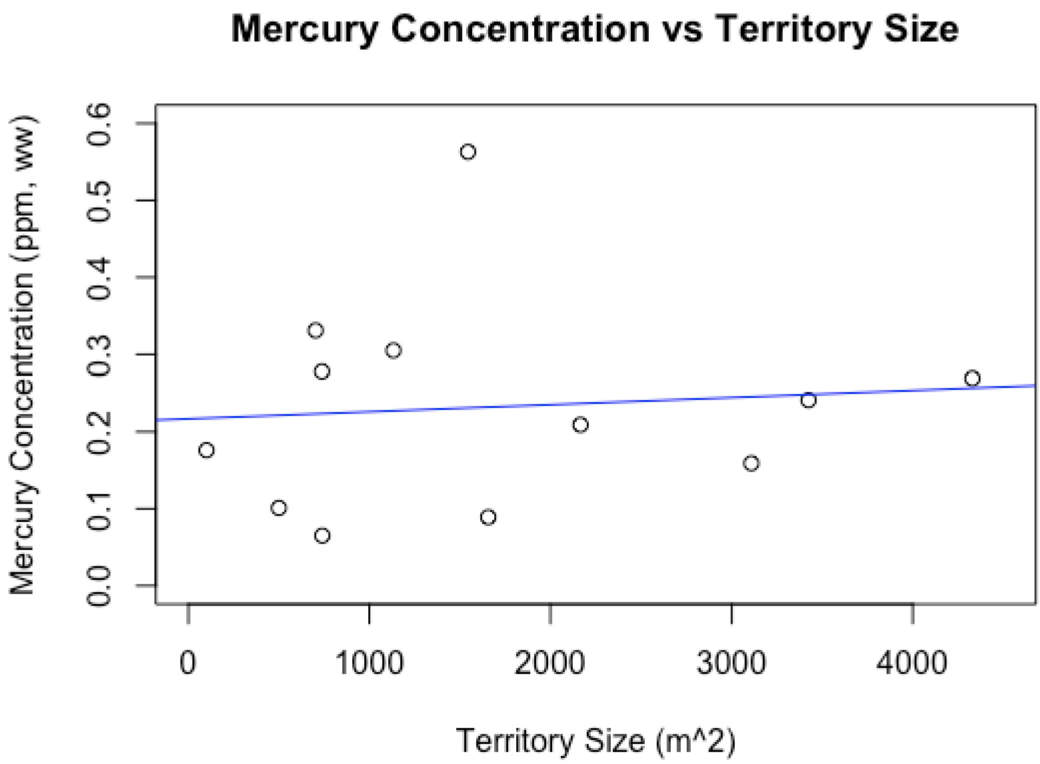


Figure 6. Blood mercury (Hg) concentration (ppm, ww) versus territory size ($m^2$) of each individual at both sites. The line in blue shows a linear regression ($r^2$= 0.04, *p*= 0.55).

The linear regression of $\log_{10}$ blood mercury on territory size ($m^2$) was not significant ($r^2$= 0.04, p= 0.55).

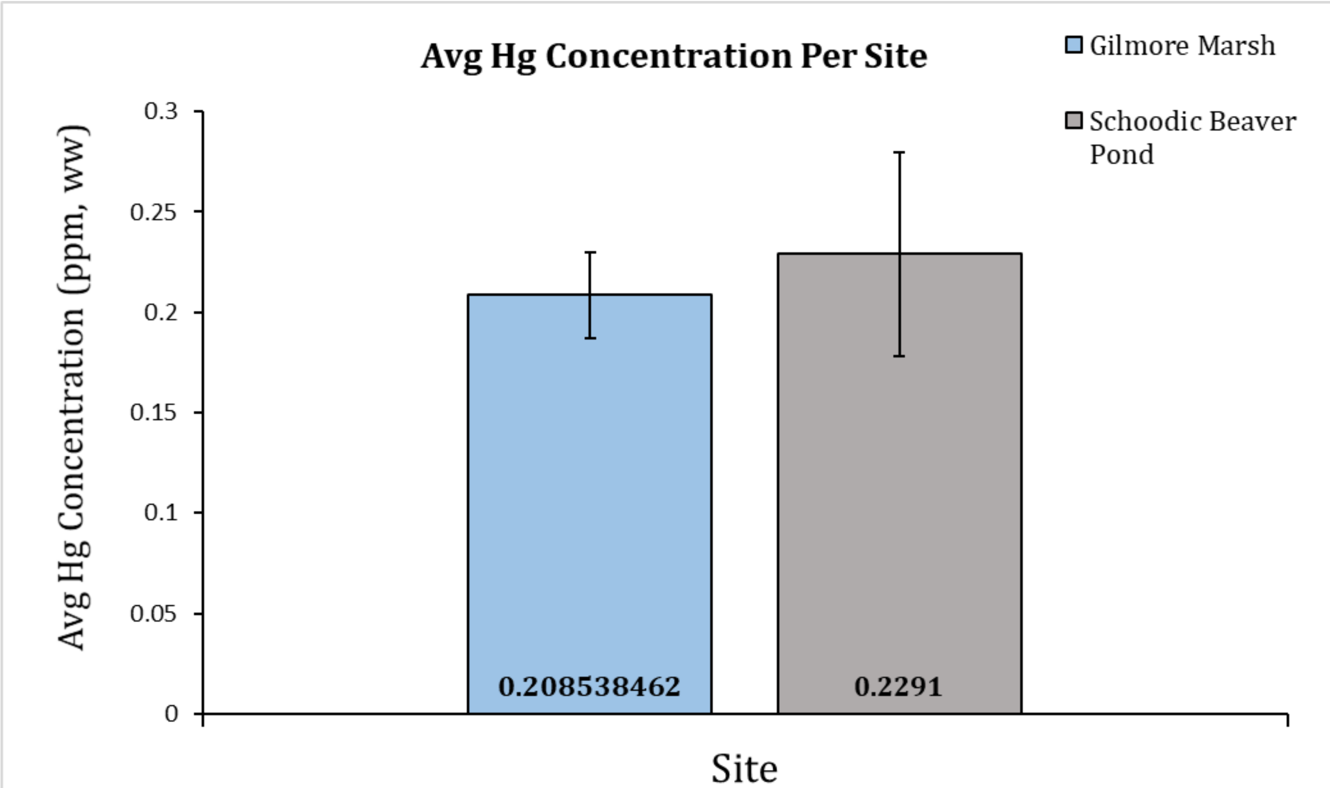


Figure 7. Average blood mercury (Hg) concentration (ppm, ww) of both sites (includes all individuals sampled). Standard error bars were also given.

Average mercury (Hg) concentrations (ppm, ww) of riparian songbirds differed slightly between Gilmore Marsh and Schoodic Beaver Pond (Figure 7), but this difference was not significant (unpaired t-test, $t$= -0.19, $p$= 0.85).

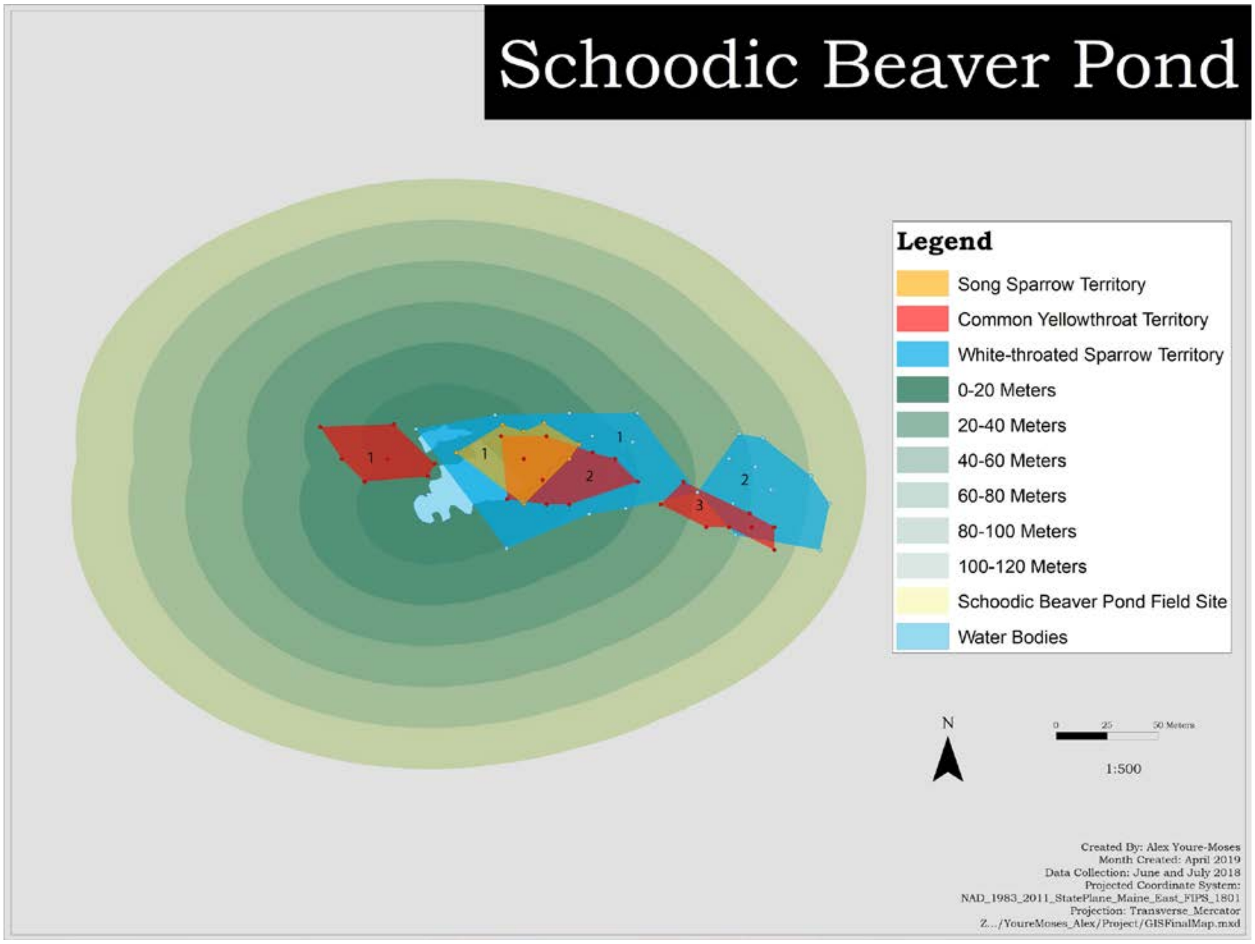


Figure 8. Observed territories of individuals at Schoodic Beaver Pond.

