

STOPOVER ECOLOGY AND THE IMPORTANCE OF  
TWO APALACHICOLA BAY BARRIER ISLANDS  
FOR NEOTROPICAL MIGRANT SONGBIRDS  
DURING SPRING MIGRATION

by

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A THESIS

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## **DEDICATION**

I dedicate this work to the memory of my brother, Christopher Mancuso (1982-2016), who showed me that even the biggest obstacles can be overcome with courage, persistence, and a dash of humor.

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# **STOPOVER ECOLOGY AND THE IMPORTANCE OF TWO APALACHICOLA BAY BARRIER ISLANDS FOR NEOTROPICAL MIGRANT SONGBIRDS DURING SPRING MIGRATION**

**Melanie Lauren Mancuso**

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## **ABSTRACT**

Nearctic-Neotropical migrant passerines are experiencing population declines due to habitat loss and degradation on both the wintering grounds and breeding grounds. A decline in suitable habitat along the migration route may also be limiting populations. Stopover habitats are essential along an ecological barrier, such as the Gulf of Mexico, so that birds can rest and refuel after a long nonstop flight. In many cases, barrier islands represent the first piece of land a northbound migrant passerine may encounter after crossing the Gulf during spring migration. However, barrier islands are under significant threats due to climate change, sea-level rise, and other anthropogenic stressors. The goal of this study was to understand the use of these barrier island ecosystems by migrant songbirds during spring migration in order to better inform management decisions as barrier islands face increasing threats.

During the 2015 and 2016 spring migration seasons, I operated banding stations on two barrier islands, St. George Island and St. Vincent Island located in Apalachicola Bay, Florida. I took measurements from all birds captured and took a blood sample from Gray Catbirds (*Dumetella carolinensis*) to be used in plasma metabolite analysis. The number of birds captured per net hour was greater on St. George Island, the smaller and more developed of the two islands. I found the two islands to be relatively equal in terms of measurements of fat scores, muscle

scores, and body mass of captured migrants, suggesting that both islands, although different in many respects, provide important stopover habitat for migrant songbirds that is nearly equal in quality. This was especially true of the Northern Waterthrush (*Parkesia novaboracensis*), which seemed to find adequate resources on both islands. I found no significant effects of sex on fat scores, muscle scores, body mass, capture date, and refueling performance of Gray Catbirds. However, I found that second-year birds were larger than after second-year birds in 2016. Age had a significant effect on capture date on St. George Island with younger birds arriving before older birds in 2015 and older birds arriving before younger birds in 2016. Refueling performance for Gray Catbirds during the entire study period was best explained by year and fat scores.

This study validates previous findings that Apalachicola Bay barrier islands are important for Neotropical migrant songbirds and that more research is needed in this geographical area. In particular, these islands may play a globally important role for transient Northern Waterthrush given the availability of isolated freshwater wetlands on both islands. Overall, both islands appear to provide important stopover habitat for migrant songbirds, although their relative importance may vary depending on annual effects of climate. In some years, one island may provide better stopover habitat for some species over the other island. Management efforts should focus on conserving these barrier islands, particularly their freshwater habitats, and other coastal habitats that are important for Neotropical migrant songbirds, considering they will be increasingly affected by sea-level rise.

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## LIST OF ABBREVIATIONS

GRCA	Gray Catbird ( <i>Dumetella carolinensis</i> )
NOWA	Northern Waterthrush ( <i>Parkesia novaboracensis</i> )
SGI	St. George Island
SVI	St. Vincent Island
BPNH	Birds per net-hour
HY	Hatching-year
AHY	After hatching-year
SY	Second-year
ASY	After second- year
NOAA	National Oceanic and Atmospheric Administration
ANERR	Apalachicola National Estuarine Research Reserve
GOM	Gulf of Mexico
TRIG	triglyceride concentration
μL	microliter
mg/dL	milligrams per deciliter
mmol/L	millimols per liter
CHD	chromo-helicase-DNA binding gene
PCR	polymerase chain reaction
TAE	Tris-acetate-EDTA buffer
AIC	Akaike Information Criterion

## **CHAPTER I**

### **INTRODUCTION**

The northern Gulf of Mexico (hereafter GOM) contains approximately half of the United States' wetlands and is home to over twenty million Americans (National Ocean Service 2008). However, this region is under increasing threats from sea-level rise, erosion, and storm events. Functioning as a protective first line of defense for this area, sit a network of barrier islands throughout the northern GOM. GOM barrier islands vary in age, composition, and substrate, but they were all formed during the Pleistocene or Holocene epochs from deposits of gravel, sand, and shell (National Ocean Service 2008; Rosati and Stone 2009). These islands protect coastal communities and ecosystems from the barrage of storm surge that is created during intense storm events (Stone and McBride 1998). They also form physical barriers that separate oceanic ecosystems from inland wetlands, creating the structure needed to support the GOM's economically lucrative fishing and oyster industries (Edmiston 2008). Therefore, these barrier islands represent valuable economic resources for the GOM.

Northern GOM barrier islands are important ecological resources as well. For example, each summer, Apalachicola Bay barrier islands provide nesting habitat for many sea turtles, including some threatened and endangered species (Edmiston 2008). Shorebirds use GOM barrier islands as both wintering habitats and stopover habitats during migration (Withers 2002). Several threatened, endangered, and rare bird species use GOM barrier islands as nesting areas (Edmiston 2008). Barrier islands are also an important resource for millions of migratory songbirds that use these islands as a stopover site during their migration across the GOM (Mehlman et al. 2005).

Sea-level rise and climate change pose new threats to these economically and ecologically significant barrier island ecosystems. This adds to the anthropogenic threats they are currently facing such as increased development and artificial stabilization (Feagin et al. 2010). Many barrier islands have already experienced land loss, migration, and breaching due to climate change and sea-level rise (Morton 2008). In addition, barrier islands, which absorb the brunt of tropical cyclones, may face higher erosion rates from an expected increase in intensity and frequency of tropical weather systems (Emanuel 2005). For example, the Chandeleur Islands off the coast of Louisiana lost 85% of their surface area during Hurricane Katrina alone (Sallenger et al. 2005).

Current sea-level rise scenarios range from 0.26 m to 0.82 m by the year 2100 due to the warming and expansion of the oceans and the loss of mass from glaciers and ice sheets near the poles (IPCC 2013). This is in addition to the 0.19 m of mean global sea-level rise experienced between 1901 and 2010 (IPCC 2013). Not all regions experience sea-level rise equally. The Apalachicola Bay of Florida, in particular, has a high vulnerability ranking based on its high erosion rates and low slope (Thieler and Hammar-Klose 2006). Over long periods of time, barrier islands can migrate with changes in sea-levels. However, under the current predictions of rapid sea-level rise, these naturally dynamic islands will experience dramatic alterations including saltwater intrusion, loss of sand, and landward migration (Fitzgerald et al. 2008). For example, St. George Island, a barrier island bordering the Apalachicola Bay, may experience a loss of approximately 44% of the forest cover under a scenario of 0.82 m of sea-level rise (Lester et al. 2016). Because of the impending loss or degradation of these barrier island ecosystems, it is imperative to understand the full extent of their functioning as a global resource.

Specifically, little is known about the use of northeastern GOM barrier islands by northbound migrant songbirds. While the eastern Gulf is generally viewed in the literature as less important for migrant songbirds (Moore et al. 1995), a recent study has challenged this idea (Lafleur et al. 2016). Many migratory songbird populations are experiencing declines, and these populations face increasing threats due to habitat loss or degradation throughout their breeding and wintering ranges (Robbins et al. 1989). Furthermore, these birds may struggle to find high-quality stopover habitat during their migrations.

The current study focuses on the functioning of barrier island ecosystems within Apalachicola Bay, Florida, as it relates to northbound passerines during the spring migration season. My objective was to examine the use of two differing barrier islands, St. George and St. Vincent, within the Apalachicola National Estuarine Research Reserve in Florida. In 2015 and 2016, I ran a spring migration banding station on these islands, collecting data from captured migrants. Data presented here examines differences in capture rates, arrival condition, and refueling performance of migratory birds between the two islands as well as temporal differences in physical condition and refueling performance. My goal was to evaluate the importance of these barrier islands for migratory birds and provide data that will help guide management decisions, especially as these islands undergo future anthropogenic alterations and sea-level rise.

## **CHAPTER II**

### **STOPOVER ECOLOGY OF MIGRANT SONGBIRDS ON TWO FLORIDA GULF COAST BARRIER ISLANDS**

#### **2.1 Introduction**

##### **2.1.1 Conservation of Nearctic-Neotropical Songbirds**

Over the past several decades, population sizes of many Nearctic-Neotropical (hereafter Neotropical) migratory songbirds have shown downward trends (Robbins et al. 1989; Holmes and Sherry 2001; Ballard et al. 2003; Lloyd-Evans and Atwood 2004; Rich et al. 2004). Reasons for these declines have been attributed to both changes in the breeding grounds and the wintering grounds (Robbins et al. 1989), with more studies reporting that deforestation in the tropics is the main culprit (Robbins et al. 1989; Rappole and McDonald 1994; Norris et al. 2004; Keller and Yahner 2006).

However, the importance of habitats used by birds during migration should not be overlooked. Migratory birds can spend approximately one-third of their annual cycle migrating (Mehlman et al. 2005), and it can be the most vulnerable period of their life cycle (Silllett and Holmes 2002). Therefore, the habitats that they use during migration are vital to their survival (Moore and Woodrey 1993; Moore et al. 1995). The ability of a bird to quickly reach the breeding grounds in the spring increases its chances of successful reproduction (Smith and Moore 2005).

Climate change and rising sea levels pose new threats to birds, especially during the vulnerable migration season. Sea-level rise threatens to alter coastal habitats, such as barrier islands, that may prove to be valuable for migratory birds. Changes in precipitation in the tropics

can alter the timing of departure date for north-bound migratory songbirds (Studds and Marra 2011). Migrating birds will also need to adjust their migration timing with a warming climate in order to arrive in the breeding grounds with optimal resource abundance. Previous studies have shown the ability of many species to adjust their migration timing to account for warming spring temperatures (Marra et al. 2005; Hurlbert and Liang 2012), but some species are less flexible with migration timing than others (Hurlbert and Liang 2012). These species will be more vulnerable in the future with a warming climate.

Urbanization and human development are growing threats for migratory birds as well. Anthropogenic stressors such as collisions with man-made objects, feral cat predation, and pollution are estimated to cause the deaths of 500 million to over one billion birds annually in the United States (Erickson et al. 2005). During the migration seasons, birds are often observed stopping over in urban areas including city parks, residential gardens, cemeteries, or small wood lots (Mehlman et al. 2005; Bonter et al. 2009). As human population sizes increase, these areas may become threatened. Recent studies have suggested that these urban areas have the potential to provide high-quality stopover habitats for migratory birds (Matthews and Rodewald 2010; Seewagen et al. 2011). Therefore, small, fragmented forests in migratory corridors can still maintain suitable resources for migratory birds, and they should be protected from further development.

#### 2.1.2 Spring migration and stopover

During the spring migration season, birds travel great distances from their wintering grounds in the tropics and South America to their breeding grounds throughout the temperate and boreal forests of North America. For many birds, this is a long and arduous endeavor, and many fail to return successfully. For example, return rates for a breeding population of Wood Thrush

(*Hylocichla mustelina*) in Delaware averaged 45% for females and 59% for males (Roth and Johnson 1993). Birds can experience starvation or predation during their migration, and many succumb to death due to environmental factors (Moore et al. 1990). Migration is the most vulnerable period in a bird's lifetime (Silllett and Holmes 2002) yet it is essential to their survival and reproduction. Migration can be especially perilous for individuals that must cross an ecological barrier. In Neotropical migrant species, one of the greatest ecological barriers is the Gulf of Mexico. Some birds avoid this obstacle entirely by circumnavigating the Gulf either via the Caribbean islands and Florida or Mexico and Texas (Williams 1945). Others fly directly over the Gulf (Lowery 1946; Stevenson 1957; Gauthreaux 1971), completing the trip with one long flight. Some species will fly over both the GOM and the Caribbean Sea (Heckscher et al. 2011). While crossing a large ecological barrier is more energetically demanding, evolution has favored this migration route in many Neotropical species. Birds that use the trans-Gulf pathway must be prepared with adequate fuel reserves for an extended period of fasting and flight.

Most birds do not complete their entire migration with one long flight, but, rather, make several, short flights, stopping along the way to rest and refuel (Mehlman et al. 2005). Stopover habitats provide a place for these birds to rest, refuel, and avoid predators before continuing their migration (Mehlman et al. 2005). Stopover sites vary in quality and can range from an expansive forest to a residential garden. Additionally, the quality of a stopover site can vary drastically for an individual bird depending on several factors including the condition of the individual, the surrounding environment, and the weather.

Weather conditions greatly influence migration and stopover habitat use by birds. During certain circumstances, a storm or strong winds will make conditions unfavorable for continued flight. This creates a "fallout," in which many migrants, regardless of their physiological

condition, are forced to the ground until favorable conditions resume (Moore and Kerlinger 1987; Simons et al. 2004). During fallout conditions, more migrants are observed at stopover sites (Yaukey and Powell 2008; Kneidel 2016).

Some stopover sites are not normally used by birds, but are critically important during emergency situations. These sites have been classified in the literature as “fire escapes” (Mehlman et al. 2005). Birds use “fire escapes” during inclement weather, or when migrants find themselves in a physiologically stressed condition.

Managing and conserving stopover sites has become a priority for the conservation of Neotropical migratory species (Donovan et al. 2002; Mehlman et al. 2005). Therefore, studies of stopover ecology for migrants are necessary in order to better understand factors that affect their migration, especially as the global climate and environment faces multiple anthropogenic changes.

### 2.1.3 The use of Gulf coast barrier islands as stopover sites

For migrant birds that migrate directly across the GOM in the spring, barrier islands represent the first place that these individuals are able to make landfall. Many birds are capable of continued flight to more suitable stopover sites (Gauthreaux and Belser 1999; Buler and Moore 2011). However, barrier islands are similar to “fire escape” stopover sites in which an individual may only use the island during unfavorable weather conditions or when the bird is physiologically stressed. Indeed, most birds that land on barrier island stopover sites arrive with reduced fuel reserves (Kuenzi et al. 1991; Yong and Moore 1997; Kneidel 2016). Those individuals are forced to make landfall, regain their fat reserves, and possibly rest before continuing northward to their destination.

Several studies of migrating passerines along the north coast of the GOM have examined patterns of arrival as well as the physical condition of migrants upon arrival. Peak spring migration is generally between late-April and early May (Moore et al. 1990), with most birds arriving during the mid-day or early afternoon hours (Gauthreaux and Belser 1999). Typically males arrive along the Gulf coast earlier in the season than females (Moore et al. 1990). This is most likely due to the reproductive advantage of males establishing territories before females reach the breeding grounds. Many lean birds that arrive on island stopover sites will remain there for a longer period of time as compared to fatter birds (Yong and Moore 1997, Cohen et al. 2014). Often they will have an increased foraging rate compared to birds storing more fat (Yong and Moore 2005). Migrants can also face competition for food at stopover sites (Moore and Yong 1991).

While numerous studies of stopover sites have been conducted on the northwestern and north-central coasts of the GOM, few studies confirm the use of the eastern Gulf as a significant stopover area. However, Lafleur et al. (2016) recently provided radar data supporting the use of the Florida panhandle by migrant birds. They suggest that the high density of forested habitats within close proximity to the Florida Gulf coast is an attractive stopover option for migrants traveling either across the Gulf or those that have been blown off course from their Caribbean flights. Therefore, more study is needed on the use of barrier island stopover sites off the coast of the Florida panhandle in order to aid in the goal of conserving Neotropical migrant species.