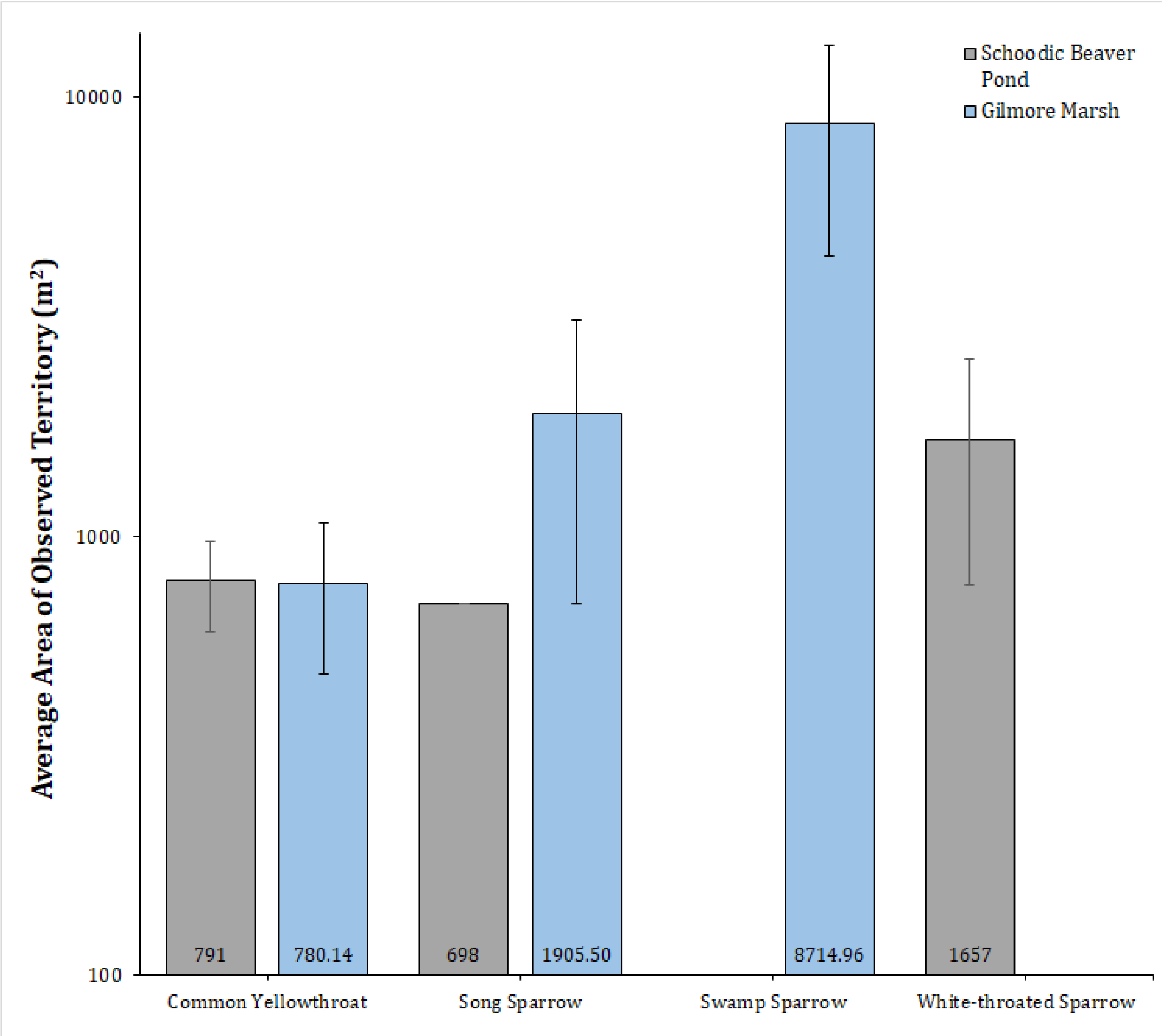


Figure 9. The average area ($m^2$) was calculated for each species at both Gilmore Marsh and Schoodic Beaver Pond. Standard error bars for the means were also given.

For each individual sampled at Schoodic Beaver Pond, the coordinate points were connected and placed on a site map as shown in Figure 8. The area of each polygon was calculated and depicted in Table 2. Territory sizes vary between individuals of the same species, as well as individuals across species. The three Common Yellowthroat Warbler individuals and the one Song Sparrow individual had notably smaller territory sizes, on average, than the White-throated Sparrow individuals, as shown in Figure 9.

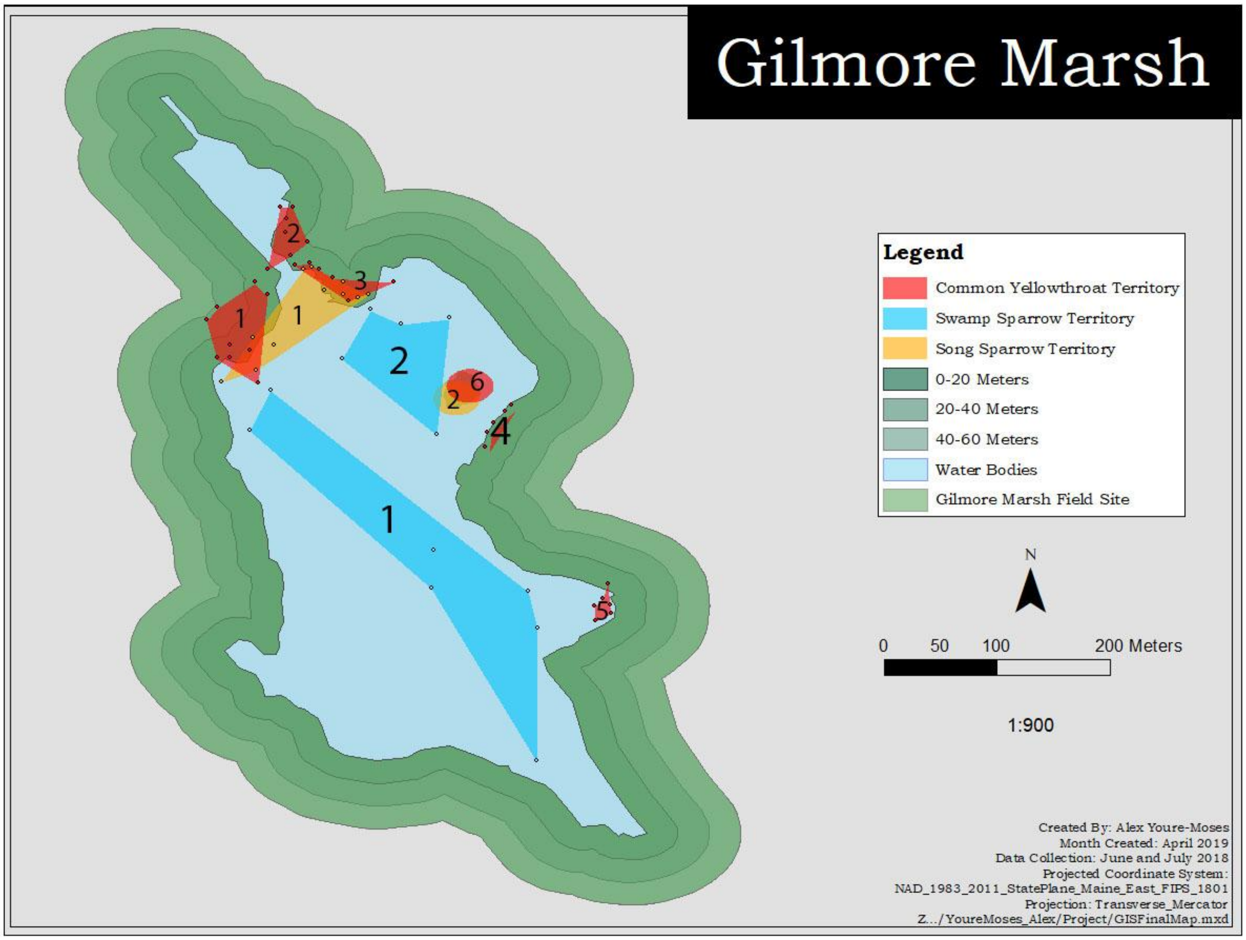


Figure 10. Observed territories of individuals at Gilmore Marsh.

| Gilmore Marsh | Polygon Number | Territory Size ($m^2$) | Acres | Hectares (ha) |
|---|---|---|---|---|
| Swamp Sparrow | 1 | 13100.38 | 3.24 | 1.31 |
| Swamp Sparrow | 2 | 4329.53 | 1.07 | 0.43 |
| Song Sparrow | 1 | 3108 | 0.77 | 0.31 |
| Common Yellowthroat | 1 | 2164 | 0.53 | 0.22 |
| Common Yellowthroat | 2 | 738 | 0.18 | 0.07 |
| Common Yellowthroat | 6 | 703 | 0.17 | 0.07 |
| Song Sparrow | 2 | 703 | 0.17 | 0.07 |
| Common Yellowthroat | 3 | 630 | 0.16 | 0.06 |
| Common Yellowthroat | 5 | 345.83 | 0.09 | 0.03 |
| Common Yellowthroat | 4 | 100 | 0.02 | 0.01 |

Table 1. Territory sizes (in $m^2$, acres, and hectares) of individual birds at Gilmore Marsh. Polygon numbers represent number assigned to each individual located in Figure 10.

| Schoodic Beaver Pond | Polygon Number | Territory Size ($m^2$) | Acres | Hectares (ha) |
|---|---|---|---|---|
| White-throated Sparrow | 1 | 3424 | 0.85 | 0.34 |
| White-throated Sparrow | 2 | 1657 | 0.41 | 0.17 |
| Common Yellowthroat | 2 | 1133 | 0.28 | 0.11 |
| Common Yellowthroat | 1 | 740 | 0.18 | 0.07 |
| Song Sparrow | 1 | 698 | 0.17 | 0.07 |
| Common Yellowthroat | 3 | 500 | 0.12 | 0.05 |

Table 2. Territory sizes (in $m^2$, acres, and hectares) of individual birds at Schoodic Beaver Pond. Polygon numbers represent number assigned to each individual located in Figure 8.

| Site | Individual | Number of Observations |
|---|---|---|
| Schoodic Beaver Pond | White-throated Sparrow 1 | 11 |
| Schoodic Beaver Pond | White-throated Sparrow 2 | 11 |
| Schoodic Beaver Pond | Common Yellowthroat 2 | 10 |
| Schoodic Beaver Pond | Common Yellowthroat 3 | 8 |
| Schoodic Beaver Pond | Song Sparrow 1 | 8 |
| Schoodic Beaver Pond | Common Yellowthroat 1 | 7 |
| Gilmore Marsh | Song Sparrow 1 | 11 |
| Gilmore Marsh | Common Yellowthroat 1 | 9 |
| Gilmore Marsh | Common Yellowthroat 2 | 7 |
| Gilmore Marsh | Swamp Sparrow 1 | 7 |
| Gilmore Marsh | Common Yellowthroat 3 | 6 |
| Gilmore Marsh | Common Yellowthroat 5 | 6 |
| Gilmore Marsh | Common Yellowthroat 4 | 5 |
| Gilmore Marsh | Swamp Sparrow 2 | 5 |

Table 3. Number of observations for each individual at both Schoodic Beaver Pond and Gilmore Marsh. Number of observations corresponds to number of points on Figure 8 and Figure 10, respectively.

Likewise, for each individual sampled at Gilmore Marsh, the coordinate points were connected and placed on a site map as shown in Figure 10. The area of each polygon was calculated and depicted in Table 1. Territory sizes vary between individuals of the same species, as well as individuals across species. The two Swamp Sparrows had notably higher territory sizes than each other species mapped at Gilmore Marsh, as depicted in Figure 9. Individuals of Common Yellowthroat Warblers showed variability across the field site as well.

Additionally, the number of observations was recorded for each individual at Schoodic Beaver Pond and Gilmore Marsh (Table 3). A higher number of observations represents a higher number of observations made and plotted in Figures 8 and 10.