This chapter focuses on differences between stopover habitat quality on St. George and St. Vincent Islands. To examine these differences, I collected data on capture rates, fat and muscle scores, and body mass of migrants captured during the peak of spring migration. I expected birds stopping over in a higher quality habitat to be in better physical condition.

Differences between sex and age classes for Gray Catbirds (*Dumetella carolinensis*) were also examined. Additionally, I investigated temporal differences in body mass for the two most commonly captured species. I hypothesize that St. Vincent Island, the larger and less-disturbed of the two islands, will provide a better stopover habitat for migratory songbirds. I predict that:

- 1.) Birds captured on St. Vincent Island will be in better condition in terms of their body mass, fat scores, and muscle scores.
- 2.) Male Gray Catbirds will arrive earlier than females. Male migrant songbirds have been found to arrive on Gulf coast island stopover sites before females (Moore et al. 1990).
- 3.) Male Gray Catbirds will also be in better condition due to the higher time sensitivity of their migration.
- 4.) I do not expect to find differences in physical condition between SY (second-year) and ASY (after second-year) Gray Catbirds. This hypothesis is supported by Woodrey and Moore (1997) who found no differences in fat scores for HY (hatching-year) and ASY Gray Catbirds during fall migration on the Gulf coast.
- 5.) St. George Island will have a higher capture rate than St. Vincent Island, due to the large size of St. Vincent and the higher diversity of habitat types compared to St. George.
- 6.) I expect to find a relationship between time of day and body mass with heavier birds captured later in the day.
- 7.) I hypothesize that there will be no relationship between body mass and date.

## **2.2 Methods**

### **2.2.1 Study Sites**

Research was conducted at two locations within the Apalachicola National Estuarine Research Reserve (ANERR) in Florida during the 2015 - 2016 spring migration seasons.

ANERR is one of 29 coastal reserves throughout the country established through the Coastal Zone Management Act with the purpose of protecting and researching estuarine habitats. The ANERR encompasses over 240,000 acres of the Apalachicola Bay, barrier islands, and portions of the lower Apalachicola River and its associated floodplains, tidal creeks, and marshes (Edmiston 2008). Fresh water input is provided to the bay via the Apalachicola River, which receives water from the Chattahoochee and Flint Rivers, draining portions of Alabama and Georgia. The reserve is managed by multiple federal and state agencies including the US Fish and Wildlife Service and the Florida Department of Environmental Protection.

The first study site, St. George Island, is located southeast of the mouth of the Apalachicola River (Figure 2.1). St. George Island is approximately 34 km long, 0.5 km wide, and contains approximately 30 km<sup>2</sup> of land and 5 km<sup>2</sup> of marsh (ANERR 1998; Edmiston 2008). Connected to the mainland via the Bryan Patton Bridge, this island has undergone rapid human development in recent years (ANERR 1998). Much of the island is currently developed with a few exceptions. St. George Island State Park, located on the eastern side of the island, contains approximately 3 km<sup>2</sup> of land (Edmiston 2008). Additionally, there are a few smaller tracts of land within the boundaries of ANERR including Unit 4 and Nick's Hole.

Research took place on Unit 4 of St. George Island (29.671634°N, 84.845448°W, Figure 2.1). Unit 4 is managed by Florida's Department of Environmental Protection. It contains 86 acres of mesic flatwoods, scrub, and tidal marsh (ANERR 1998). The forest canopy is dominated by slash pine (*Pinus eliottii*), and the thick understory is composed mostly of saw palmetto (*Serenoa repens*), live oak (*Quercus virginiana*), sand live oak (*Quercus geminata*), and wax myrtle (*Myrica cerifera*). The ground cover contains a variety of herbaceous plants and grasses. Closer to the bay, the habitat transforms into a tidal marsh dominated by black

needlerush (*Juncus roemerianus*) and a few other grasses and herbaceous plants interspersed. Unit 4 has a history of human disturbance. It has been heavily fragmented by roads and hiking trails. It is bordered by residential developments and has historically been used as an illegal dumping ground for garbage and construction waste. The native pine forest is fire-dependent; however, a complete prescribed burn has not been conducted on Unit 4 since 1999. A prescribed burn was initiated, but not completed, in 2012, (K. Wren, ANERR Stewardship Coordinator, personal communication, 2015). Management efforts have focused on limiting human disturbance with future plans to conduct prescribed burning (ANERR 1998).

St. Vincent Island was chosen as a second field site for this study (Figure 2.1). The island is vastly different from St. George and offers an interesting comparison. Differing from the typical long and thin barrier islands, St. Vincent is triangular in shape. It is approximately 14 km long and 6 km wide totaling 50 km<sup>2</sup> (Edmiston 2008). St. Vincent Island was acquired by the U.S. Fish and Wildlife Service in 1968 and is currently managed as a National Wildlife Refuge. The island has a well-developed dune and swale topography resulting in a series of parallel ridges ranging from 1 to 2 m in height (Edmiston 2008). The plant communities of the island are diverse and result from the island's unique edaphic conditions and topography. Some of those communities include beaches, tidal marsh, freshwater wetlands, scrub, and pine flatwoods.

My research was conducted in a pine flatwoods habitat bordering a palustrine marsh at the intersection of roads 5 and C (29.648565°N, 85.140796°W; Figure 2.1). The forest canopy of this area is composed of mostly slash pine (*Pinus elliottii*) with a midstory of live oak (*Quercus virginiana*) and cabbage palm (*Sabal palmetto*). The dense understory contains saw palmetto (*Serenoa repens*), *Lyonia* spp., gallberry (*Ilex coriacea*), *Baccharis* spp., and yaupon

(*Ilex vomitoria*). Towards the northeast, the elevation decreases, and the vegetation transforms into marsh grasses. While this island has been spared from most development, it has experienced some human disturbance through hunting, cattle ranching, and logging (US Fish and Wildlife Service 2012). There is a network of gravel and dirt roads that run across the island and a few buildings and structures for management purposes. St. Vincent Island is open to the public, but because no bridges connect it to the mainland, access is limited. St. Vincent is still used for the recreational hunting of sambar deer (*Rusa unicolor*), an introduced species from Asia. Invasive plants and animals such as cogongrass (*Imperata cylindrica*) and feral hogs (*Sus scrofa*) are threats to St. Vincent Island and its plant communities. Refuge staff implement management activities such as prescribed burning, invasive plant control, and hog trapping to protect the island's ecological communities (US Fish and Wildlife Service 2012).

### 2.2.2 Mist-netting and data collection

Our research team ran spring migration banding stations on St. George and St. Vincent Islands in 2015 and 2016. Banding took place during the following time intervals: 13 April – 8 May 2015 and 6 April – 8 May 2016. During 2015, we alternated banding between the two islands every three days, and in 2016, we banded birds on both islands simultaneously with help from a research team at University of Massachusetts at Amherst. We used a combination of 6 m, 9 m, and 12 m nets (approximately 120 m; 36 mm mesh size) as recommended by Ralph et al. (1993) for a well-trained two-person crew. We opened nets every morning approximately thirty minutes prior to sunrise, weather permitting, and closed nets during the midday hours when birds were less active. Under favorable conditions, we reopened nets during the evening hours and closed them prior to sunset. We did not operate nets during rain, high winds, or temperature extremes (The North American Banding Council 2001). We checked mist-nets every 10

minutes, extracted all captured birds, placed them in cloth bags, and transported them back to the banding table for processing.

Each bird was identified to species before being fit with an individually numbered aluminum USGS leg band. Individuals were aged and sexed according to Pyle (1997). Other measurements included fat content on an 8-point scale (DeSante 2015), muscle score (Redfern and Clark 2001), unflattened wing chord (to nearest mm), and body mass (to 0.1 g).

### 2.2.3 Collecting and storing avian blood

I collected a blood sample from the brachial vein of captured Gray Catbirds to be used in molecular sex determination and plasma metabolite analysis. Prior to collection, I used an alcohol swab to move away feathers and sanitize the puncture site. I collected less than the 1% of the body mass or less than 10% of the blood volume as recommended by Fair et al. (2010) and Owen (2011) using a 26-gauge needle (BD precisionGlide) and heparinized micro-hematocrit capillary tubes (Fisherbrand; Guglielmo et al. 2002). This usually amounted to 50-150  $\mu$ L of whole blood, depending on the physical condition of the bird and the ease of blood collection. After I collected blood, I applied pressure until the bleeding stopped. I transferred whole blood immediately to a 1.5 mL plastic centrifuge tube and labeled each vial with the individual's band number, the date, site code, and a designated lab number using a fine lab-quality marker. I stored samples in a cooler in the field with ice packs until banding concluded for the day (usually no more than eight hours).

I recorded capture time and bleed time for each individual. In the event that a Gray Catbird was seen hitting the net, the exact time was recorded. Due to the time-sensitivity of plasma triglycerides (Zajac et al. 2006), I did not collect blood samples if more than twenty minutes had passed since the bird flew into the net (Seewagen et al. 2011).

After mist-netting was concluded for the day, I centrifuged each blood sample for 15 minutes at 2000xG to separate the plasma from the blood cells. I removed the plasma using a micropipette and placed it into a 1 mL Nalgene cryogenic screw-top vial. I labeled each vial with the individual's band number, the 4-letter species code, date, site, an estimate of how much plasma the vial contained, and a designated lab number with a lab-quality fine marker. Since plasma metabolites are sensitive to freezing-thawing (Owen 2011), I stored samples in liquid nitrogen until the conclusion of the banding season. At the end of the season, samples were packed in dry ice for transport from Florida to Delaware. I stored samples in -80° C freezer at Delaware State University, Dover, Delaware until analysis was completed.

I used DNA from the remaining blood cells for determining the sex of each bird. Before the start of the field season, I prepared Queen's Lysis Buffer solution for use in storing residual red blood cells (Seutin et al. 1991). This solution was prepared by combining 1.21 g Tris, 0.58 g NaCl, 20 mL EDTA (0.5M) pH 8.0, and 10 g n-lauroylsarcosine. I added 800 mL of double-distilled water to bring the volume up to 1 L. I stirred the mixture until all particles were dissolved and autoclaved the solution. The final concentrations of this buffer solution was 0.01 M Tris, 0.01 M NaCl, 0.01 M sodium-EDTA, and 1% n-lauroylsarcosine, pH=8.0.

After I removed plasma from each sample, I added either 1mL (2015) or 200 µL (2016) of Queen's Lysis Buffer to the residual blood cells. These vials were stored at room temperature (Seutin et al. 1991) for the remainder of the field season and then transferred to a -20° C freezer upon return to Delaware State University.

#### 2.2.4 Molecular sex determination

Residual blood cells stored with Queen's Lysis Buffer were used for determining the sex of Gray Catbirds since this species is sexually monomorphic during migration (Pyle 1997).

Molecular sex determination was attempted for all blood samples of Gray Catbirds in 2015 and all blood samples from St. Vincent Island in 2016. Blood samples taken from Gray Catbirds captured on St. George Island in 2016 were not used for molecular sex determination.

Griffiths et al. (1998) describe the discovery of two chromo-helicase-DNA-binding genes (CHD) and their use in determining the sex of most avian species. The test they describe employs Polymerase Chain Reaction (PCR) to amplify the CHD-W gene, which is located on the W chromosome and found only in female birds, and the CHD-Z gene, which is found on the Z chromosome and present in both sexes. The process for molecular sex determination begins with DNA extraction, followed by PCR, and finalized with visualization using gel electrophoresis. Due to low initial success rates with molecular sex determination, I used different methods throughout the process. Appendix I summarizes successes and failures of the process in order to better inform future molecular sex determinations.

I extracted DNA using either of two different methods. For the first method, I used Zygem prepGEM blood DNA extraction kit following the Quick-start Guide (Zygem). I first brought all blood samples to room temperature. Using a thin-walled 0.2 mL PCR tube, I combined 76.5  $\mu$ L of PCR-grade pure water, 5  $\mu$ L of 10 mM  $\text{CaCl}_2$ , 10  $\mu$ L of 10x buffer, 1  $\mu$ L of prepGEM, and 7.5  $\mu$ L of blood sample. Each tube was thoroughly mixed and placed in a thermocycler (Eppendorf Nexus Gradient or Bio-rad Mycycler). The samples ran in the thermocycler at 75° C for 15 minutes followed by 95° C for 5 minutes. Samples were removed and centrifuged at 14,000xG for 8 minutes. I carefully removed the supernatant from the tube without disturbing the pellet. I determined DNA concentrations using a spectrophotometer (NanoDrop Lite). The supernatant, which contained DNA, was stored in a -20° C freezer until PCR could be completed.

Due to the low success rate of DNA extraction using the previous method, I used a second method on samples for which I was unable to determine sex. I used the QIAGEN QIAamp DNA mini kit (cat. no. 51304) and followed the methods for the spin protocol as outlined in the QIAamp DNA Mini and Blood Mini Handbook (QIAGEN 2016). To a 1.5 mL microcentrifuge tube, I added 20  $\mu$ L of proteinase K, 200  $\mu$ L blood sample, and 200  $\mu$ L of Buffer AL. I mixed thoroughly by pulse-vortexing for fifteen seconds. Samples were then placed in a hot water bath for ten minutes at 56°C. Next, I added 200  $\mu$ L ethanol, mixed by pulse-vortexing, and briefly centrifuged the samples. This mixture was added to a spin-column with a 2 mL collection tube and centrifuged for one minute. I discarded the filtrate, added 500  $\mu$ L of Buffer AW1 to the spin column, and centrifuged for one minute. Again, I discarded the filtrate, added 500  $\mu$ L of Buffer AW2 to the spin column, and centrifuged for three minutes. The filtrate was discarded and I centrifuged spin columns for one minute. I placed spin columns in labeled 1.5 mL centrifuge tubes and added 200  $\mu$ L Buffer AE to each spin column. I allowed the buffer solution to incubate at room temperature for five minutes before I centrifuged the columns for another minute. This final filtrate contained sample DNA. I determined DNA concentrations using a spectrophotometer (NanoDrop Lite) and froze samples in a -20°C freezer until PCR was completed.

After DNA extraction, I employed PCR to amplify the CHD-W and CHD-Z genes using a thermocycler (Eppendorf Nexus Gradient or Bio-rad Mycycler). The two primers that Griffiths et al. (1998) used were custom-ordered for the current study from Invitrogen Life Technologies (P8 5'-CTC CCA AGG ATG AGR AAY TG-3' and P2 5'-TCT GCA TCG CTA AAT CCT TT-3'). I used two different methods for PCR using either OneTaq 2x Master Mix with Standard Buffer (New England BioLabs Inc. M0482) or LongAmp Taq DNA Polymerase (New England

Biolabs Inc. M0323S). For the first method, a 25  $\mu$ L reaction was made combining 9.5  $\mu$ L PCR-grade pure water, 0.5  $\mu$ L 10 mM P2 primer, 0.5  $\mu$ L 10 mM P8 primer, 12.5  $\mu$ L One*Taq* 2X Master Mix with Standard Buffer, and 2  $\mu$ L template DNA. Each tube was mixed thoroughly and placed in the thermocycler. The reaction was started with an initial denaturation at 94° C for 30 seconds, then 30 cycles of 94° C for 20 seconds, 55° C for 30 seconds, and 68° C for 60 seconds, then a final extension at 68° C for five minutes.