Comparatively, Common Yellowthroat Warbler average territory sizes were similar across both sites, as shown in Figure 9. There were no significant differences between Common Yellowthroat Warbler territory sizes at Gilmore Marsh and Schoodic Beaver Pond (unpaired t-test, $t$= -0.03, $p$= 0.98). Similarly, although Song Sparrow territory size averages differed between Gilmore Marsh and Schoodic Beaver Pond, but there were too few samples collected to test for significance.

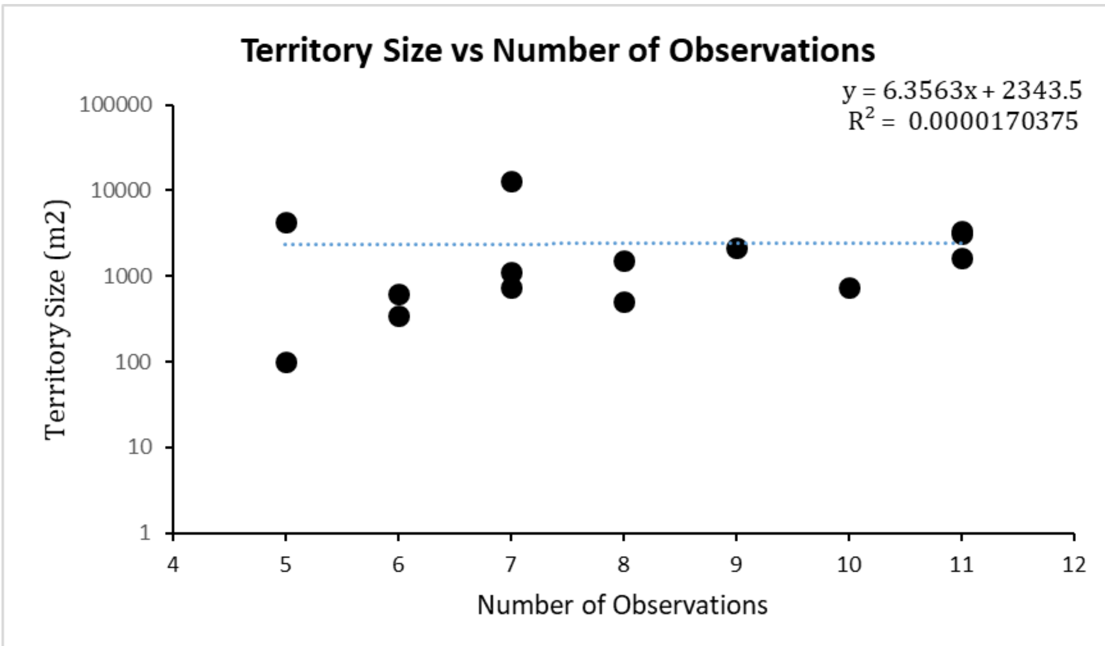


Figure 11. Relationship between number of observations and territory size ($m^2$). Y axis is on a log scale.

The linear regression of number of observations on territory size was not significant ($r^2$ = 1.7E-05, p = 0.53).

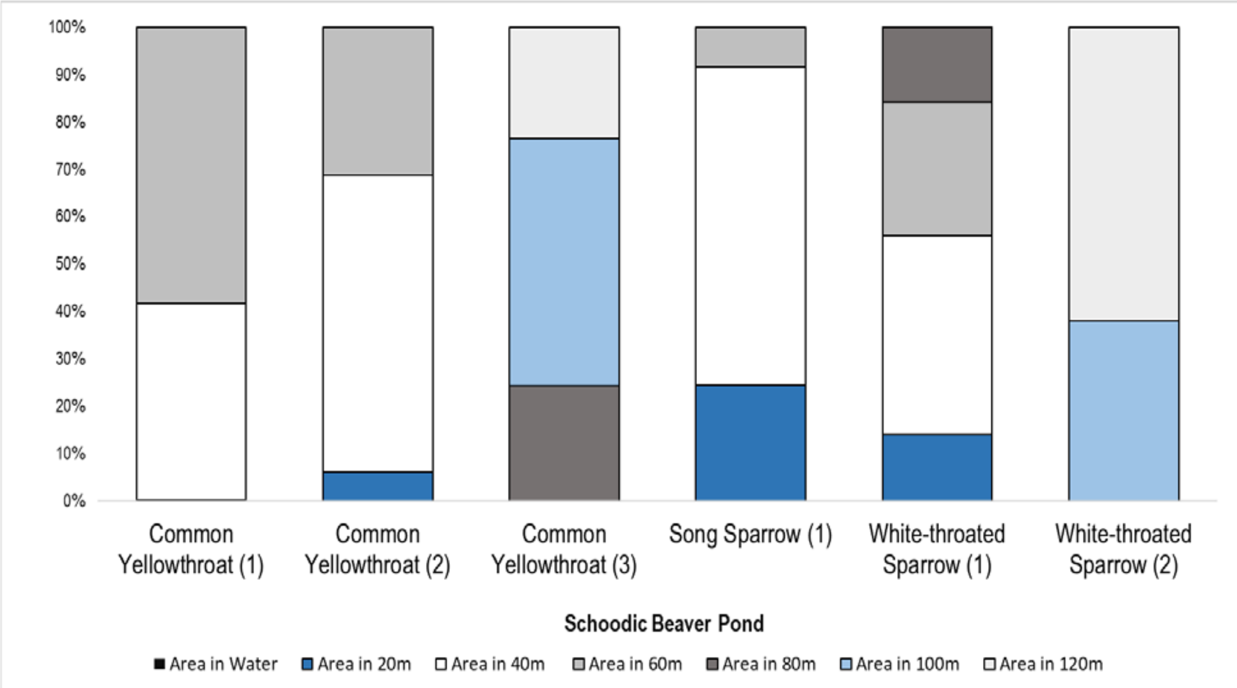


Figure 12. Territories at Schoodic Beaver Pond were separated by 20m categories based on distance from the main waterbody. The percent of territory in each buffer category was determined. Numbers next to species name represents the polygon number in Figure 3.

Individuals between species and individuals across species differed in their habitat usage at Schoodic Beaver Pond (Figure 12). Common Yellowthroat Warblers 1 and 2, Song Sparrow 1, and White-throated Sparrow 1 had a majority of their territories located between the waterbody layer and the 40m buffer. Common Yellowthroat Warbler 3 and White-throated Sparrow 2 had all of their territory located above the 60m buffer.

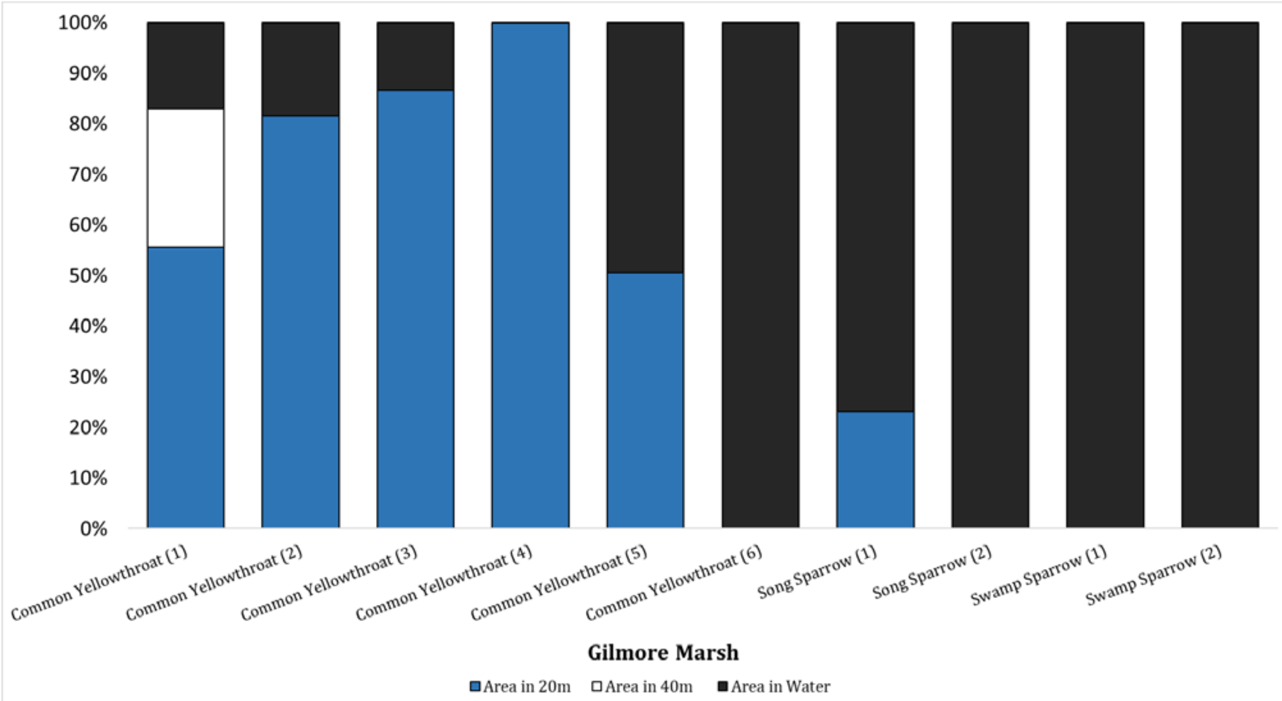


Figure 13. Territories at Gilmore Marsh were separated by 20m categories based on distance from the main waterbody. The percent of territory in each buffer category was determined. Numbers next to species name represents the polygon number in Figure 5.

Similarly, individuals between species and individuals across species differed in their habitat usage at Gilmore Marsh (Figure 13). The observed territories Swamp Sparrows 1 and 2 were both completely located within the water, whereas the observed territory for Common Yellowthroat Warbler 4 was completely located in the 20m buffer. Song Sparrow 2 was located on a small island within the waterbody. The other Common Yellowthroat Warbler individuals varied in their distance to the water.

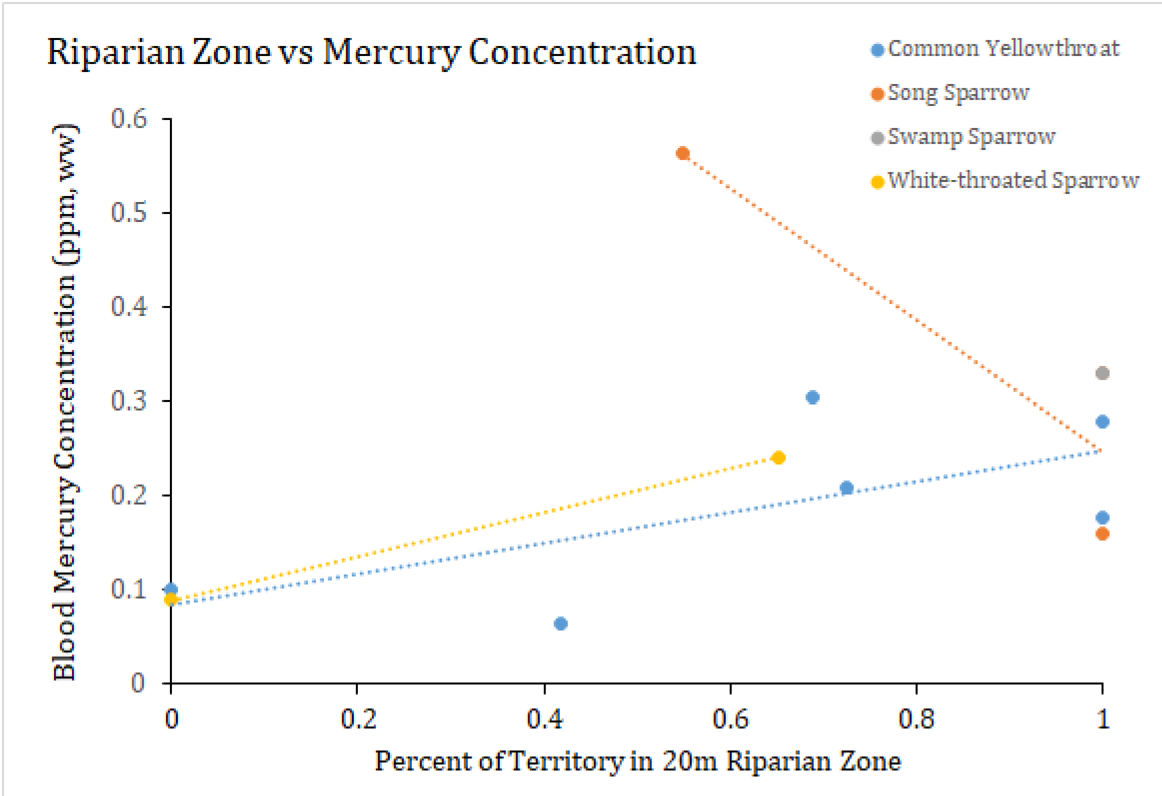


Figure 14. The portion of individual territory polygons located in the water buffer and 20m buffer were combined for both sites and set as the 'riparian zone.' This portion was compared with the total area for that polygon and calculated as a percentage. A value of one states that all of the territory is located within the riparian zone. Trendlines were added to visualize this relationship.

Trendlines for Common Yellowthroat Warbler ($r^2 = 0.43$) and White-throated Sparrow ($r^2 = 1$) individuals showed that there was less mercury found in an individual's blood as more of their territory was located outside of the riparian buffer (Figure 14). The trendline for Song Sparrows ($r^2 = 0.9$) showed this relationship in the opposite direction. The linear regression of blood mercury on percent of territory within the riparian zone was not significant for these three species ($p > 0.05$).

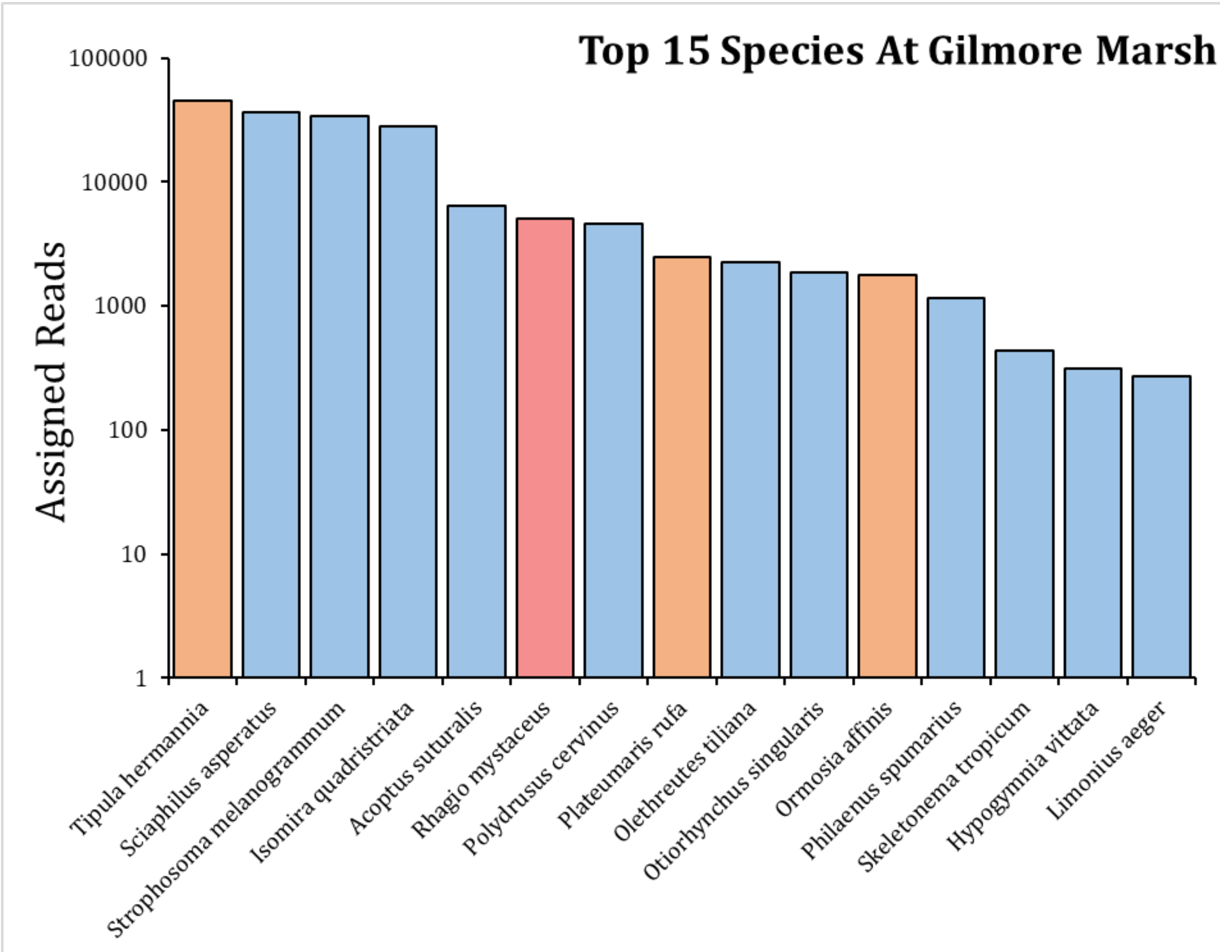


Figure 15. Bar graph of arthropod prey identified in the feces of 1 Black-throated Green Warbler (*Dendroica virens*), 1 Red-breasted Nuthatch (*Sitta canadensis*), 1 Swamp Sparrow (*Melospiza georgiana*), 1 Slate-colored Junco (*Junco hyemalis*), 2 Song Sparrow (*Melospiza melodia*), 2 Hermit Thrush (*Catharus guttatus*), and 3 Common Yellowthroat Warblers (*Geothlypis trichas*) in DNA analysis. Orange bars represent emergent aquatic insects and the red bar represents an insect that feeds on other insects. Y-axis is on a log scale. See Appendix for full species list (Table 4).

At Gilmore Marsh, we captured 47 species in the data analysis from 11 bird individuals: 1 Black-throated Green Warbler (*Dendroica virens*), 1 Red-breasted Nuthatch (*Sitta canadensis*), 1 Swamp Sparrow (*Melospiza georgiana*), 1 Slate-colored Junco (*Junco hyemalis*), 2 Song Sparrow (*Melospiza melodia*), 2 Hermit Thrush (*Catharus guttatus*), and 3 Common Yellowthroat Warblers (*Geothlypis trichas*). From all OTUs (operational taxonomic units), 15 species were highly frequent in the Gilmore Marsh dataset and represented 10.1% of all assigned reads in MEGAN6 (Figure 14). Host species accounted for 41% of all assigned reads (not included in Graph 15).

These 15 OTUs were assigned to 6 orders: Coleoptera (72% of all assigned reads), Diptera (26.8%), Lepidoptera (1.4%), Hemiptera (0.69%), Thalassiosirales (0.26%), and Lecanorales (0.18% reads). The average number of assigned reads by total taxa was 3645.9.

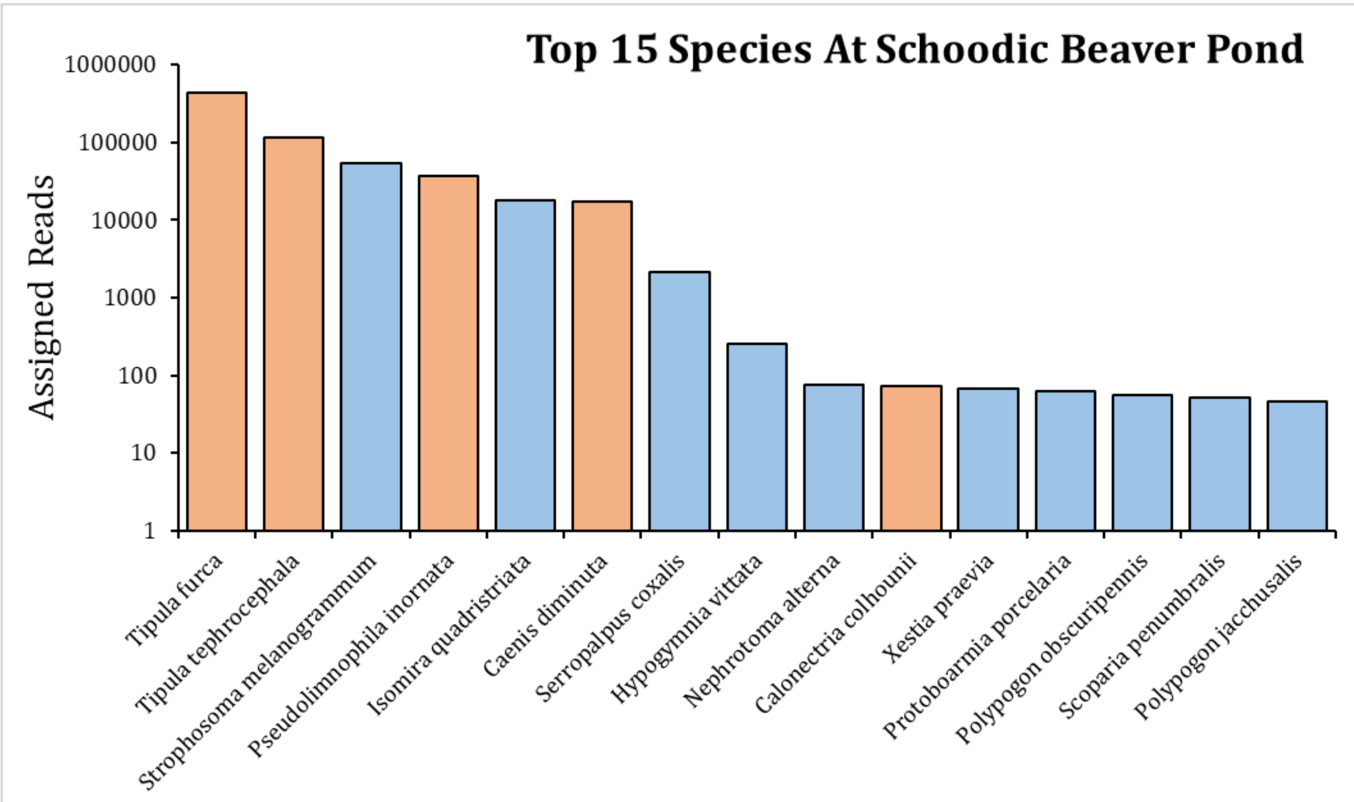


Figure 16. Bar graph of arthropod prey identified in the feces of 2 White-throated Sparrow (*Zonotrichia albicollis*), 2 Song Sparrow (*Melospiza melodia*), and 4 Common Yellowthroat Warblers (*Geothlypis trichas*) in DNA analysis. Orange bars represent emergent aquatic insects. Y-axis is on a log scale. See Appendix for full species list (Table 5).