Because of low success rates using One*Taq* I began using LongAmp *Taq* as my second method for the remainder of my samples. For the second method, a 25  $\mu$ L reaction was made by combining 11.25  $\mu$ L PCR-grade pure water, 5  $\mu$ L of 5X LongAmp *Taq* Reaction Buffer, 0.75  $\mu$ L 10 mM dNTPs, 1  $\mu$ L 10  $\mu$ M P2 primer, 1  $\mu$ L 10  $\mu$ M P8 primer, 1  $\mu$ L LongAmp *Taq* DNA Polymerase, and 5  $\mu$ L template DNA. I thoroughly mixed samples and placed them in a thermocycler. The reaction began with an initial denaturation step at 94° C for 30 seconds, followed by 30 cycles of 94° C for 20 seconds, 55° C for 30 seconds, and 65° C for 60 seconds, and a final extension at 65° C for 10 minutes. For both methods, after completion, the reactions were held at 4° C until removed from the thermocycler. If gel electrophoresis was not completed immediately after PCR, samples were stored in a -20° C freezer.

I performed gel electrophoresis to visualize the results. 2% agarose gels were prepared by combining 1 g of agarose with 50 mL of 1x Tris-acetate-EDTA buffer solution (TAE) in an Erlenmeyer flask. I microwaved this mixture for approximately two minutes, stirring occasionally until the agarose was completely dissolved. I added 2  $\mu$ L of Ethidium Bromide (EtBr) and allowed this mixture to cool for five minutes before pouring into a casting tray with a comb in place to form wells. Once gels were solidified, I carefully removed the comb and

poured 1x TAE buffer over the top of the gel, filling the chamber and submerging the gel completely.

I combined 3  $\mu\text{L}$  of a 100-basepair DNA ladder with 2  $\mu\text{L}$  of loading dye and carefully added this to the first and last wells using a micropipette. For each of the remaining wells, I loaded 5  $\mu\text{L}$  of PCR product and 2  $\mu\text{L}$  of loading dye. This process was repeated for each sample. Once all wells were full, I placed the lid on the electrophoresis chamber and plugged it into a power supply. Gels were run at 100 volts for approximately 60 minutes. Once complete, I visualized gels using a BioDoc-It Imaging System (Ultra-Violet Products Ltd). I determined samples with one band to be males and samples with two bands to be females.

#### 2.2.5 Data analysis

I performed all data analysis using R version 3.2.3 (R Core Team 2015), and I determined results to be significant when  $P \leq 0.05$ . All values were first tested for normality using the Shapiro-Wilk test. All data presented are taken from the first capture of each individual bird.

To determine differences between islands in migrant capture numbers, I used a chi-squared goodness-of-fit test. Because the effort between the two islands differed, I first adjusted capture rates of Neotropical migrants to reflect the unequal effort between the two islands. Effort was calculated as net-hours, where one net-hour is a standard 12 m net open for one hour. I averaged the net hours of the two sites and then divided the actual effort by this number to calculate a correction factor (Swanson et al. 2003; Seewagen et al. 2011). I then multiplied the number of captured migrants at each site by this correction factor and compared the two values using a chi-squared test. That analysis was completed for both the 2015 and 2016 seasons.

Since a Shapiro-Wilk test confirmed that values for fat and muscle scores of migrants were not normally distributed, I performed a Wilcoxon rank-sum test to determine differences between fat and muscle scores between islands for both 2015 and 2016.

Observations on St. Vincent Island indicated that many of the birds classified as Neotropical migrants were possibly breeding within the study site. For example, Prothonotary Warblers (*Protonotaria citrea*), White-eyed Vireos (*Vireo griseus*), and Summer Tanagers (*Piranga rubra*) showed breeding characteristics or were observed exhibiting territorial behavior. Since it was difficult to separate breeding individuals from migrants, I chose to examine the two most commonly captured species, Gray Catbird and Northern Waterthrush (*Parkesia novaboracensis*), separately as representative species. The Gray Catbird breeds in temperate forests of North America and is only found on the Gulf coast during the migration and winter seasons (Ryder et al. 2011). The Northern Waterthrush migrates through the Gulf coast and is, therefore, only present during spring and fall migration seasons (Whitaker and Eaton 2014). I compared fat and muscle scores of both species between the two islands using a Wilcoxon rank-sum test.

I used a t-test to confirm that there was no significant difference in wing chord between islands and years in the two most commonly captured migrants. Then, I used a simple linear regression to examine the relationship between mass and wing chord in the two species. A significant relationship meant that the body mass must be adjusted to correlate with body size before subsequent analysis. I followed methods outlined by Pieg and Green (2009) to adjust body mass using the scaled mass model as follows:

$$\tilde{M}_i = M_i \left[ \frac{L_o}{L_i} \right]^{b_{SMA}}$$

where  $M_i$  is the mass of individual  $i$ ;  $L_i$  is the wing chord of individual  $i$ ;  $L_o$  is the arithmetic mean of wing chord for the species; and  $b_{SMA}$  is the scaling exponent estimated by standardized major axis regression of  $M$  on  $L$  (Peig and Green 2009). The value of  $b_{SMA}$  was determined using online software (Bohonak and van der Linde 2004). While other metrics of body size such as tarsus or culmen length can be used, I did not collect that data on all birds. Wing chord measurements were used because this measurement was taken on nearly all individuals.

I compared size-adjusted body mass of the two species between islands using a t-test. I also examined the relationship between size-adjusted body mass and ordinal date as well as the relationship between size-adjusted body mass and capture time using a simple linear regression. Capture time was reported as hours passed since sunrise, and exact times of sunrise were determined using the NOAA Solar Calculator (Earth System Research Laboratory). These analyses were completed for both islands and both years.

For Gray Catbirds only, I used a Wilcoxon rank-sum test to determine the effects of age and sex on fat scores, muscle scores, and capture date. I used a t-test to examine the effects of age and sex on size-adjusted body mass. I also examined the effects of age and sex on capture dates using a Wilcoxon rank-sum test. Since it can be difficult to classify Northern Waterthrushes by sex or age class (Pyle 1997), no analysis was completed on the effect of age or sex on any variables.

## 2.3 Results

### 2.3.1 Capture rates between islands

A summary of effort and captures is provided in Table 2.1. In 2015, mist-netting effort on St. George Island totaled 645.5 net hours. I captured 147 individual birds of 29 different species. Of those, 129 individuals of 24 species were Neotropical migrants. Birds per net hour (BPNH) for Neotropical migrants on St. George Island totaled 0.20. Mist-netting effort on St. Vincent Island in 2015 totaled 702.96 net hours. I captured 116 total individuals of 28 species, including 70 Neotropical migrants from 16 different species. BPNH for Neotropical migrants on St. Vincent Island was 0.10. Adjusting for unequal effort, St. George Island had significantly more Neotropical migrants captured than St. Vincent Island in 2015 ( $\chi^2 = 22.64$ ,  $P < 0.001$ ).

In 2016 on St. George Island, mist-netting effort completed by the research crew with the University of Massachusetts at Amherst totaled 1877.75 net-hours. The crew captured 309 total individual birds of 41 species. From those captured, 243 individuals from 26 species were considered Neotropical migrants. BPNH for Neotropical migrants on St. George Island was 0.13. On St. Vincent Island, mist-netting effort totaled 1941.09 net-hours in 2016. I captured 178 total individuals from 26 species on the island, including 105 individuals from 15 Neotropical migrant species. BPNH for Neotropical migrants on St. Vincent Island in 2016 was 0.05. St. George Island also had significantly more Neotropical migrants captured in 2016 as compared to St. Vincent, adjusting for effort ( $\chi^2 = 59.25$ ,  $P < 0.001$ ).

The most abundant species captured on both islands and in both years was the Gray Catbird. In 2015, 55 Gray Catbirds were captured on St. George Island and 19 were captured on St. Vincent Island. In 2016, with increased effort, 93 were captured on St. George Island, and 33 were captured on St. Vincent. The second most common captured species on both islands and in

both years was the Northern Waterthrush. In 2015, 14 Northern Waterthrushes were captured on both St. George Island and St. Vincent, and in 2016, 32 were captured on St. George Island and 21 on St. Vincent.

### 2.3.2 Fat and muscle scores of migrants

Table 2.2 provides a summary of the comparison of fat and muscle scores of all Neotropical migrant birds, Gray Catbirds, and Northern Waterthrushes captured during the spring migration season in 2015 on St. George and St. Vincent Islands, Florida. Table 2.3 provides the same data for the 2016 season.

In 2015, Neotropical migrants on St. George Island had significantly higher fat scores ( $W = 5450.5$ ,  $P = 0.01$ ; Figure 2.2) and lower muscle scores ( $W = 3848$ ,  $P = 0.04$ ; Figure 2.3) as compared to those captured on St. Vincent Island. In 2016, there was no statistical difference between fat scores ( $W = 12561$ ,  $P = 0.99$ ; Figure 2.2) or muscle scores ( $W = 12225$ ,  $P = 0.68$ ; Figure 2.3) between islands.

Gray Catbirds captured during the 2015 season showed no significant differences between fat scores ( $W = 563$ ,  $P = 0.61$ ; Figure 2.4) or muscle scores ( $W = 534$ ,  $P = 0.85$ ; Figure 2.5) between islands. In 2016, there was no statistical difference in fat scores between islands for Gray Catbirds ( $W = 1233.5$ ,  $P = 0.10$ ; Figure 2.4). However, Gray Catbirds on St. George Island had significantly higher muscle scores ( $W = 1828.5$ ,  $P = 0.04$ ; Figure 2.5).

In 2015, Northern Waterthrushes showed no significant differences in either fat scores ( $W = 124$ ,  $P = 0.22$ ; Figure 2.6) or muscle scores ( $W = 102.5$ ,  $P = 0.83$ ; Figure 2.7) between the two islands. 2016 results were similar, with no significant differences detected in fat scores ( $W = 315$ ,  $P = 0.70$ ; Figure 2.6) or muscle scores ( $W = 311.5$ ,  $P = 0.60$ ; Figure 2.7) for Northern Waterthrushes between islands.

Because Northern Waterthrushes showed no differences between islands, I combined the data to determine if they were different between years. Mean fat scores in 2015 were 1.19, and mean muscle scores were 1.67. In 2016, mean fat scores were 1.43, and mean muscle scores were 1.70. There was no significant difference in either fat scores ( $W = 637$ ,  $P = 0.28$ ) or muscle scores ( $W = 711.5$ ,  $P = 0.72$ ) between years.

### 2.3.3 Size-adjusted body mass for Gray Catbirds

Because there was no significant difference in the wing chords of all Gray Catbirds captured between islands (2015:  $t = 1.56$ ,  $df = 27.43$ ,  $P = 0.13$ ; 2016:  $t = -0.40$ ,  $df = 59.60$ ,  $P = 0.69$ ) and between years ( $t = 1.179$ ,  $df = 152.12$ ,  $P = 0.24$ ), I adjusted the body mass of all Gray Catbirds using the following equation:  $\tilde{M}_i = M_i[88.79/L_i]^{3.232}$ .

In 2015, the average size-adjusted body mass for Gray Catbirds captured on St. George Island was  $34.10 \pm 4.28$ , and the average for St. Vincent Island was  $36.73 \pm 4.67$ . Gray Catbirds on St. Vincent Island had significantly higher size-adjusted body mass ( $t = -2.11$ ,  $df = 27.21$ ,  $P = 0.04$ , Figure 2.8). In 2016, Gray Catbirds on St. George Island had average size-corrected body masses of  $34.50 \pm 4.10$ , and those on St. Vincent Island were  $35.12 \pm 3.92$ . There was no significant difference between islands in 2016 ( $t = -0.77$ ,  $df = 58.65$ ,  $P = 0.45$ , Figure 2.8).

There was no significant relationship between the size-corrected body mass of Gray Catbirds and the date of capture on St. George Island ( $F_{1,52} = 0.59$ ,  $R^2 = 0.01$ ,  $P = 0.44$ ) or St. Vincent Island ( $F_{1,16} = 3.85$ ,  $R^2 = 0.19$ ,  $P = 0.07$ ) in 2015 (Figure 2.9). Similarly, there was no significant relationship in 2016 (St. George:  $F_{1,91} = 0.50$ ,  $R^2 = 0.005$ ,  $P = 0.48$ ; St. Vincent:  $F_{1,31} = 3.50$ ,  $R^2 = 0.10$ ,  $P = 0.07$ ; Figure 2.10).

While all Gray Catbirds captured in both years and on both islands had a positive relationship between size-adjusted body mass and capture time, the results were not statistically

significant (St. George Island, 2015:  $F_{1,52} = 1.01$ ,  $R^2 = 0.02$ ,  $P = 0.32$ ; St. Vincent Island, 2015:  $F_{1,16} = 0.07$ ,  $R^2 = 0.004$ ,  $P = 0.80$ ; Figure 2.11; St. George Island, 2016:  $F_{1,91} = 0.58$ ,  $R^2 = 0.01$ ,  $P = 0.45$ ; St. Vincent Island, 2016:  $F_{1,31} = 3.26$ ,  $R^2 = 0.10$ ,  $P = 0.08$ ; Figure 2.12; Table 2.4).

#### 2.3.4 Effects of age and sex in Gray Catbirds

A summary of captures classified by age and sex is provided in Table 2.5. In 2015, nearly equal amounts of age classes and sexes were captured on both islands. In 2016, there was a slightly lower percentage of ASY Gray Catbirds on both islands (44% on St. George and 45% on St. Vincent), but the values were not significantly different ( $\chi^2 = 0.67$ ,  $P = 0.41$ ). In 2015, the sex of approximately 72% of Gray Catbirds was successfully determined. In 2016, approximately 48% of blood samples from St. Vincent Island were successfully processed. I did not attempt to determine the sex for Gray Catbirds captured on St. George Island in 2016.

Age and sex had no significant effects on fat or muscle scores of captured Gray Catbirds. Since fat and muscle scores in 2015 did not differ between islands, I pooled the data to examine the effects of age and sex on fat and muscle scores. In 2015, there was no difference between the fat ( $W = 585$ ,  $P = 0.27$ ) or muscle scores ( $W = 722$ ,  $P = 0.57$ ) between SY and ASY Gray Catbirds. Sex also had no effect on fat ( $W = 272$ ,  $P = 0.75$ ) or muscle scores ( $W = 287$ ,  $P = 1$ ) in 2015. In 2016, there was no effects of age on either island for fat scores (St. George Island:  $W = 892.5$ ,  $P = 0.66$ ; St. Vincent Island:  $W = 104.5$ ,  $P = 0.27$ ) or muscle scores (St. George Island:  $W = 1034$ ,  $P = 0.40$ ; St. Vincent Island:  $W = 127.5$ ,  $P = 0.39$ ). On St. Vincent Island, sex had no effects on fat scores ( $W = 15.5$ ,  $P = 0.46$ ) or muscle scores (all birds in which sex was determined had muscle scores of 2).

Because there was a difference between the size-adjusted body mass between islands in 2015, I examined the effects of age (Figure 2.13) and sex (Figure 2.14) on size-adjusted body

mass on each island separately for 2015. There was no effects of age on size-adjusted body mass for either island in 2015 (St. George Island:  $t = 0.79$ ,  $df = 50.80$ ,  $P = 0.43$ ; St. Vincent Island:  $t = -0.93$ ,  $df = 14.35$ ,  $P = 0.37$ ). There was also no effect of sex on the size-adjusted body mass of Gray Catbirds on either island (St. George Island:  $t = -0.72$ ,  $df = 32.97$ ,  $P = 0.47$ ; St. Vincent Island:  $t = 1.20$ ,  $df = 6.54$ ,  $P = 0.27$ ). In 2016, SY birds were significantly heavier after adjusting for size ( $t = 3.19$ ,  $df = 113.87$ ,  $P = 0.002$ ). Sex had no effect on size-adjusted body mass on St. Vincent Island in 2016 ( $t = -1.05$ ,  $df = 10.85$ ,  $P = 0.32$ ).

I found that age had more of an effect on capture date than sex, although the effect was different between years (Figure 2.15). Capture dates did not differ between the sexes on either island or year (2015-St. George Island:  $W = 165$ ,  $P = 0.92$ ; 2015-St. Vincent Island:  $W = 27$ ,  $P = 0.15$ ; 2016-St. Vincent Island:  $W = 28.5$ ,  $P = 0.32$ ). However, in 2015, SY birds were captured on average five days earlier than ASY birds on St. George Island ( $W = 220.5$ ,  $P = 0.008$ ). This effect was not seen on St. Vincent Island ( $W = 36.5$ ,  $P = 0.51$ ). In 2016, SY birds were captured approximately 2.5 days after ASY birds on St. George Island ( $W = 1216$ ,  $P = 0.03$ ). Again, this effect was not seen on St. Vincent Island ( $W = 155$ ,  $P = 0.48$ ).

### 2.3.5 Size-adjusted body mass for Northern Waterthrushes

Northern Waterthrushes showed no difference in wing chord measurements between islands in 2015 ( $t = -0.31$ ,  $df = 23.26$ ,  $P = 0.76$ ), but wing chord measurements were significantly larger on St. George Island in 2016 ( $t = 2.36$ ,  $df = 36.38$ ,  $P = 0.02$ ). In 2015, Northern Waterthrushes showed no significant correlation between body mass and wing chord in all sampled birds from both islands combined ( $F_{1,26} = 0.16$ ,  $R^2 = 0.01$ ,  $P = 0.69$ ). Therefore, body mass values did not need to be adjusted to body size. Northern Waterthrushes captured on St. Vincent Island in 2016 showed no significant relationship between body mass and wing chord

( $F_{1,19} = 1.82$ ,  $R^2 = 0.09$ ,  $P = 0.19$ ), but birds captured on St. George Island showed a positive correlation ( $F_{1,30} = 5.29$ ,  $R^2 = 0.15$ ,  $P = 0.03$ ). Therefore, only body masses from Northern Waterthrushes captured on St. George Island in 2016 were adjusted using the following equation:

$$\tilde{M}_i = M_i[74.06/L_i]^{3.474}.$$

In 2015, the average mass of Northern Waterthrushes captured on St. George Island was  $15.77 \pm 1.88$ , and the average mass of Northern Waterthrushes captured on St. Vincent Island was  $14.99 \pm 1.54$ . There was no significant difference in the body masses of Northern Waterthrushes between islands ( $t = 1.21$ ,  $df = 24.99$ ,  $P = 0.24$ , Figure 2.16). In 2016, the average size-corrected body mass of Northern Waterthrushes captured on St. George Island was  $15.04 \pm 1.91$ , and the average body mass of Northern Waterthrushes captured on St. Vincent Island was  $14.73 \pm 1.52$ . Similarly, there was no significant difference in 2016 between the body masses of Northern Waterthrushes between the two islands ( $t = 0.64$ ,  $df = 48.98$ ,  $P = 0.52$ , Figure 2.16). Combining Northern Waterthrushes from both islands showed that there was no significant difference between the body masses and size-adjusted body masses between years ( $t = 1.13$ ,  $df = 55.78$ ,  $P = 0.26$ ).

Body mass had no significant relationship with ordinal date for Northern Waterthrushes captured on St. George Island in 2015 ( $F_{1,12} = 2.01$ ,  $R^2 = 0.14$ ,  $P = 0.18$ ; Figure 2.17). However, body mass was negatively correlated with ordinal date on St. Vincent Island in 2015 ( $F_{1,12} = 28.91$ ,  $R^2 = 0.71$ ,  $P < 0.001$ ; Figure 2.17). In 2016, Northern Waterthrushes captured on both islands showed no significant relationship between size-adjusted body mass and ordinal date (St. George Island:  $F_{1,30} = 0.003$ ,  $R^2 < 0.001$ ,  $P = 0.96$ ; St. Vincent Island:  $F_{1,19} = 3.79$ ,  $R^2 = 0.17$ ,  $P = 0.07$ ; Figure 2.18).

Time of capture had no significant effect on body mass of Northern Waterthrushes on either island in 2015 (St. George Island:  $F_{1,12} = 3.61$ ,  $R^2 = 0.23$ ,  $P = 0.08$ ; St. Vincent Island:  $F_{1,12} = 0.97$ ,  $R^2 = 0.07$ ,  $P = 0.34$ ; Figure 2.19; Table 2.4). However, in 2016, birds with larger body mass were captured later in the day on St. Vincent Island ( $F_{1,19} = 24.72$ ,  $R^2 = 0.57$ ,  $P < 0.001$ , Figure 2.20; Table 2.4). There was no significant relationship for St. George Island in 2016 ( $F_{1,30} = 0.08$ ,  $R^2 = 0.002$ ,  $P = 0.78$ , Figure 2.20).

### 2.3.6 Tables and Figures

**Table 2.1** Summary of effort and Neotropical migrant songbird captures on St. George Island (SGI) and St. Vincent Island (SVI), Apalachicola National Estuarine Research Reserve, Florida, during the spring migration seasons in 2015 and 2016.

	SGI, 2015	SVI, 2015	SGI, 2016	SVI, 2016
Birds banded	129	70	243	105
Species	24	16	26	15
Net Hours	645.5	702.96	1877.75	1941.09
Birds per net hour	0.20	0.10	0.13	0.05

**Table 2.2** Fat and muscle scores for all Neotropical migrant songbirds, Gray Catbirds (GRCA), and Northern Waterthrushes (NOWA) in 2015 on both St. George Island (SGI) and St. Vincent Island (SVI) in Apalachicola National Estuarine Research Reserve, Florida.

	SGI	SVI	$P$ value <sup>1</sup>
Migrant mean fat score $\pm$ SE	$1.52 \pm 0.11$	$1.10 \pm 0.14$	0.01*
Migrant mean muscle score $\pm$ SE	$1.71 \pm 0.05$	$1.89 \pm 0.06$	0.05*
GRCA mean fat score $\pm$ SE	$2.09 \pm 0.16$	$1.95 \pm 0.28$	0.61
GRCA mean muscle score $\pm$ SE	$1.87 \pm 0.06$	$1.84 \pm 0.09$	0.85
NOWA mean fat score $\pm$ SE	$1.64 \pm 0.45$	$0.71 \pm 0.19$	0.22
NOWA mean muscle score $\pm$ SE	$1.71 \pm 0.16$	$1.64 \pm 0.13$	0.83

<sup>1</sup> Wilcoxon Rank-Sum Test

\* Significant results,  $P < 0.05$

**Table 2.3** Fat and muscle scores for all Neotropical migrant songbirds, Gray Catbirds (GRCA), and Northern Waterthrushes (NOWA) in 2016 on both St. George Island (SGI) and St. Vincent Island (SVI) in Apalachicola National Estuarine Research Reserve, Florida.

	St. George Island	St. Vincent Island	<i>P</i> value <sup>1</sup>
Migrant mean fat score $\pm$ SE	1.42 $\pm$ 0.08	1.46 $\pm$ 0.13	0.99
Migrant mean muscle score $\pm$ SE	1.80 $\pm$ 0.05	1.86 $\pm$ 0.05	0.68
GRCA mean fat score $\pm$ SE	2.00 $\pm$ 0.14	2.45 $\pm$ 0.25	0.10
GRCA mean muscle score $\pm$ SE	2.18 $\pm$ 0.06	1.97 $\pm$ 0.03	0.04*
NOWA mean fat score $\pm$ SE	1.38 $\pm$ 0.22	1.52 $\pm$ 0.29	0.70
NOWA mean muscle score $\pm$ SE	1.66 $\pm$ 0.12	1.76 $\pm$ 0.10	0.60

<sup>1</sup> Wilcoxon rank-sum test

\* Significant results, *P* < 0.05

**Table 2.4** Daily mass change of Gray Catbirds and Northern Waterthrushes on St. George Island (SGI) and St. Vincent Island (SVI), Apalachicola National Estuarine Research Reserve, Florida, in 2015 and 2016. Size- corrected body mass (SCBM) is reported as mean  $\pm$  SE.

Species	Year	Island	N	SCBM	Slope	R <sup>2</sup>	P value <sup>1</sup>
Gray Catbird	2015	SGI	52	34.10 $\pm$ 0.58	0.36	0.02	0.32
		SVI	18	36.73 $\pm$ 1.10	0.14	0.004	0.80
	2016	SGI	93	34.50 $\pm$ 0.43	0.23	0.01	0.45
		SVI	33	35.12 $\pm$ 0.68	0.65	0.10	0.08
Northern Waterthrush	2015	SGI	14	15.77 $\pm$ 0.50	0.42	0.23	0.08
		SVI	14	14.99 $\pm$ 0.41	0.19	0.07	0.34
	2016	SGI	32	15.04 $\pm$ 0.34	0.05	0.002	0.78
		SVI	21	14.73 $\pm$ 0.33	0.39	0.57	<0.001*

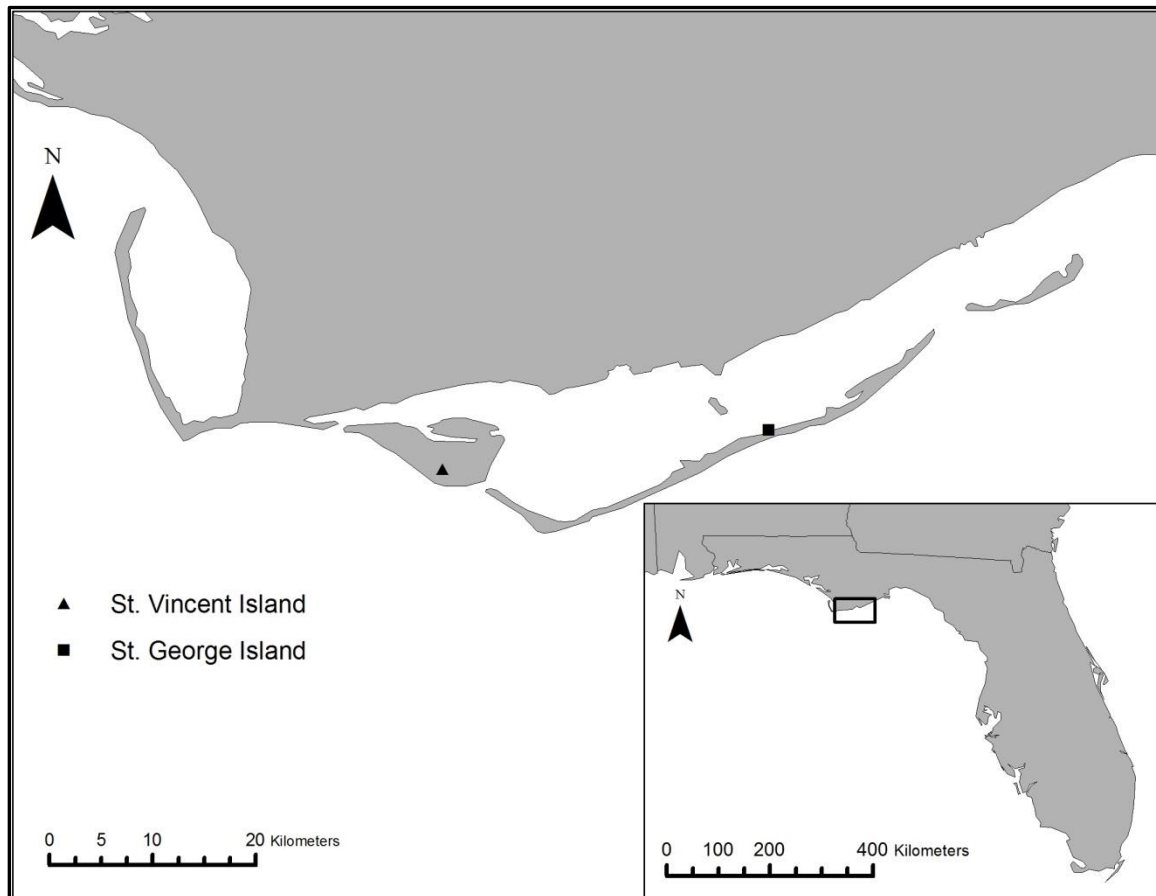
<sup>1</sup> Linear regression

\* Significant results, *P* < 0.05

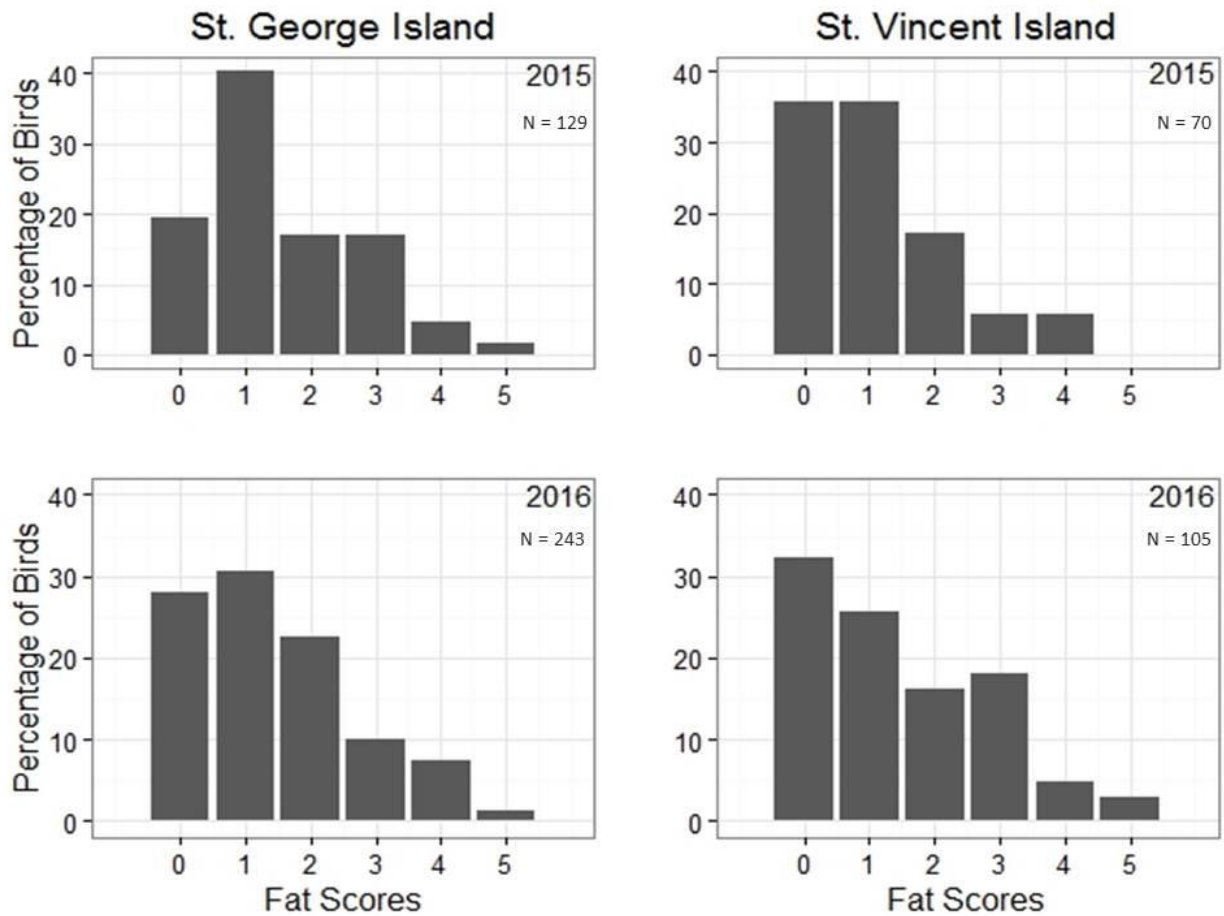
**Table 2.5** Numbers of captured Gray Catbirds on St. George Island (SGI) and St. Vincent Island (SVI), Apalachicola National Estuarine Research Reserve, Florida, in 2015, 2016, and both years combined. Age = SY (second-year), ASY (after second-year), or U (unknown, combined after-hatching year and ages that were not determined). A sex of unknown represents individuals that were unsuccessful with molecular sex determination or those that were not attempted.