We captured 45 species in the data analysis from 8 bird individuals at Schoodic Beaver Pond: 2 White-throated Sparrow (*Zonotrichia albicollis*), 2 Song Sparrow (*Melospiza melodia*), and 4 Common Yellowthroat Warblers (*Geothlypis trichas*). From all OTUs (operational taxonomic units), 15 species were highly frequent in the Schoodic Beaver Pond dataset and represented 83.2% of all assigned reads in MEGAN6 (Figure 16). Two host species (*Melospiza melodia* and *Geothlypis trichas*) accounted for 10.4% of all assigned reads (not included in Figure 16). These 15 OTUs were assigned to 6 orders: Diptera (71.9% of all assigned reads), Coleoptera

(9.1%), Lepidoptera (0.03%), Ephemeroptera (2.12%) Lecanorales (0.0092%), and Hypocreales (0.009%). The average number of assigned reads by total taxa was 17,597.

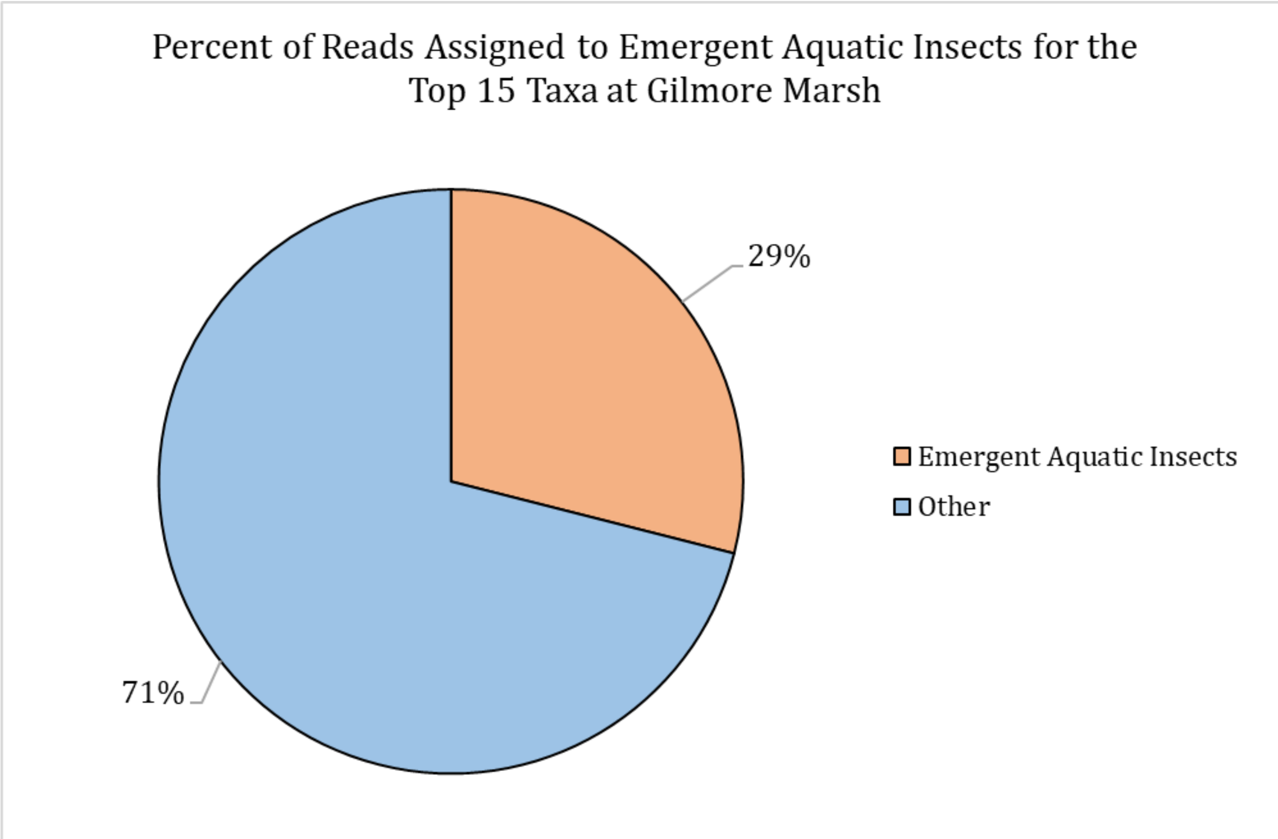


Figure 17. Percent of reads assigned to emergent aquatic insects for the top 15 taxa at Gilmore Marsh.

29% of the reads assigned to the top 15 taxa at Gilmore Marsh were assigned to emergent aquatic insects (Figure 17).

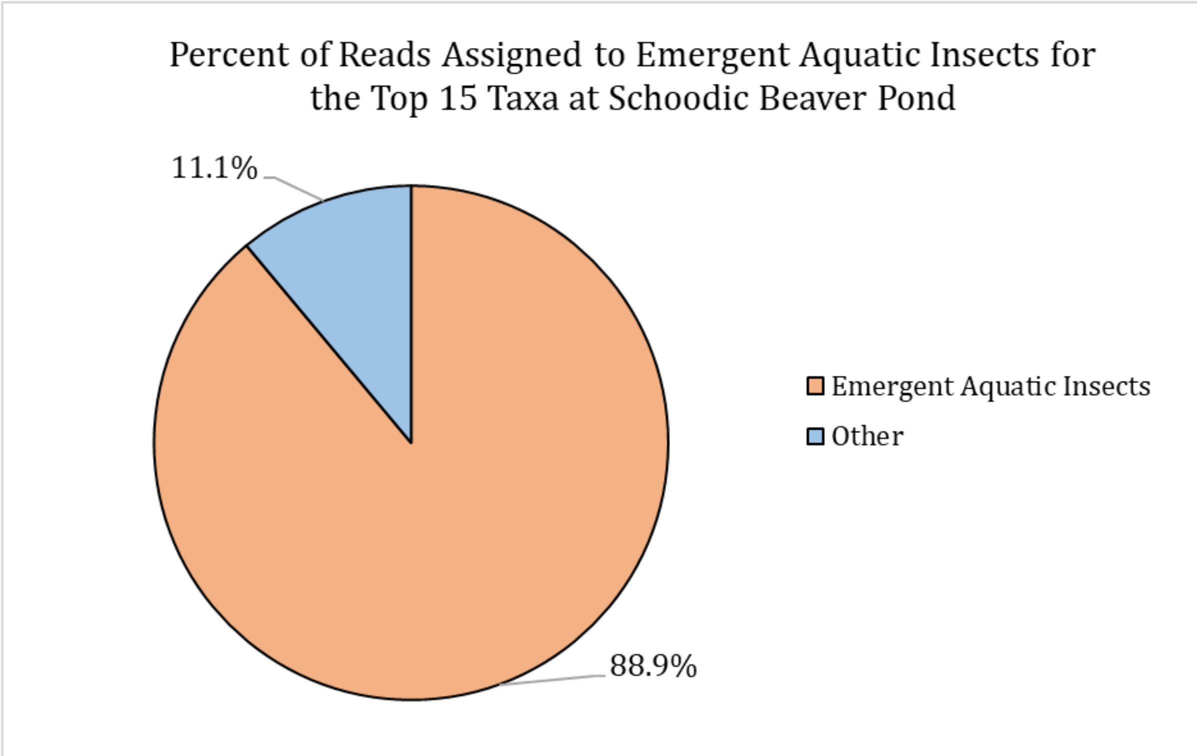


Figure 18. Percent of reads assigned to emergent aquatic insects for the top 15 taxa at Schoodic Beaver Pond.

88.9% of the reads assigned to the top 15 taxa at Schoodic Beaver Pond were assigned to emergent aquatic insects (Figure 18).

# Discussion

The goal of this study was to relate habitat usage and diet to blood mercury concentrations in four riparian songbirds, the Common Yellowthroat Warbler, White-throated Sparrow, Song Sparrow, and Swamp Sparrow. We found that different species vary in their mercury levels and individuals of the same species appear to vary in their mercury levels between sites, although these differences were not statistically significant. We also found that territory sizes differed for individuals at each site, which may be due to individual preferences, food availability, and competition. Diet analyses also showed that riparian songbirds are eating emergent aquatic insects, which may aid to the biomagnification of methylmercury within the food web. However, the results demonstrate that further research is necessary to determine relationships between habitat usage, diet, and mercury in riparian songbirds.

**Mercury**

Prior mercury research conducted for the Dragonfly Mercury Project at Acadia National Park suggested that dragonflies, which are top aquatic predators and potential vectors of methylmercury, may be contributing to mercury levels in riparian songbirds (Eagles-Smith et al., 2018). The Dragonfly Mercury Project utilized Aunt Betty's Pond and Schoodic Beaver Pond as sampling sites to collect dragonfly larvae. For this study, Schoodic Beaver Pond and Gilmore Marsh were chosen as sampling sites. Gilmore Marsh was an easier-to-access site and shares the same watershed as Aunt Betty's Pond, so it was chosen as one of the sampling sites for this project. The data from 2014-2016 for both Aunt Betty's Pond and Gilmore Marsh showed that there were relatively high levels of mercury in dragonfly larvae (Figure 2). Schoodic Beaver Pond showed higher mercury levels than Aunt Betty's Pond, so it was hypothesized that there would also be high levels of mercury seen in birds sampled at this site. However, as Figure 6 shows, there was no significant difference between mercury concentrations of sampled riparian songbirds. In fact, the average mercury was slightly lower at Schoodic Beaver Pond (mean = 0.23 ppm, ww) than it was at Gilmore Marsh (mean = 0.21 ppm, ww). This suggests that mercury levels dragonflies may not be a good indicator of mercury levels in riparian songbirds.

Mercury concentrations in riparian songbirds did vary between sites. The Black-throated Green Warbler, Red-breasted Nuthatch, and Swamp Sparrow had higher blood mercury averages than the Hermit Thrush, Common Yellowthroat Warblers, and Song Sparrows (Figure 8). Although, it is hard to make a generalization based on this data because there were only two species, the Common Yellowthroat Warbler and Song Sparrow, that had more than one individual in the sample. Therefore, this may not be a good representation of blood mercury concentration for the other three species. However, all three species sampled at Schoodic Beaver Pond, the Common Yellowthroat Warbler, the Song Sparrow, and the White-throated Sparrow, had more than one individual analyzed for blood mercury concentrations. The Song Sparrows had higher levels of mercury in their blood, on average, than the Common Yellowthroat Warblers and White-throated Sparrows (Figure 4). The Song Sparrows at Schoodic Beaver Pond had higher levels of mercury, on average, than the Song Sparrows at Gilmore Marsh (Figure 4). This difference was not significant, but it these differences show that individuals of this species vary between sites.

Likewise, the Common Yellowthroat Warblers at Gilmore Marsh had slightly higher levels of mercury in their blood than Common Yellowthroat Warblers at Schoodic Beaver Pond. However, this difference was also not significant. Additionally, there was no significant difference found for blood mercury concentrations between any of the four target species. It is also important to note that the average mercury concentrations of the four target species at Schoodic Beaver Pond and Gilmore Marsh are lower than other mercury levels found in bird species sampled across Acadia National Park and eastern North America (Jackson et al. 2015; Longcore et al., 2007b).