		<b>SGI</b>	<b>SVI</b>	<b>Totals</b>
<b>2015</b>				
Captures		55	19	<b>74</b>
Age	SY	28	10	38
	ASY	27	9	36
Sex	M	21	6	27
	F	20	6	26
	U	14	7	21
<b>2016</b>				
Captures		93	33	<b>126</b>
Age	SY	47	18	65
	ASY	41	15	56
	U	5	0	5
Sex	M		10	10
	F		6	6
	U	93	17	17
<b>Total</b>				
Captures		148	52	<b>200</b>
Age	SY	75	28	103
	ASY	68	24	92
	U	5	0	5
Sex	M	21	16	37
	F	20	12	32
	U	107	24	131

**Figure 2.1** The Florida Gulf coast and barrier islands within the Apalachicola Bay, Apalachicola National Estuarine Research Reserve, Florida. Locations of banding stations on St. George Island and St. Vincent Island are marked.



**Figure 2.2** Percentages of fat scores for all Neotropical migrant songbirds captured on St. George and St. Vincent Islands, Apalachicola National Estuarine Research Reserve, Florida, in 2015 and 2016. In 2015, birds on St. George Island had significantly higher fat scores ( $P = 0.01$ ), but this result was not seen in 2016 ( $P = 0.99$ ).



**Figure 2.3** Percentages of muscle scores for all Neotropical migrant songbirds captured on St. George and St. Vincent Islands, Apalachicola National Estuarine Research Reserve, Florida, in 2015 and 2016. In 2015, birds on St. George Island had significantly lower muscle scores ( $P = 0.04$ ), but this result was not seen in 2016 ( $P = 0.68$ ).

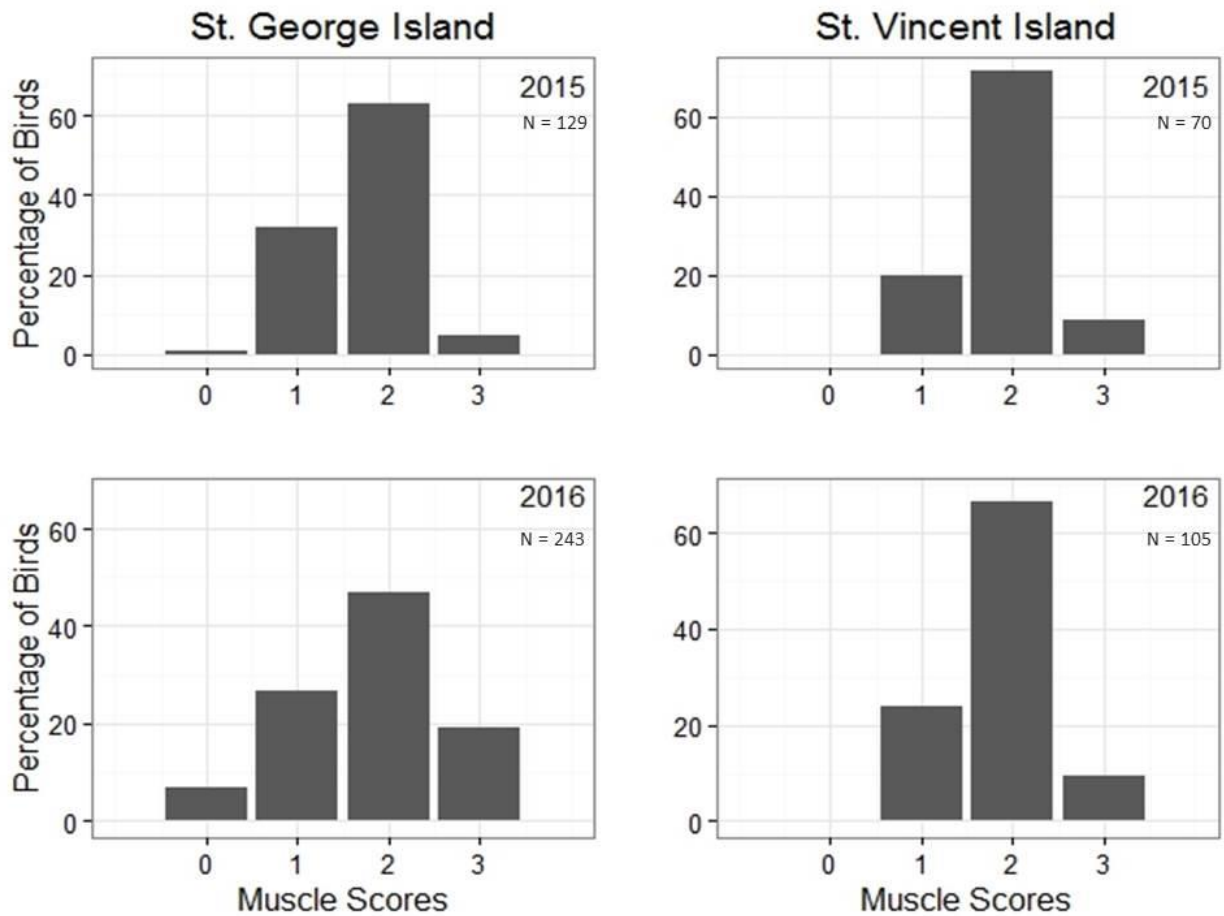
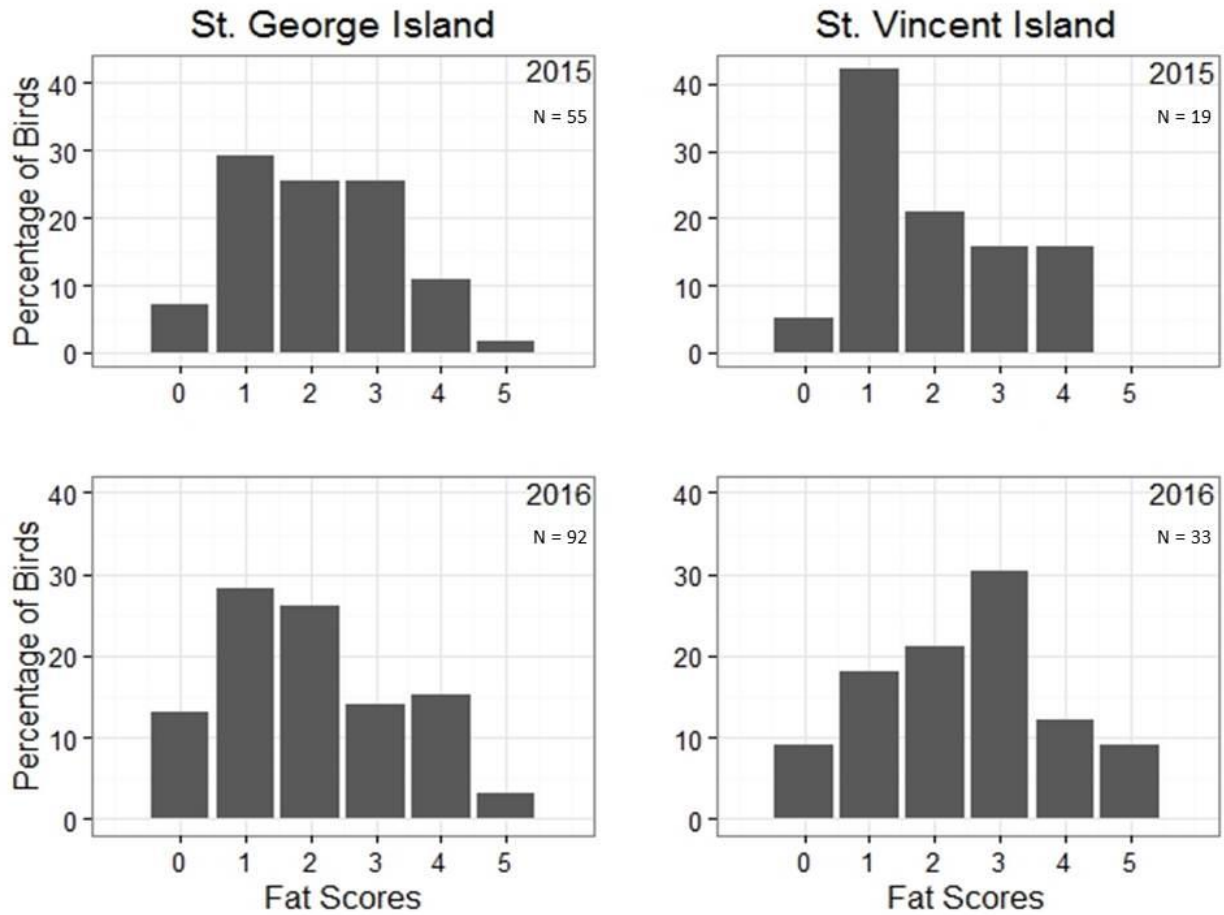
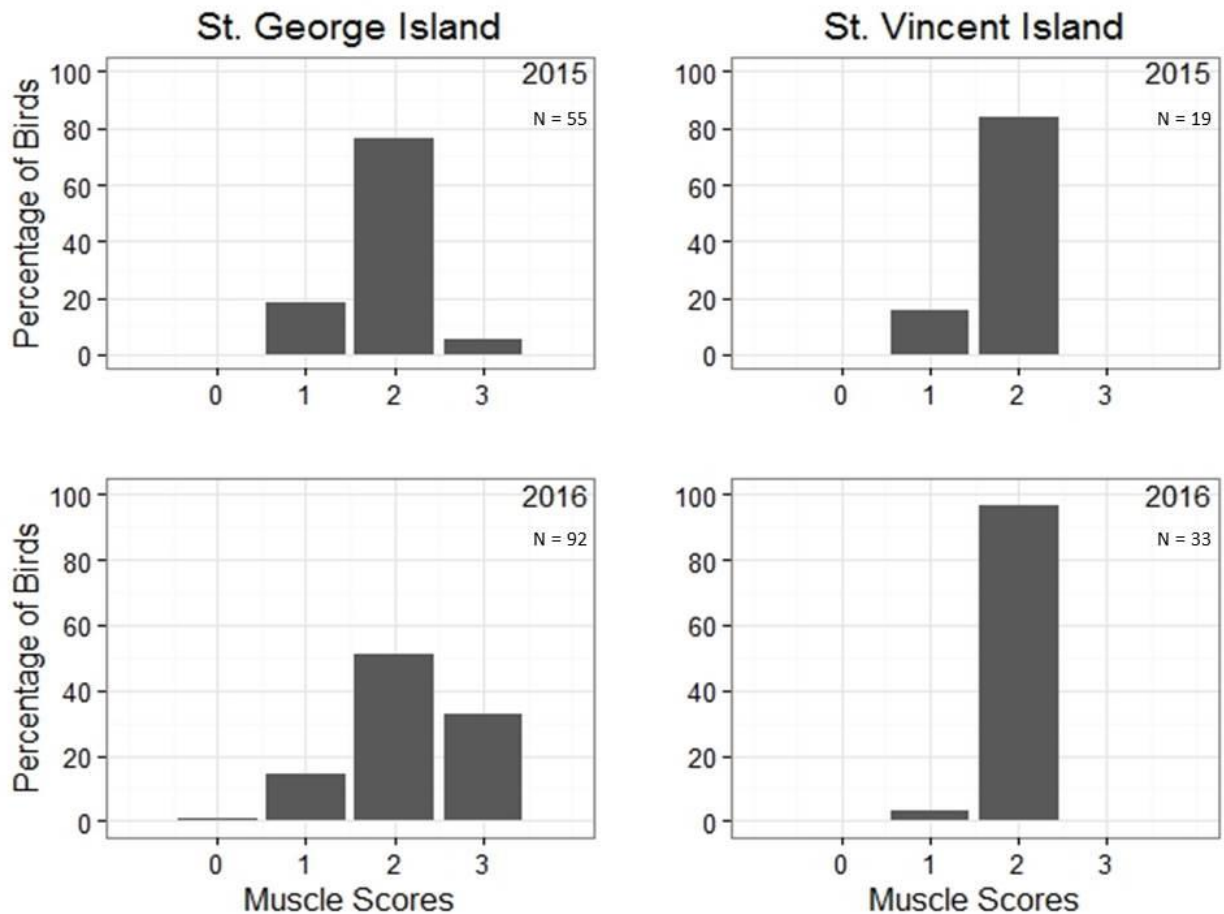


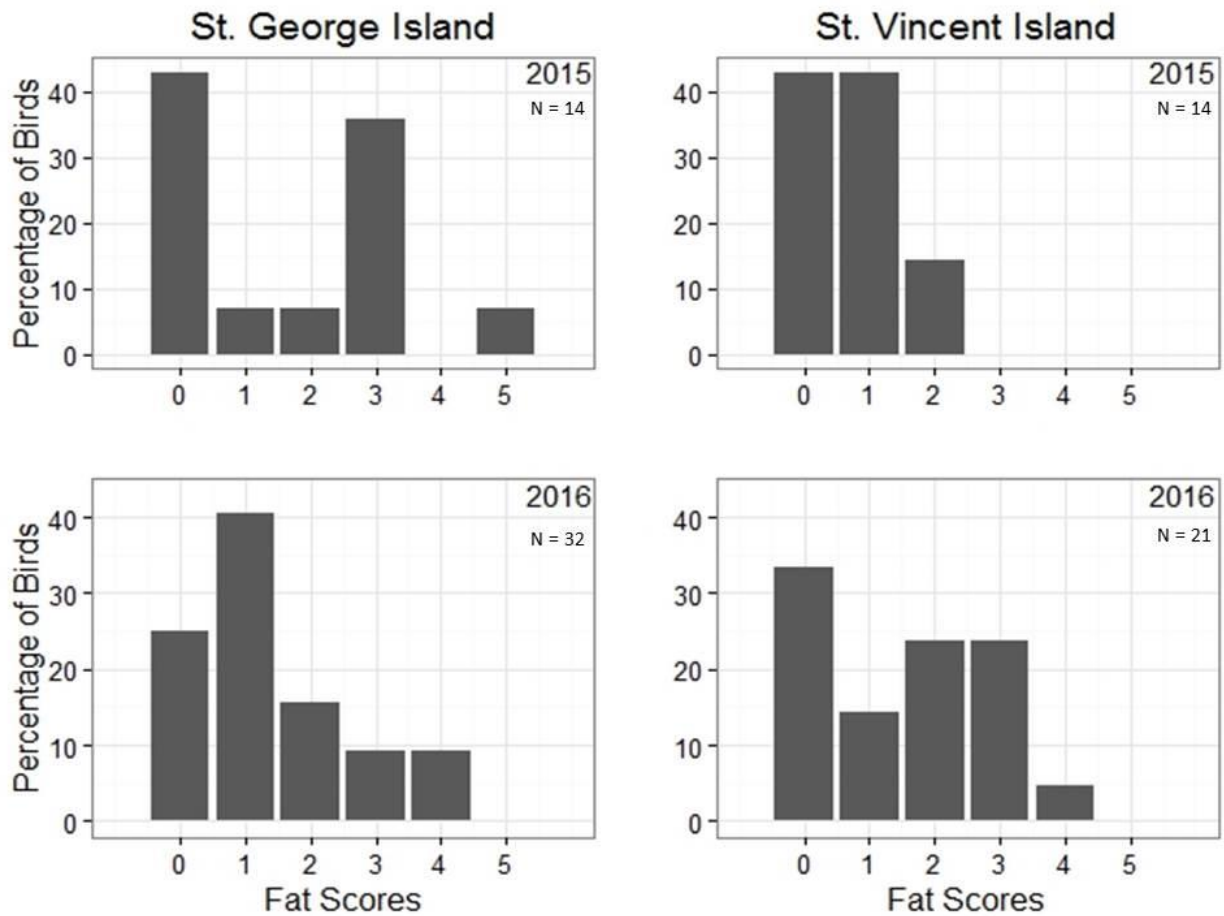
Figure 2.4 Percentages of fat scores of all Gray Catbirds captured on St. George and St. Vincent Islands, Apalachicola National Estuarine Research Reserve, Florida, in 2015 and 2016. There was no significant difference between the fat scores of Gray Catbirds between the two islands in either 2015 ( $P = 0.61$ ) or 2016 ( $P = 0.10$ ).



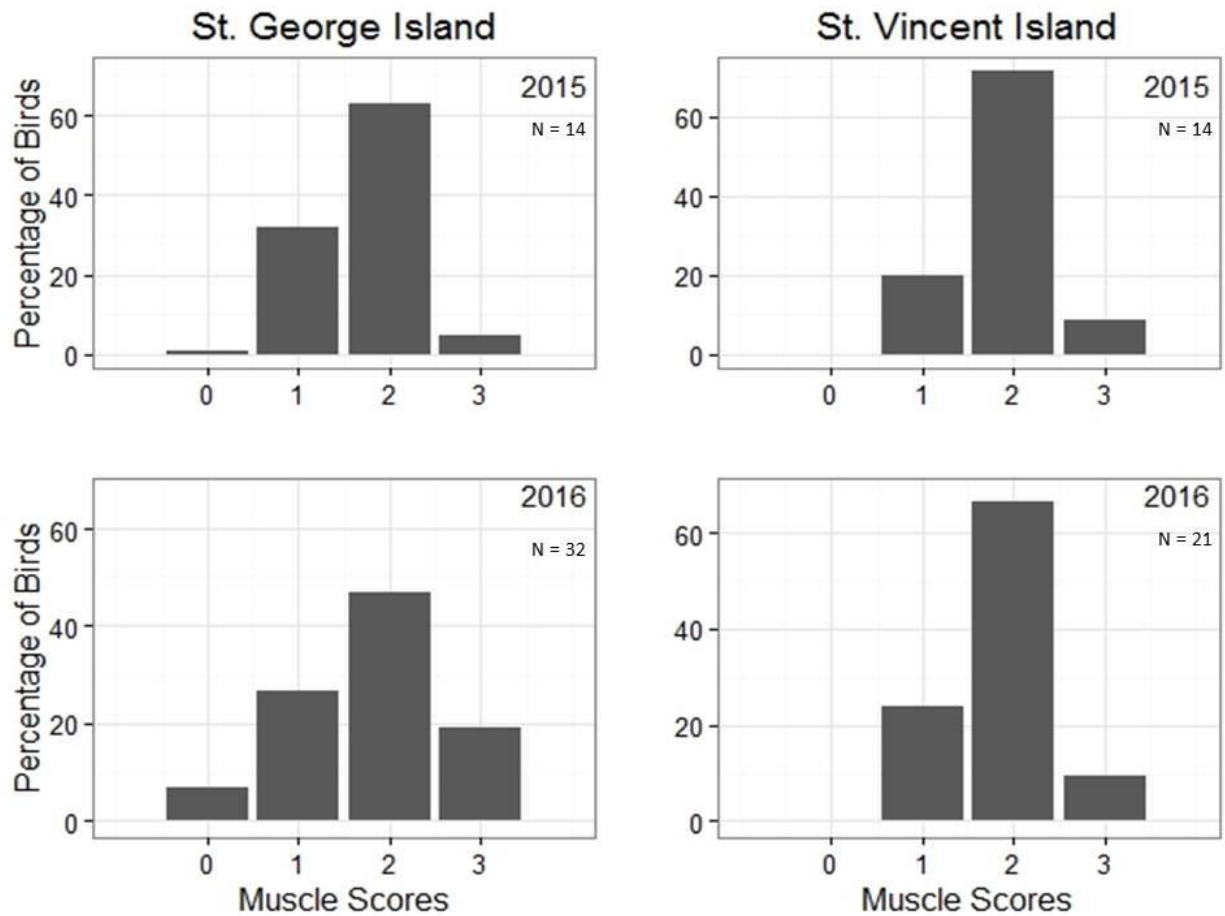
**Figure 2.5** Percentages of muscle scores of all Gray Catbirds captured on St. George and St. Vincent Islands, Apalachicola National Estuarine Research Reserve, Florida, in 2015 and 2016. There was no significant difference between the muscle scores of Gray Catbirds in 2015 ( $P = 0.85$ ). In 2016, Gray Catbirds on St. George Island had higher muscle scores ( $P = 0.04$ ).



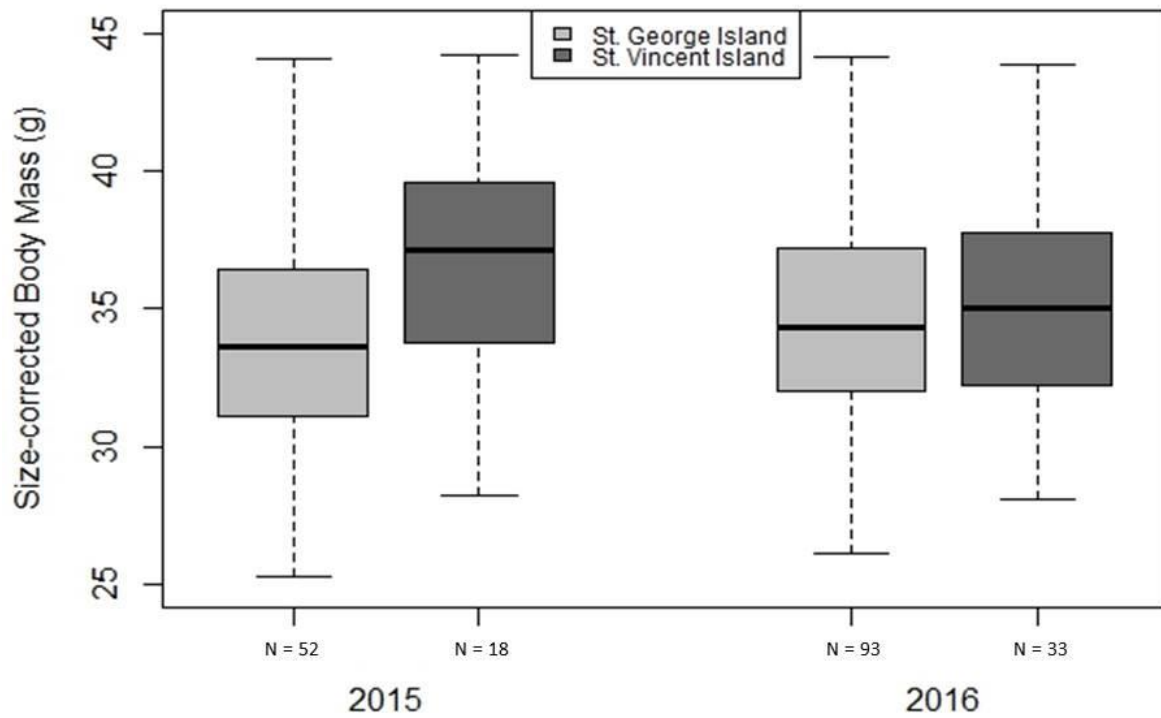
**Figure 2.6** Percentages of fat scores of all Northern Waterthrushes captured on St. George and St. Vincent Islands, Apalachicola National Estuarine Research Reserve, Florida, in 2015 and 2016. There was no significant difference between the fat scores of Northern Waterthrushes between the two islands in either 2015 ( $P = 0.22$ ) or 2016 ( $P = 0.70$ ).



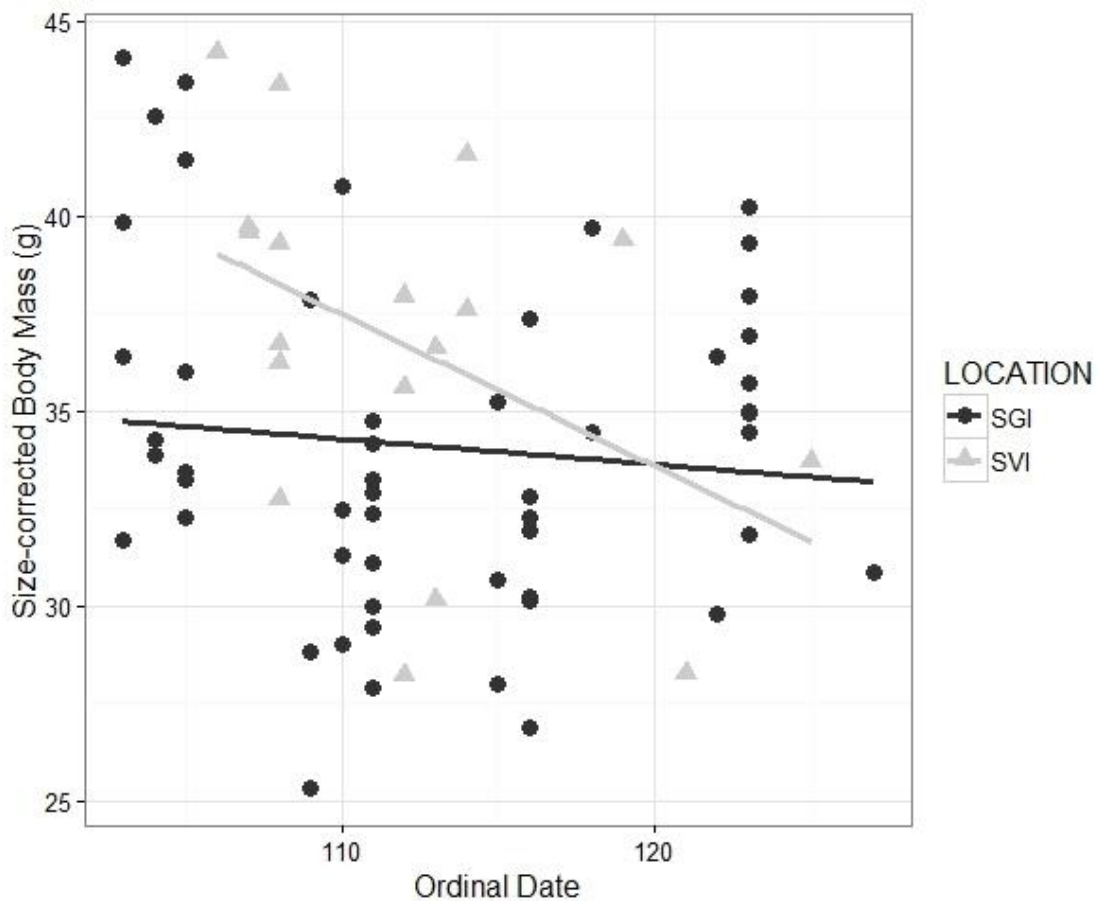
**Figure 2.7** Percentages of muscle scores of all Northern Waterthrushes captured on St. George and St. Vincent Islands, Apalachicola National Estuarine Research Reserve, Florida, in 2015 and 2016. There was no significant difference between the muscle scores of Northern Waterthrushes between the two islands in either 2015 ( $P = 0.83$ ) or 2016 ( $P = 0.60$ ).



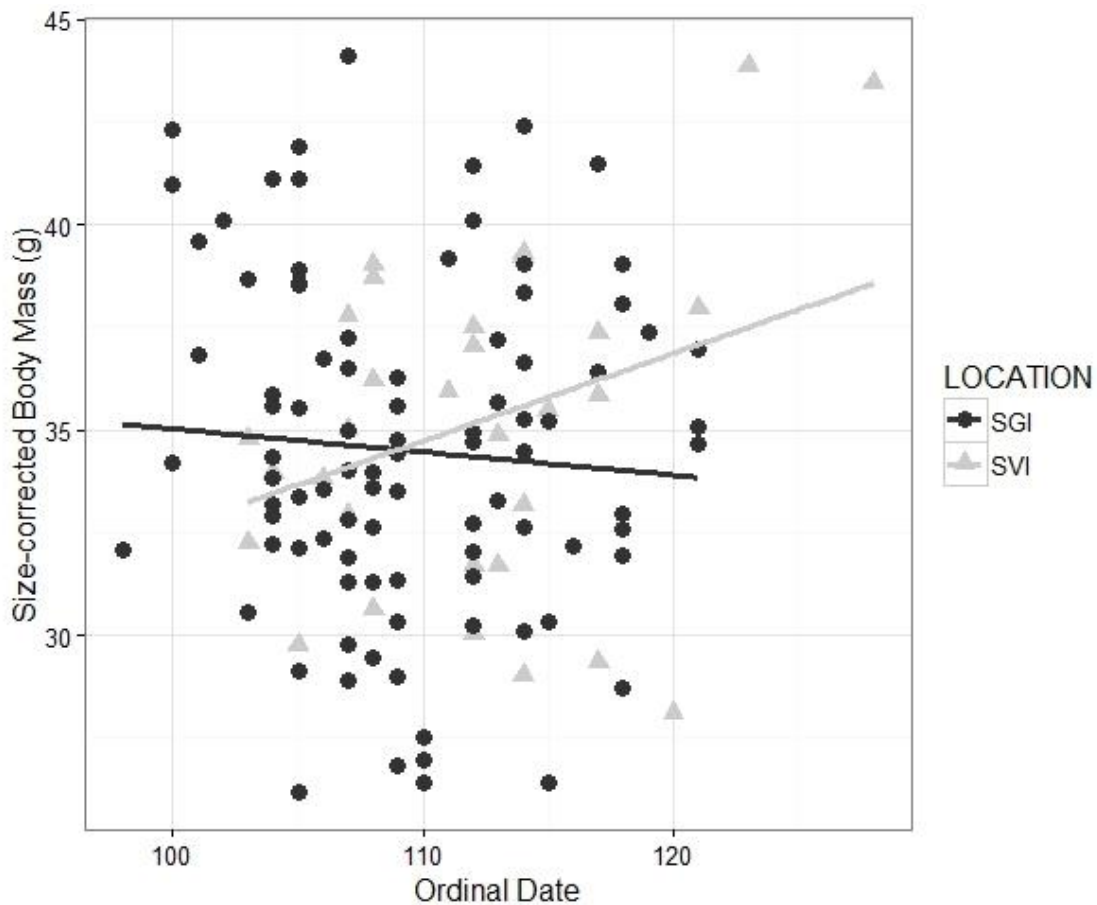
**Figure 2.8** Size-corrected body mass (g) of Gray Catbirds captured on St. George and St. Vincent Islands, Apalachicola National Estuarine Research Reserve, Florida, in 2015 and 2016. Birds captured on St. Vincent were significantly heavier in 2015 ( $P = 0.04$ ). There was no difference between islands in 2016 ( $P = 0.45$ ).