Additionally, the relationship between blood mercury concentrations and bird mass (g) was not significant (Figure 5). It is important to note that the songbirds were sampled during the months of June and July, when fat reserves may have been low due to spatiotemporal variations in the importance of body fattening and energy reserves (Blem, 1976, Blem 1986). During migration and overwintering, birds may regulate their energy by fattening these reserves in order to survive during periods when food availability is low (Ekman and Hake, 1990). Therefore, sampling songbirds over a year long period may show variations in mass and blood mercury concentrations, but more research needs to be conducted.

**Territory Size, Number of Observations, and Distance from the Water**

Territory sizes also differed between and across species at both sites. After points were collected and connected on maps (shown in Figures 8 and 10), the area ($m^2$) of observed territories were calculated and compared in Tables 1 and 2. At Schoodic Beaver Pond, the White-throated Sparrows had larger territories than the Common Yellowthroat Warblers and the Song Sparrow. White-throated Sparrow 1, White-throated Sparrow 2, and Common Yellowthroat Warbler 3 had observed territories greater than 1000 $m^2$ (Table 2). Additionally. White-throated Sparrow 1, Song Sparrow 1, and Common Yellowthroat Warblers 2 all had overlapping territories near the waterbody buffer (Figure 8). White-throated Sparrow 2 and Common Yellowthroat 3 had territories that were only located above the 40m buffer, whereas Common Yellowthroat Warbler 1, Common Yellowthroat Warbler 2, Song Sparrow 1, and White-throated Sparrow 1 had territories that were located below the 60m buffer (Figure 12). At Gilmore Marsh, Swamp Sparrows 1 and 2 had higher territory sizes than all other individuals (Table 1) and were only observed in the waterbody (Figure 10). The individuals of the two other species, the Common

Yellowthroat Warbler and the Song Sparrow, showed high variability across the site. Common Yellowthroat Warblers 2, 3, and 4 had territories primarily located in the 20m buffer (Figure 13).

Several studies have attempted to quantify territory sizes for these four target species. On average, the White-throated Sparrow had territories ranging from 0.22-3.26 ha (Formica et al., 2004; Jones, 1987h; Lainevool, 1966; and Tuttle, 1993). This is similar to the two White-throated Sparrows that were sampled at Schoodic Beaver Pond. White-throated Sparrow 1 had a territory of 0.34 ha, while White-throated Sparrow 2 had a territory of 0.17 ha (Table 1). Territories for Common Yellowthroat Warblers vary by location, but studies have reported sizes ranging from 0.2-1.2 ha (Foster, 1977; Hofslund, 1959; and Powell and Jones, 1978). The range of territory sizes for Common Yellowthroat Warblers sampled for both sites is 0.01-0.11 ha, with a mean of 0.08 ha (Table 1 and Table 2). These territories are consistent with the prior research. Territory sizes for Song Sparrows vary annually, but research has suggested that at high density (roughly 25 pairs/ha), territories can be as small as 0.02 ha, while at low density (2 pairs/ha), territories can be as high as 0.5 ha (Arcese, 1989b). Song Sparrows at Schoodic Beaver Pond and Gilmore Marsh had a range of 0.07-0.31 ha, which is on the lower side of the spectrum, considering there was no intraspecific competition (Table 1 and Table 2). Lastly, research has stated that Swamp Sparrow territories range from 0.03-0.61 ha (Reinert and Golet, 1979), which is consistent with the two Swamp Sparrow territories mapped in Figure 10. At Gilmore Marsh, Swamp Sparrow 1 had a territory size of 1.31 ha, while Swamp Sparrow 2 had a territory size of 0.43 ha (Table 1).

Differences in territory sizes can be due to individual preferences in habitat, food availability, predation, and competition (Cody, 1981; Tinbergen, 1957), however some studies suggest that territory can vary day to day during the breeding season (Stenger, 1958). Likewise, birds select sites during this time that allow for maximum reproductive success (Murray and Best, 2014). These choices are often nonrandom and are based on a number of different factors and activities, such as foraging and nesting (Clark and Shutler, 1999). Because coordinate points were collected during the breeding season at the end of June and beginning of July, it is important to take this into consideration when discussing territory distribution.

Song Sparrows are a territorial species that are generalists in respect to habitat preference, but tend to favor thickets, brushes, and marshes (Chesser et al., 2018). Their preferences in habitat selection, especially during the breeding season, may be a result of nest-predation (Chase, 2002). As such, nests that are hidden in dense, shrubby vegetation may be more successful than nests in grazed vegetation. Song Sparrows at both Schoodic Beaver Pond and Gilmore Marsh were seen in denser shrub patches surrounding the water bodies, however habitat structure was not a factor that was looked at in this study. At Gilmore Marsh specifically, Song Sparrows were hardly seen leaving this area and only foraged near the outskirts of the waterbody. At Gilmore Marsh, a large portion of Song Sparrow 1's territory was located in the water. However, this Song Sparrow individual spent a majority of its time in the dense shrub patches surrounding the waterbody (Table 3 and Figure 10). Additionally, at both sites, the Song Sparrow individuals had territory sizes that were, on average, larger than that of surrounding individuals of other species, which may be due to the lack of intraspecific competition.

Similarly, Common Yellowthroat Warblers are also habitat generalists, with a wide-range of tolerance for a number of different habitats (Stauffer and Best, 1980). Although they are habitat generalists, Common Yellowthroat Warblers tend to breed in wet habitats and prefer low, dense vegetation (Kendeigh, 1945). This is consistent with the number of observations made for Common Yellowthroat Warblers at both sites (Figure 8 and Figure 10). More observations were made for Common Yellowthroat individuals in areas that are surrounding the waterbody and had dense vegetation, although Common Yellowthroat 3 at Schoodic Beaver Pond had all of their territory above the 40m buffer. This was probably due to intraspecific competition near the water body, shown in Figure 3.

Swamp Sparrows have a strong preference for wetland habitats, especially during the breeding season (Greenberg, 1988). Greenberg, in this paper, suggests that Song Sparrows and Swamp Sparrows have non-overlapping territories and exhibit strong interspecific aggression. Although interspecific aggression was not noted in this study, the territories of Song Sparrows and Swamp Sparrows did not overlap at Gilmore Marsh (Figure 10). Additionally, Greenberg (1988) hypothesizes that Swamp Sparrows are capable of wading in the water to search for prey items, but Song Sparrows have difficulty foraging in the water and choose to stay on land to forage. This hypothesis is consistent with the location of observations for both species at Gilmore Marsh (Figure 10). Swamp Sparrows were primarily seen in the water, while Song Sparrows were seen foraging around the water's surface, on moist land. Additionally, the territories of Swamp Sparrow 1 and Swamp Sparrow 2 did not overlap (Figure 8). In respect to intraspecific competition, Swamp Sparrow 1 had a larger territory than Swamp Sparrow 2, which may indicate that it is the more dominant individual.

The White-throated Sparrow is another habitat generalist that favors low, dense shrubs (Rousseau et al., 2012). White-throated Sparrows exhibit intraspecific competition and will chase out competitors (Falls, 1975). This is consistent with the documented territories of White-throated Sparrows at Schoodic Beaver Pond, depicted in Figure 3. White-throated Sparrow 1 was the more dominant of the two, having a larger territory size than White-throated Sparrow 2 (Table 2). The territory occupied by White-throated Sparrow 1 may be of better quality, in relation to food abundance and the amount of cover or nesting habitat (Formica et al., 2004).

Like competition, food availability also has an influence on territory. One theory, called the food value theory, states that individuals may space themselves out in order to obtain enough food to rear their young (Stenger, 1958). This theory implies that territory size and food availability are correlated. This theory also relates heavily to the concept of cross-ecosystem subsidies, in which energy and nutrients are transferred from one ecosystem to another. An important cross-ecosystem subsidy is the transfer of aquatic prey into terrestrial systems, in the form of emergent aquatic prey (Cristol et al., 2008). This subsidy occurs annually during the defoliation period and may contribute to riparian songbird diet (Nakano and Murakami, 2001). The influx of emergent aquatic insects is most frequently seen in the transition period of spring and summer.

During the breeding season, riparian songbirds rely heavily on emergent aquatic insects to feed their young, which may be due to scarce terrestrial resources, such as seeds, berries, and invertebrates. If aquatic insect biomass is highest during the breeding season, the territory sizes of individual birds that are closer to the water may be smaller due to the increase in available food.

Only studying these differences in habitat preference and utilization over a long period of time can allow for broader generalizations to be made about a species, especially because there may be annual variation in factors that influence site selection such as competition, food availability, local weather conditions, and foliage structure (Petit et al., 1990). However, territory size may be related to these factors, but more studies need to be conducted in order to be able to make these generalizations.

**Mercury and Territory**

This study failed to find a significant relationship between blood mercury concentrations and territory size ($m^2$), as depicted in Figure 11. Territory sizes, as stated above, are influenced by many different factors, whereas mercury concentrations may be dependent solely on the availability of aquatic and predator prey items. However, regressions for the relationship between blood mercury concentrations on percent of territory within the riparian zone suggest there may be a trend (Figure 14). Due to low sample sizes, these trends need to be further investigated.

**Diet**

Prior diet research conducted on over 400 Song Sparrows states that Coleoptera (mostly being weevils, ground beetles, and leaf beetles) contributed to 9% of their diet, Lepidoptera contributed to 6%, and Diptera (mostly midges and craneflies), Hemiptera, and Ephemeroptera all contributed less than 3% each (Judd, 1901b). Diet analysis conducted in the month of May for White-throated Sparrows suggest that about 90% of their diet is made up of insects in the orders of Coleoptera, Diptera, and Lepidoptera (Falls and Kopachena, 2010). According to research, the Red-breasted Nuthatch primarily eats beetles in the family of Curculionidae, Chrysomelidae, Scarabaeidae, and Elateridae during the breeding season (Anderson, 1976b). Unfortunately, no specific diet analyses on the Hermit Thrush have been conducted, but a study stated that over 1,694 insects, including beetles, moths, mayflies, damselflies, caddisflies, and various diptera, were brought to a nest site in Iowa (Shaver, 1918). Likewise, little research has been conducted on the diet of Swamp Sparrows, but studies have shown that during the spring and early summer, over 80% of the diet is contributed to insects, such as beetles, ants, and caterpillar (Wetherbee, 1968). Additionally, a long term diet analysis of 269 Dark-eyed Juncos stated that 24% of their diet was attributed to insects, including Coleoptera, Lepidoptera, Hymenoptera, and Diptera (Beal, 1907). Lastly, studies have suggested that Lepidoptera, Diptera, Coleoptera, Hemiptera, and Hymenoptera all contribute to the diet of Black-throated Green Warblers during the breeding season (Bent, 1953b; Smith et al., 1998c).