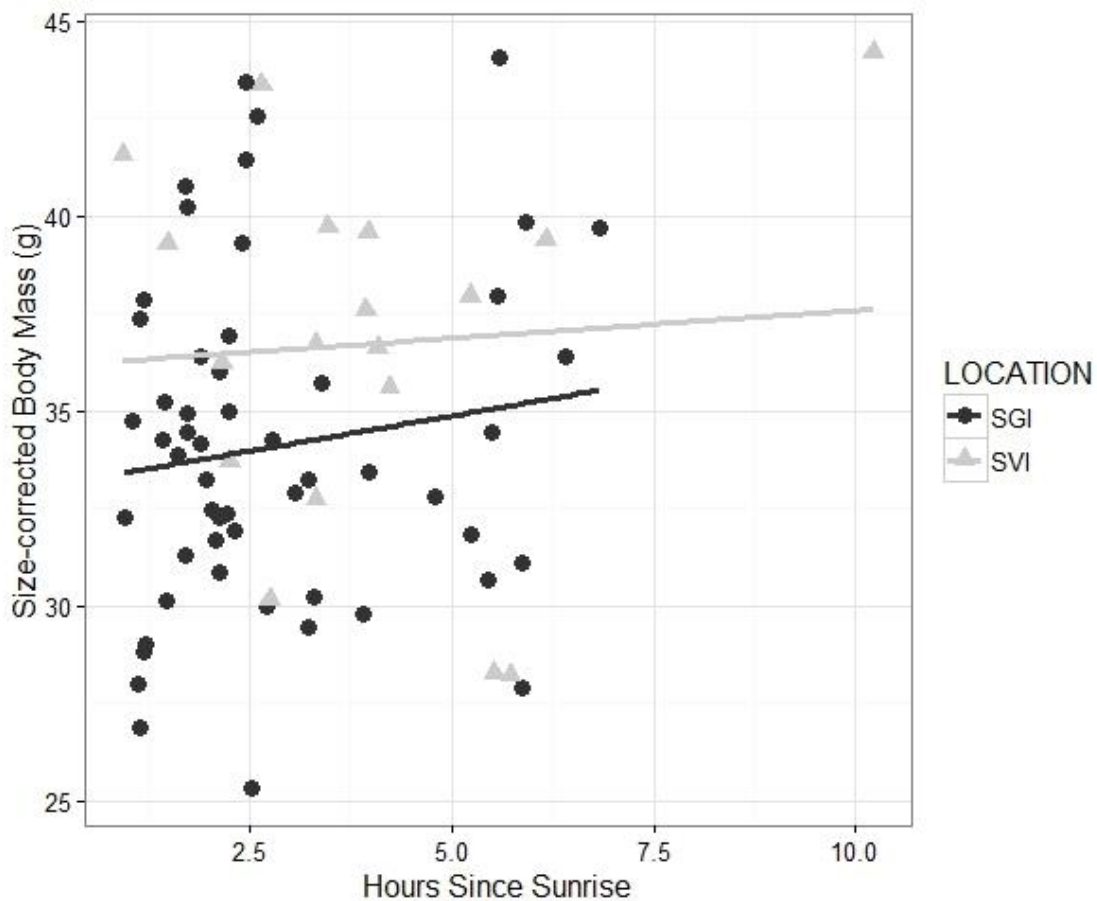
**Figure 2.9** Relationship between size-corrected body mass (g) of Gray Catbirds and ordinal date on St. George Island (SGI) and St. Vincent Islands (SVI), Apalachicola National Estuarine Research Reserve, Florida, in 2015. There was no significant relationship between the two variables for St. George Island ( $P = 0.44$ ). St. Vincent Island trended towards a negative direction ( $P = 0.07$ ).



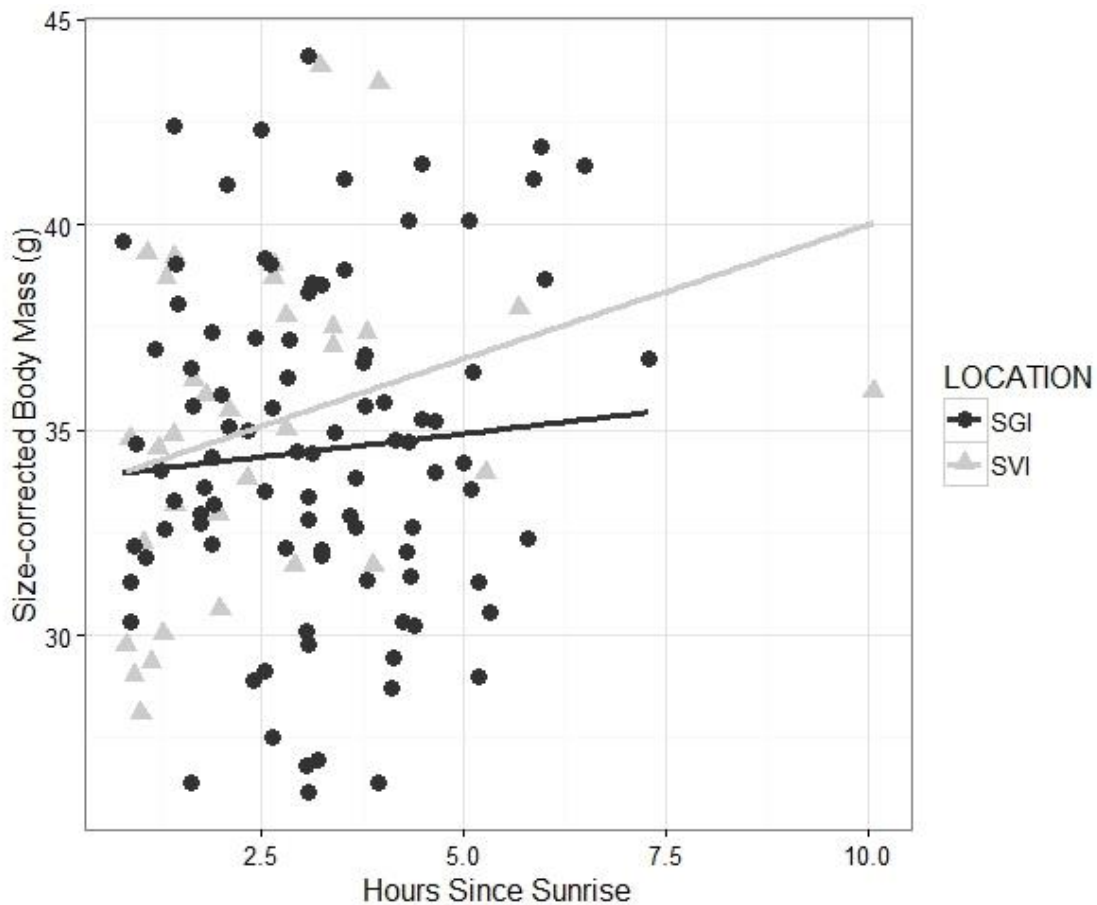
**Figure 2.10** Relationship between size-corrected body mass (g) of Gray Catbirds and ordinal date on St. George Island (SGI) and St. Vincent Island (SVI), Apalachicola National Estuarine Research Reserve, Florida, in 2016. The relationship was not significant for St. George Island ( $P = 0.48$ ), but the relationship trended towards a positive correlation for St. Vincent Island ( $P = 0.07$ ).



**Figure 2.11** Relationship between size-adjusted body mass (g) of Gray Catbirds and time of capture for St. George Island (SGI) and St. Vincent Island (SVI), Apalachicola National Estuarine Research Reserve, Florida, in 2015. Time is measured as hours passed since sunrise. There was no significant relationship for either St. George Island ( $P = 0.32$ ), or St. Vincent Island ( $P = 0.80$ ) in 2015.



**Figure 2.12** Relationship between size-adjusted body mass (g) of Gray Catbirds and time of capture for St. George Island (SGI) and St. Vincent Island (SVI), Apalachicola National Estuarine Research Reserve, Florida, in 2016. Time is measured as hours passed since sunrise. There was no significant relationship for St. George Island ( $P = 0.45$ ), but the relationship trended towards a positive correlation for St. Vincent Island ( $P = 0.08$ ) in 2016.



**Figure 2.13** Size-corrected body mass (g) for second-year (SY) and after second-year (ASY) Gray Catbirds captured on St. George and St. Vincent Islands, Apalachicola National Estuarine Research Reserve, Florida, in 2015 and 2016. Age did not significantly affect size-corrected body mass in 2015 (St. George Island:  $P = 0.43$ ; St. Vincent Island:  $P = 0.37$ ), but SY birds were significantly heavier, after adjusting for size, in 2016 ( $P = 0.002$ ).

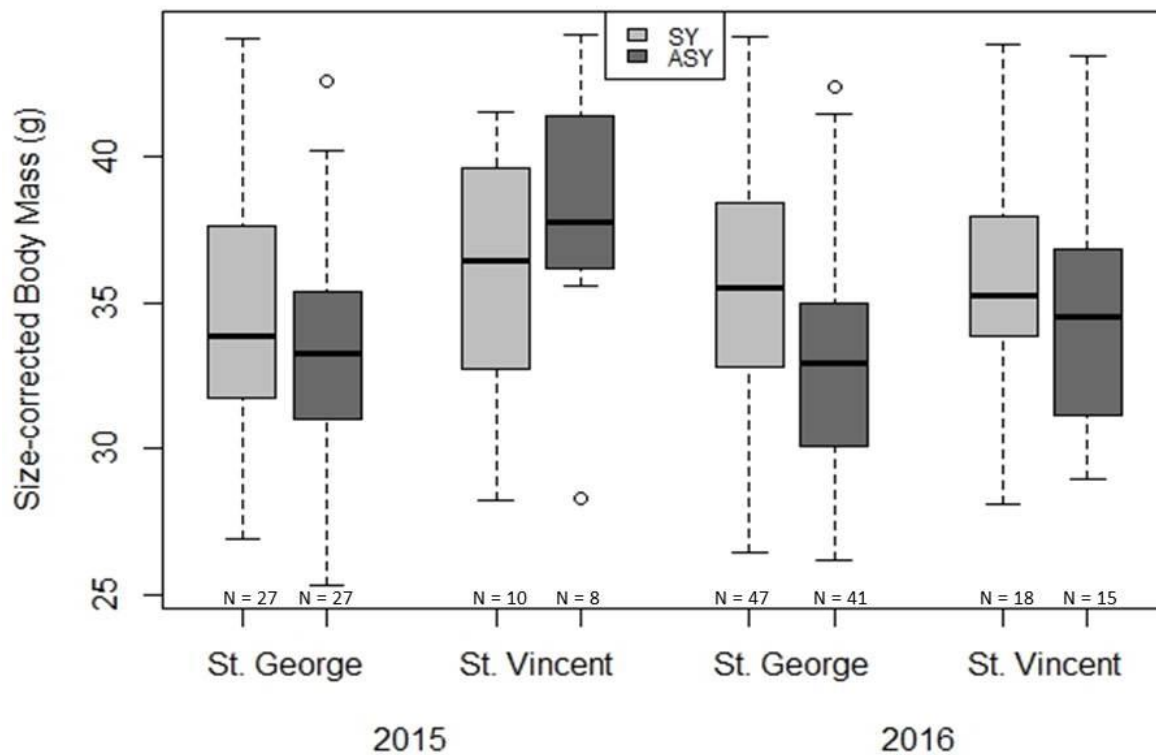


Figure 2.14 Size-corrected body mass (g) for male and female Gray Catbirds captured in Apalachicola National Estuarine Research Reserve, Florida, on both St. George and St. Vincent Islands in 2015 and St. Vincent Island only in 2016. The sex of Gray Catbirds on St. George Island in 2016 was not determined. Sex had no significant effects on size-corrected body mass (St. George Island:  $P = 0.47$ ; St. Vincent Island- 2015:  $P = 0.27$ ; St. Vincent Island- 2016:  $P = 0.32$ ).