Although we lost the individuality of our samples when we pooled them together for the diet analysis, Coleoptera, Diptera, Lepidoptera, Hemiptera, Thalassiosirales, and Lecanorales all showed up in the analysis for species sampled at Gilmore Marsh, which included fecal samples from 1 Black-throated Green Warbler, 1 Red-breasted Nuthatch, 1 Swamp Sparrow, 1 Slate-colored Junco, 2 Song Sparrow, 2 Hermit Thrush , and 3 Common Yellowthroat Warblers. The top fifteen represented taxa from Gilmore Marsh were *Tipula hermannia*, *Sciaphilus asperatus*, *Strophosoma melanogrammum*, *Isomira quadristriata*, *Acoptus suturalis*, *Rhagio mystaceus*, *Polydrusus cervinus*, *Plateumaris rufa*, *Olethreutes tiliana*, *Otiorhynchus singularis*, *Ormosia* *affinis*, *Philaenus spumarius*, *Skeletonema tropicum*, *Hypogymnia vittata*, and *Limonius aeger*. These 15 taxa represent 11 families: Aphrophoridae, Chrysomelidae, Curculionidae, Elateridae, Limoniidae, Parmeliaceae, Rhagionidae, Skeletonemataceae, Tenebrionidae, Tipulidae, and Tortricidae. Based on the prior research, our results were consistent for the diet analyses of the seven songbird species in the Gilmore Marsh sample.

Similarly, Diptera, Coleoptera, Lepidoptera, Ephemeroptera, Lecanorales, and Hypocreales showed up in the analysis for the species sampled at Schoodic Beaver Pond, which included fecal samples from 2 White-throated Sparrow, 2 Song Sparrow, and 4 Common Yellowthroat Warblers. The top fifteen represented taxa from Schoodic Beaver Pond were *Tipula furca*, *Tipula tephrocephala*, *Strophosoma melanogrammum*, *Pseudolimnophila inornata*, *Isomira quadristriata*, *Caenis diminuta*, *Serropalpus coxalis*, *Hypogymnia vittata*, *Nephrotoma alterna*, *Calonectria colhounii*, *Xestia praevia*, *Protoboarmia porcelaria*, *Polypogon obscuripennis*, *Scoparia penumbralis*, and *Polypogon jacchusalis*. These 15 taxa represent 12 families: Caenidae, Crambidae, Curculionidae, Erebidae, Geometridae, Limoniidae, Melandryidae, Nectriaceae, Noctuidae, Parmeliaceae, Tenebrionoidea, and Tipulidae. Based on the prior research, our results were consistent for the diet analyses of the three songbird species in the Schoodic Beaver Pond sample.

Unfortunately, diet analyses conducted on individual songbird species are severely lacking. Additionally, most diet analyses for songbirds rely on observational techniques and stomach content analysis, therefore more research using molecular methods needs to be conducted on the diets of riparian songbirds.

Next Generation Sequencing, compared to other methods of diet analysis, is a valuable tool that can aid ornithological research (Moreby and Stoate, 2000). By using both observational methods and molecular methods, the accuracy of diet analyses on riparian songbirds may be amplified. This could be useful in determining the diets of individual songbird species, for which research is few and far between. Additionally, the foraging guild classifications of riparian songbirds seem to be outdated. For instance, a paper published on foraging guilds of North American birds stated that Song Sparrows were granivores (Degraaf et al., 1985), yet other sources stated that Song Sparrows have been seen feeding nestlings insect prey during the breeding season (Girard et al., 2012). Further diet analyses of riparian songbirds could aid species foraging guild classifications that may be outdated or too specific.

**Diet and Territory**

As stated above, territory sizes and location can change based on food availability. With food availability changing seasonally, based on the defoliation and leafing periods of terrestrial vegetation (Nakano and Murakami, 2001), territory size and location may also change seasonally, although some studies suggest that territory sizes can shift daily (Stenger, 1958). The molecular methods utilized in this study do not quantify abundances of arthropod prey, therefore using observational methods with molecular methods may be useful in determining if food availability directly influences territory size and location.

**Mercury and Diet**

Although the individuality was lost when the fecal samples were pooled by site, we were still able to detect emergent aquatic insects in the diet of these birds. Of the top 15 taxa at Schoodic Beaver Pond, five species were emergent aquatic insects: *Tipula furca*, *Tipula tephrocephala*, *Pseudolimnophila inornata*, *Nephrotoma alterna*, and *Caenis diminuta*. The first four species listed were crane flies and the last species was a mayfly. Unfortunately, we cannot determine the stage of development of these insects (which would be helpful in understanding how much mercury may be transferred), but we can speculate that, regardless of stage, they may be contributing to the concentration of mercury seen within songbirds at Schoodic Beaver Pond. Of the top 15 taxa at Gilmore Marsh, three were emergent aquatic insects (*Tipula hermannia*, *Ormosia affinis*, and *Plateumaris rufa*) and one (*Rhagio mystaceus*) feeds on other insects. Two emergent aquatic insects were crane flies and one was an aquatic leaf beetle. On the other hand, *Rhagio mystaceus* is a snipe fly, which is known to prey on other insects, which may contribute to the biomagnification of mercury in the food web.

Studies have shown that contaminant exposure among riparian songbirds is related to the contribution of emergent aquatic insects on their diet (Walters et al., 2008). The contribution of emergent aquatic prey on the diet of songbirds can allow for a greater understanding of how mercury gets into these individuals, but it is important to note that this contribution varies between bird species due to differences in foraging behavior (Nakano and Murakami, 2001). More studies need to be conducted on the relationship of mercury levels in songbirds and their respective diets.

## Limitations and Complications

While molecular methods of diet analysis provide insight to the diet of riparian songbirds, there are a few limitations associated with the techniques. For instance, there may be a bias towards hard-bodied invertebrates due to the fast digestion of soft-bodied invertebrates (Crisol-Martinez et al., 2016). Therefore, the diet analysis may only show larger, hard-bodied arthropods. Additionally, fecal matter only shows the diet items of the individual's most recent meal (Ohem et al., 2011; Warner, 1981). In order to accurately assess an individual's diet, fecal samples need to be collected frequently and abundantly throughout the entire year.

In addition to this, there were some complications in the field. Although fecal samples were kept in a freezer for long-term storage, the freezer was constantly opened and closed, which may have degraded the samples. Fecal samples were also stored in ethanol, which may interact with the uric acid within the feces. We also used two kits, the DNA Stool Mini Kit (Qiagen #51604) and the Quick DNA Fecal/Soil Microbe Microprep Kit (Zymo #D6012). The DNA Stool Mini Kit produced DNA yields that were consistently low. Since our samples sizes were so small, with fecal pellets weighing between 0.0092-0.1118 g, it might have been more helpful to use the Quick DNA Fecal/Soil Microbe Microprep Kit from the start. Additionally, holding the captured songbirds in the sterilized bag for longer periods of time may have allowed for better fecal samples to be collected.

Upland samples, including fecal, blood, and observed territory, of songbirds may have also aided this research. Having upland comparisons would have allowed us to determine how far emergent aquatic insects are traveling and if they are contributing to the diet and mercury levels seen in a more diverse set of birds in temperate, deciduous forests.

## Implications and Future Research Directions

Many questions remain about how territory and diet relate to mercury concentration in riparian songbirds and the results of this study illustrate the need for more research. Future research should continue to examine the trophic transfer of mercury as a result of influxes in emergent aquatic prey, as well as how territory is influenced by this increase in food availability. Diet analysis using molecular methods, with the help of observational diet observations, may prove to be a useful tool in determining the contribution of emergent aquatic prey in the diet of riparian songbirds.

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# Appendix

**Table 4:** Full list of species found in the fecal samples collected at Schoodic Beaver Pond.

| Schoodic Beaver Pond | | | | | | |
|---|---|---|---|---|---|---|
| Order | Family | Species | Percent Identity | Assigned Reads | Sequence Length | E Value |
| Diptera | Tipulidae | Tipula tephrocephala | 98.26% | 114376.0 | 345 | 5.00E-169 |
| Passeriformes | Passerellidae | Melospiza melodia | 98.68% | 67391.0 | 378 | 0 |
| Coleoptera | Curulionidae | Strophosoma melanogrammum | 99.71% | 54402.0 | 343 | 3.00E-176 |
| Diptera | Tipulidae | Tipula furca | 98.83% | 48271.0 | 343 | 3.00E-171 |
| Diptera | Tipulidae | Tipula furca | 98.67% | 42212.0 | 301 | 6.00E-148 |
| Diptera | Limoniidae | Pseudolimnophila inornata | 99.14% | 36932.0 | 349 | 3.00E-176 |
| Diptera | Tipulidae | Tipula furca | 98.55% | 31732.0 | 345 | 1.00E-170 |
| Coleoptera | Tenebrionoidea | Isomira quadristriata | 98.84% | 18006.0 | 344 | 8.00E-172 |
| Passeriformes | Parulidae | Geothlypis trichas | 98.41% | 17726.0 | 378 | 0 |
| Ephemeroptera | Caenidae | Caenis diminuta | 98.23% | 17450.0 | 339 | 1.00E-165 |
| Passeriformes | Turdidae | Catharus guttatus | 98.45% | 12565.0 | 388 | 0 |
| Passeriformes | Passerellidae | Junco hyemalis | 98.41% | 11339.0 | 377 | 0 |
| Passeriformes | Passerellidae | Melospiza georgiana | 98.67% | 3273.0 | 376 | 0 |
| Coleoptera | Melandryidae | Serropalpus coxalis | 92.57% | 2148.0 | 350 | 9.00E-127 |
| Lecanorales | Parmeliaceae | Hypogymnia vittata | 100% | 209.0 | 347 | 4.21E-180 |
| Diptera | Tipulidae | Nephrotoma alterna | 100% | 75.0 | 341 | 1.13E-176 |
| Hypocreales | Nectriaceae | Calonectria colhounii | 100% | 73.0 | 333 | 2.23E-172 |
| Lepidoptera | Noctuidae | Xestia praevia | 100% | 66.0 | 341 | 1.32E-174 |
| Lepidoptera | Geometridae | Protoboarmia porcelaria | 100% | 62.0 | 340 | 2.86E-176 |
| Lepidoptera | Erebidae | Polypogon obscuripennis | 99.50% | 55.0 | 371 | 0.00E+00 |
| Lepidoptera | Crambidae | Scoparia penumbralis | 99.10% | 51.0 | 315 | 8.16E-157 |
| Lepidoptera | Erebidae | Polypogon jacchusalis | 99.70% | 46.0 | 339 | 4.88E-174 |
| Diptera | Limoniidae | Pseudolimnophilia inornata | 100% | 46.0 | 338 | 3.68E-175 |
| Diptera | Sphaeroceridae | Terrilimosina schmitzi | 99.70% | 45.0 | 340 | 1.33E-174 |
| Capnodiales | Mycospaerellaceae | Cercospora sojina | 99.00% | 40.0 | 349 | 5.28E-174 |
| Hypocreales | Cordycipitaceae | Beauveria caledonica | 100% | 34.0 | 351 | 0 |
| Hypocreales | Cordycipitaceae | Lecanicillium lecanii | 100% | 23.0 | 357 | 0 |
| Lecanorales | Parmeliaceae | Hypogymnia vittata | 100% | 23.0 | 359 | 0 |
| Hypocreales | Nectriaceae | Cosmospora gigas | 100% | 21.0 | 338 | 3.69E-175 |
| Hypocreales | Incertae sedis | Verticillium dahliae | 99.50% | 20.0 | 367 | 0 |
| Lecanorales | Lecanoraceae | Lecanora cinerofusca | 100% | 20.0 | 367 | 0 |
| Helotiales | Helotiaceae | Cairneyella variabilis | 100% | 19.0 | 366 | 0 |
| Chaetothyriales | Herpotrichiellaceae | Cladophialophora bantiana | 99.70% | 19.0 | 370 | 0 |
| Lecanorales | Parmeliaceae | Hypogymnia vittata | 91.80% | 19.0 | 364 | 5.38E-134 |
| Lecanorales | Parmeliaceae | Imshaugia aleurites | 100% | 14.0 | 336 | 0 |
| Incertae sedis | Incertae sedis | Leohumicola verrucosa | 100% | 13.0 | 368 | 0 |
| Lecanorales | Parmeliaceae | Parmotrema stuppeum | 100% | 12.0 | 340 | 2.94E-176 |
| Incertae sedis | Pseudeurotiaceae | Pseudogymnoascus destructans | 98.60% | 11.0 | 363 | 1.20E-180 |
| Eurotiales | Trichocomaceae | Talaromyces marneffei | 100% | 11.0 | 348 | 1.04E-180 |
| Hypocreales | Hypocreaceae | Trichoderma hamatum | 100% | 10.0 | 354 | 0 |
| Lecanorales | Parmeliaceae | Parmotrema ultralucens | 100% | 10.0 | 366 | 0 |
| Hypocreales | Cordycipitaceae | Beauveria bassiana | 100% | 7.0 | 334 | 6.65E-173 |
| Onygenales | Myxotrichaeceae | Myxotrichum deflexum | 100% | 7.0 | 340 | 2.91E-176 |
| Hypocreales | Hypocreaceae | Trichoderma asperellum | 100% | 7.0 | 366 | 0 |
| Hypocreales | Cordycipitaceae | Lecanicillium saksenae | 100% | 6.0 | 348 | 1.17E-180 |
| Lepidoptera | Nymphalidae | Charaxes jasius | 100% | 6.0 | 332 | 7.99E-172 |
| Lepidoptera | Noctuidae | Xestia badicollis | 100% | 6.0 | 337 | 1.32E-174 |
| Hypocreales | Hypocreaceae | Trichoderma gamsii | 100% | 5.0 | 314 | 8.96E-162 |
| Hypocreales | Incertae sedis | Verticillium nonalfalfae | 96.20% | 5.0 | 339 | 4.42E-150 |
| Coleoptera | Curulionidae | Strophosoma melanogrammum | 98.10% | 5.0 | 363 | 2.34E-177 |
| Lecanorales | Parmeliaceae | Parmotrema stuppeum | 100% | 4.0 | 340 | 2.82E-176 |

**Table 5:** Full list of species found in the fecal samples collected at Gilmore Marsh.

| Gilmore Marsh | | | | | | |
|---|---|---|---|---|---|---|
| **Order** | **Family** | **Species** | **Percent Identity** | **Assigned Reads** | **Sequence Length** | **E Value** |
| Passeriformes | Passerellidae | Melospiza melodia | 97.64% | 598,436 | 381 | 0.0 |
| Passeriformes | Passerellidae | Zonotrichia albicollis | 98.41% | 76,785 | 377 | 0.0 |
| Diptera | Tipulidae | Tipula hermannia | 99.41% | 44,906 | 315 | 4E-159 |
| Coleoptera | Curculionidae | Sciaphilus asperatus | 99.42% | 36,464 | 346 | 3E-176 |
| Coleoptera | Curculionidae | Strophosoma melanogrammum | 99.42% | 33,803 | 346 | 3E-176 |
| Coleoptera | Tenebrionidae | Isomira quadristriata | 98.84% | 28,220 | 344 | 8E-172 |
| Passeriformes | Parulidae | Geothlypis trichas | 98.40% | 15,412 | 376 | 0.0 |
| Rodentia | Cricetidae | Peromyscus leucopus | 98.84% | 8,837 | 344 | 8E-172 |
| Coleoptera | Curculionidae | Acoptus suturalis | 97.12% | 6,379 | 343 | 1E-145 |
| Coleoptera | Rhagionidae | Rhagio mystaceus | 99.71% | 5,025 | 344 | 8E-177 |
| Coleoptera | Curculionidae | Polydrusus cervinus | 98.83% | 4,538 | 342 | 1E-170 |
| Coleoptera | Chrysomelidae | Plateumaris rufa | 99.41% | 2,465 | 341 | 2E-173 |
| Lepidoptera | Tortricidae | Olethreutes tiliana | 99.13% | 2,230 | 344 | 2E-173 |
| Coleoptera | Curculionidae | Otiorhynchus singularis | 99.13% | 1,860 | 343 | 6E-173 |
| Coleoptera | Limoniidae | Ormosia affinis | 99.42% | 1,766 | 347 | 8E-177 |
| Hemiptera | Aphrophoridae | Philaenus spumarius | 97.10% | 1,143 | 345 | 1E-160 |
| Lecanorales | Parmeliaceae | Hypogymnia vittata | 100% | 352 | 359 | 0 |
| Thalassiosirales | Skeletonemataceae | Skeletonema tropicum | 98.40% | 304 | 370 | 0 |
| Coleptera | Elateridae | Limonius aeger | 100% | 270 | 317 | 1.75E-163 |
| Opiliones | Phalangiidae | Rilaena triangularis | 98.60% | 227 | 341 | 1.72E-168 |
| Lecanorales | Parmeliaceae | Parmotrema stuppeum | 100% | 174 | 342 | 2.49E-177 |
| Entomobryomorpha | Entomobryidae | Entomobrya nivalis | 99.60% | 162 | 346 | 8.73E-177 |
| Thalassiosirales | Skeletonemataceae | Skeletonema tropicum | 98.40% | 135 | 368 | 0 |
| Fucales | Fucaceae | Cystoseira tamariscifolia | 100% | 99 | 318 | 6.02E-164 |
| Coleptera | Elateridae | Dalopius vagus | 100% | 79 | 329 | 3.73E-170 |
| Fucales | Fucaceae | Cystoseira tamariscifolia | 100.00% | 63 | 318 | 5.94E-164 |
| Lecanorales | Parmeliaceae | Parmotrema stuppeum | 100% | 52 | 341 | 8.98E-177 |
| Lecanorales | Parmeliaceae | Hypogymnia vittata | 98.30% | 50 | 359 | 3.26E-176 |
| Lecanorales | Parmeliaceae | Imshaugia aleurites | 100% | 49 | 367 | 0 |
| Diptera | Chironomidae | Tanytarsus mendax | 99.70% | 44 | 337 | 6.17E-173 |
| Diptera | Tipulidae | Tipula hermannia | 99.70% | 44 | 325 | 8.07E-167 |
| Lecanorales | Parmeliaceae | Imshaugia aleurites | 100% | 40 | 368 | 0 |
| Fucales | Fucaceae | Cystoseira tamariscifolia | 100% | 36 | 335 | 1.75E-173 |
| Fucales | Sargassaceae | Bifurcaria bifurcata | 98.70% | 35 | 318 | 2.40E-157 |
| Diptera | Culicidae | Culex salinarius | 100% | 32 | 315 | 2.25E-162 |
| Eustigmatales | Eustigmataceae | Nannochloropsis salina | 99.70% | 30 | 367 | 0 |
| Eustigmatales | Eustigmataceae | Nannochloropsis salina | 99.50% | 28 | 375 | 0 |
| Thalassiosirales | Skeletonemataceae | Skeletonema pseudocostatum | 98.10% | 17 | 324 | 1.12E-156 |
| Hemiptera | Nepidae | Nepa hoffmanni | 100% | 16 | 316 | 6.44E-163 |
| Diptera | Tipulidae | Tipula hermannia | 100% | 16 | 340 | 2.85E-176 |
| Ectocarpales | Acinetosporaceae | Hincksia hincksiae | 100% | 15 | 320 | 3.46E-165 |
| Fucales | Sargassaceae | Bifurcaria bifurcata | 100% | 15 | 318 | 4.45E-164 |
| Malasseziales | Malasseziaceae | Malassezia japonica | 99.90% | 14 | 362 | 0 |
| Eustigmatales | Eustigmataceae | Nannochloropsis salina | 100% | 14 | 367 | 0 |
| Sporidio | Sporidiobolaceae | Rhodotorula mucilaginosa | 100% | 14 | 365 | 0 |
| Asparagales | Orchdaceae | Erycina pusilla | 100% | 13 | 331 | 2.99E-171 |
| Sporidiobales | Sporidiobolaceae | Rhodotorula mucilaginosa | 99.10% | 13 | 358 | 3.93E-180 |
| Ectocarpales | Acinetosporaceae | Hincksia hincksiae | 100% | 12 | 319 | 1.42E-164 |
| Microstomatales | Microstromataceae | Jaminaea angkoriensis | 99.20% | 11 | 332 | 2.79E-166 |
| Fucales | Seirococcaceae | Phyllospora carollina | 98.70% | 11 | 346 | 8.68E-172 |
| Lecanorales | Parmeliaceae | Parmotrema ultralucens | 100% | 10 | 366 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Ustilaginales | Ustiliginaceae | Sporisorium scitamineum | 100% | 9 | 323 | 7.99E-169 |
| Hymenoptera | Tenthredinidae | Pristiphora bivittata | 99.70% | 9 | 367 | 0 |
| Ectocarpales | Ectocarpaceae | Pleurocladia lacustris | 100% | 9 | 367 | 0 |
| Lecanorales | Parmeliaceae | Imshaugia aleurites | 100% | 8 | 366 | 0 |
| Caryophyllales | Caryophyllaceae | Silene noctiflora | 100% | 8 | 366 | 0 |
| Thalassiosirales | Skeletonemataceae | Skeletonema cf. pseudocostatum | 99.80% | 8 | 322 | 3.49E-165 |
| Mucorales | Mucoraceae | Lichtheimia ramosa | 100% | 7 | 354 | 0 |
| Lecanorales | Parmeliaceae | Parmotrema ultralucens | 100% | 7 | 366 | 0 |
| Lecanorales | Parmeliaceae | Hypogymnia vittata | 99.70% | 7 | 366 | 0 |
| Tremellales | Tremellaceae | Tremella fuciformis | 99.30% | 6 | 366 | 0 |
| Exobasidales | Branchiodrilidae | Meira miltonrushii | 100% | 6 | 366 | 0 |
| Ustilaginales | Ustiliginaceae | Sporisorium scitamineum | 100% | 6 | 301 | 1.18E-154 |
| Blastocladiales | Blastocladiaceae | Blastocladiella emersonii | 99.90% | 5 | 368 | 0 |
| Ectocarpales | Ectocarpaceae | Pleurocladia lacustris | 100% | 5 | 367 | 0 |
| Microstomatales | Microstromataceae | Jaminaea angkoriensis | 100% | 5 | 340 | 2.82E-176 |
| Lecanorales | Peltigeraceae | Peltigera dolichorrhiza | 99.40% | 5 | 334 | 1.97E-168 |
| Mucorales | Mucoraceae | Lichtheimia ramosa | 99.70% | 5 | 305 | 1.52E-154 |
| Lecanorales | Parmeliaceae | Parmotrema stuppeum | 100% | 4 | 333 | 2.18E-172 |
| Tremellales | Tremellaceae | Hannaella oryzae | 98.50% | 4 | 357 | 1.79E-173 |
| Lecanorales | Peltigeraceae | Peltigera dolichorrhiza | 100% | 4 | 366 | 0 |
| Caryophyllales | Caryophyllaceae | Silene noctiflora | 100% | 4 | 366 | 0 |
| Tremellales | Tremellaceae | Tremella fuciformis | 100% | 4 | 363 | 0 |
| Malasseziales | Malasseziaceae | Malassezia japonica | 100% | 4 | 366 | 0 |