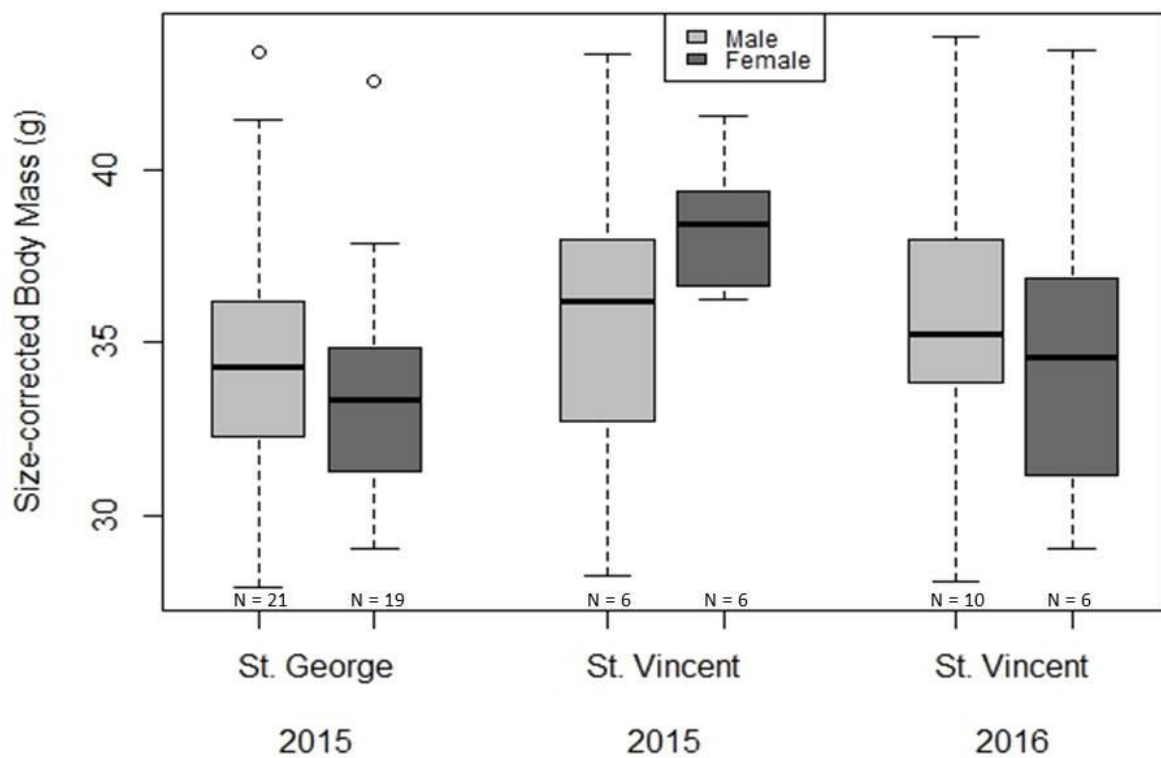
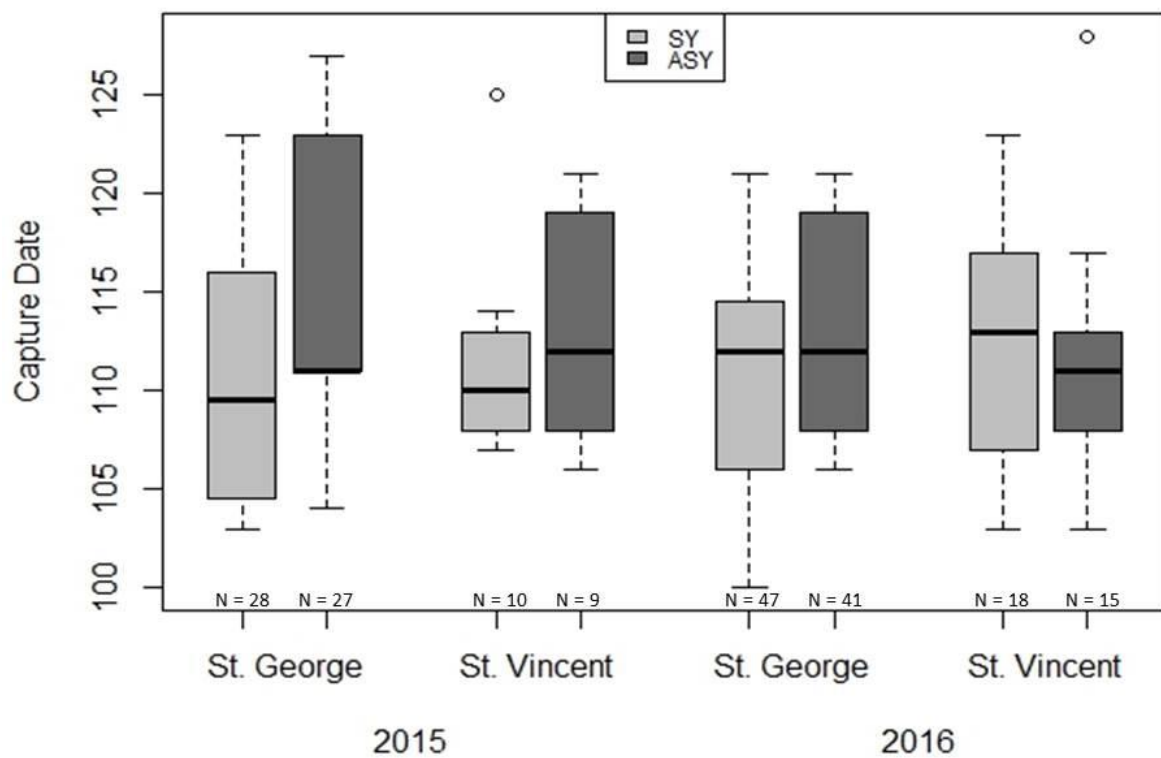
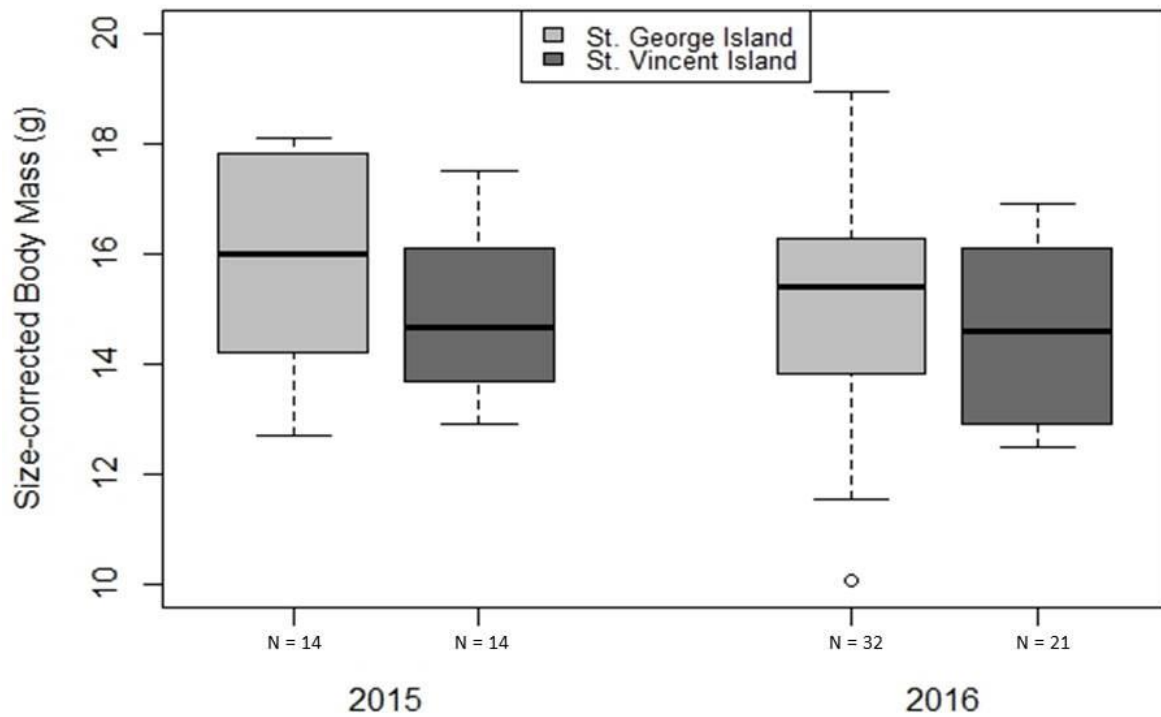


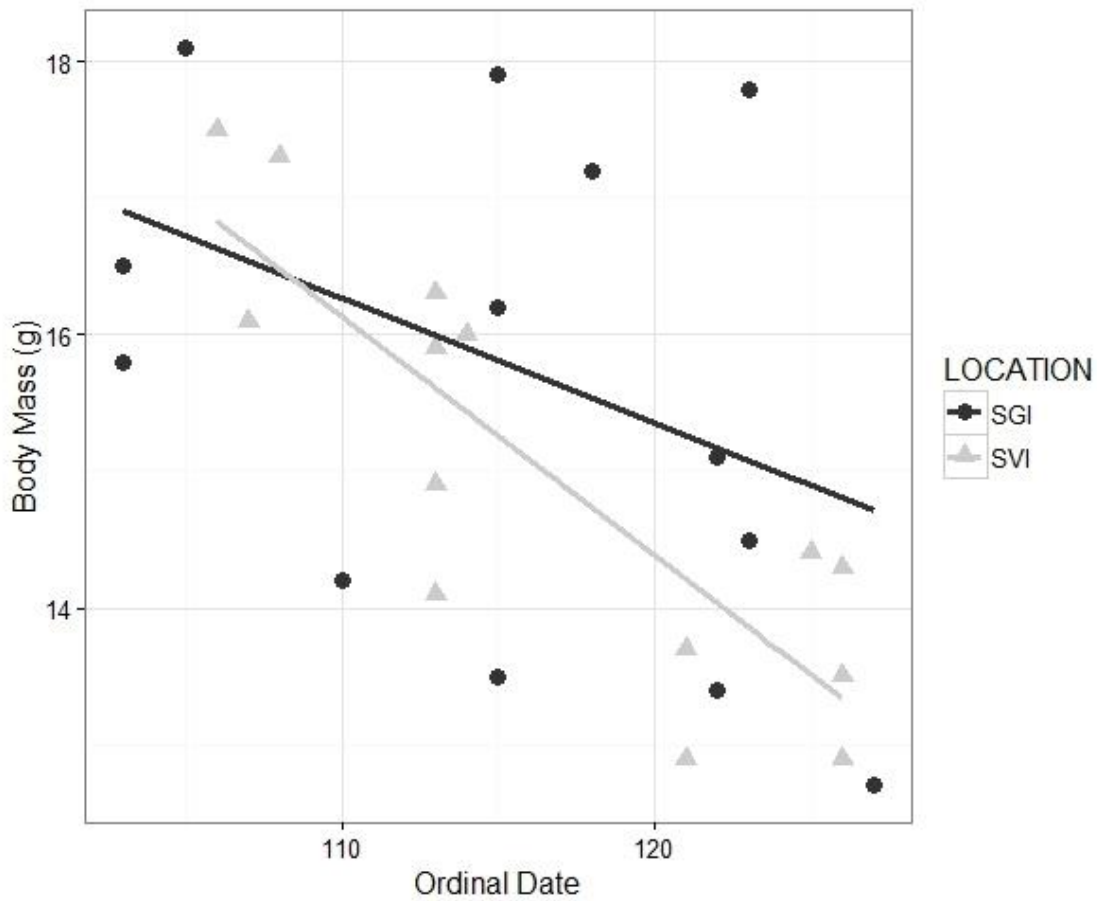
Figure 2.15 Capture dates for second-year (SY) and after second-year (ASY) Gray Catbirds captured on St. George and St. Vincent Islands, Apalachicola National Estuarine Research Reserve, Florida, in 2015 and 2016. Capture date is displayed as ordinal date. There was a significant difference between the capture dates of SY and ASY birds on St. George Island (2015:  $P = 0.008$ ; 2016:  $P = 0.03$ ), but this effect was not seen on St. Vincent Island (2015:  $P = 0.51$ ; 2016:  $P = 0.48$ ).



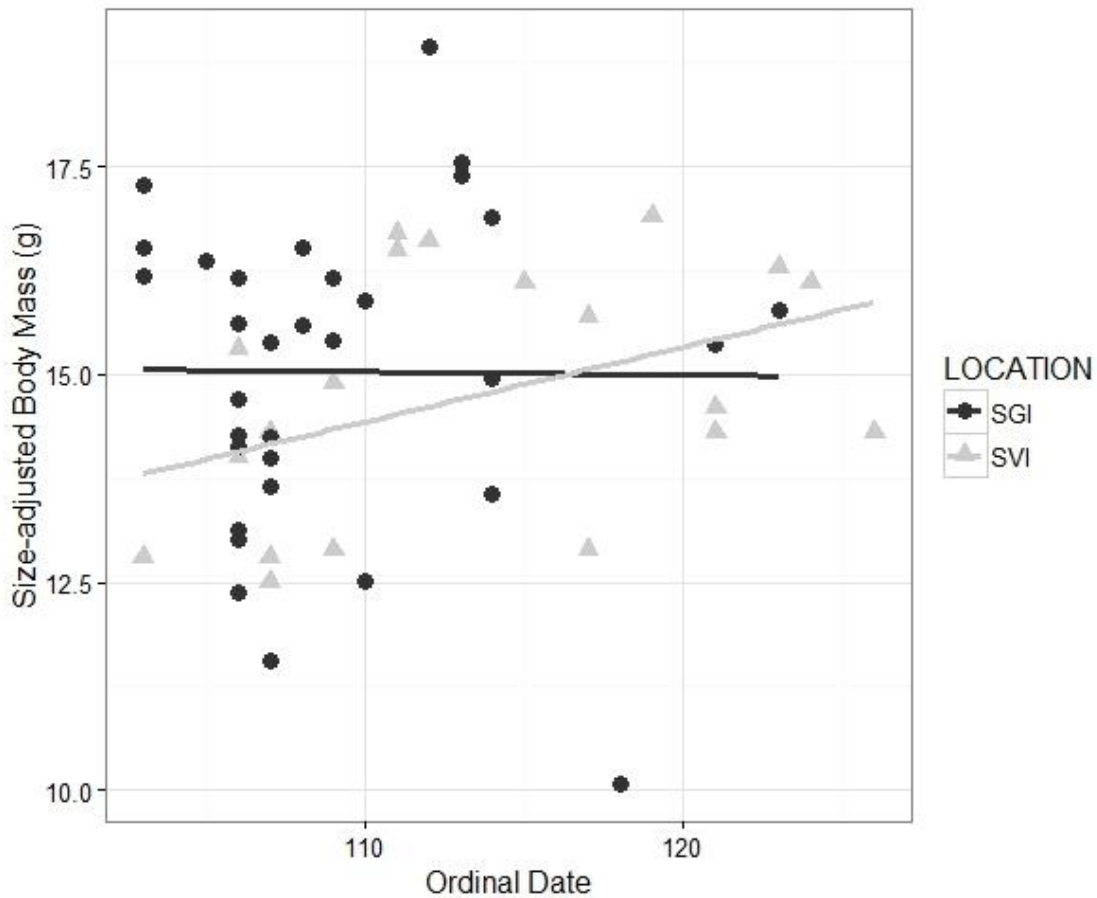
**Figure 2.16** Size-corrected body mass (g) of Northern Waterthrushes captured on St. George and St. Vincent Islands, Apalachicola National Estuarine Research Reserve, Florida, in 2015 and 2016. There was no significant difference between islands for 2015 ( $P = 0.24$ ) or 2016 ( $P = 0.52$ ).



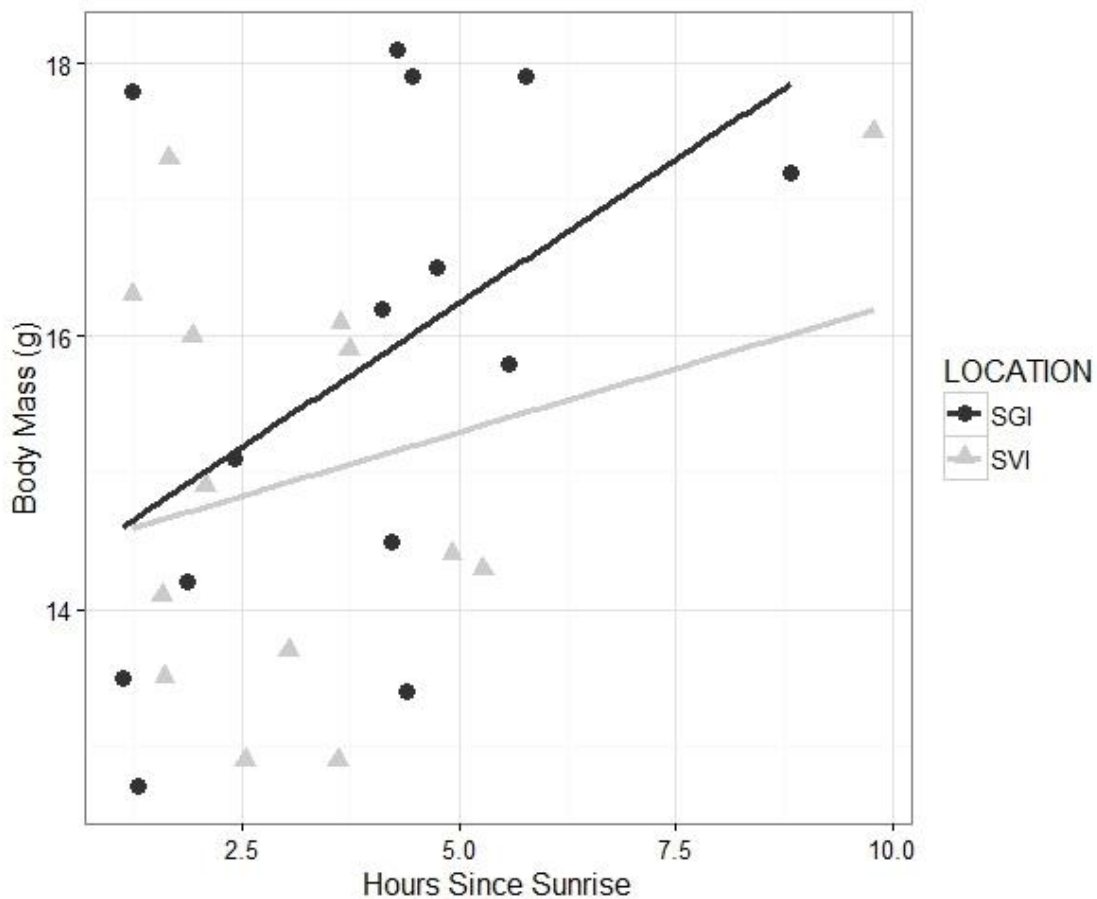
**Figure 2.17** Relationship between size-adjusted body mass (g) of Northern Waterthrushes and ordinal date on both St. George (SGI) and St. Vincent (SVI) islands, Apalachicola National Estuarine Research Reserve, Florida, in 2015. Birds on both islands were negatively correlated with ordinal date, but only the relationship on St. Vincent Island was significant (St. George Island:  $P = 0.18$ ; St. Vincent Island:  $P < 0.001$ ).



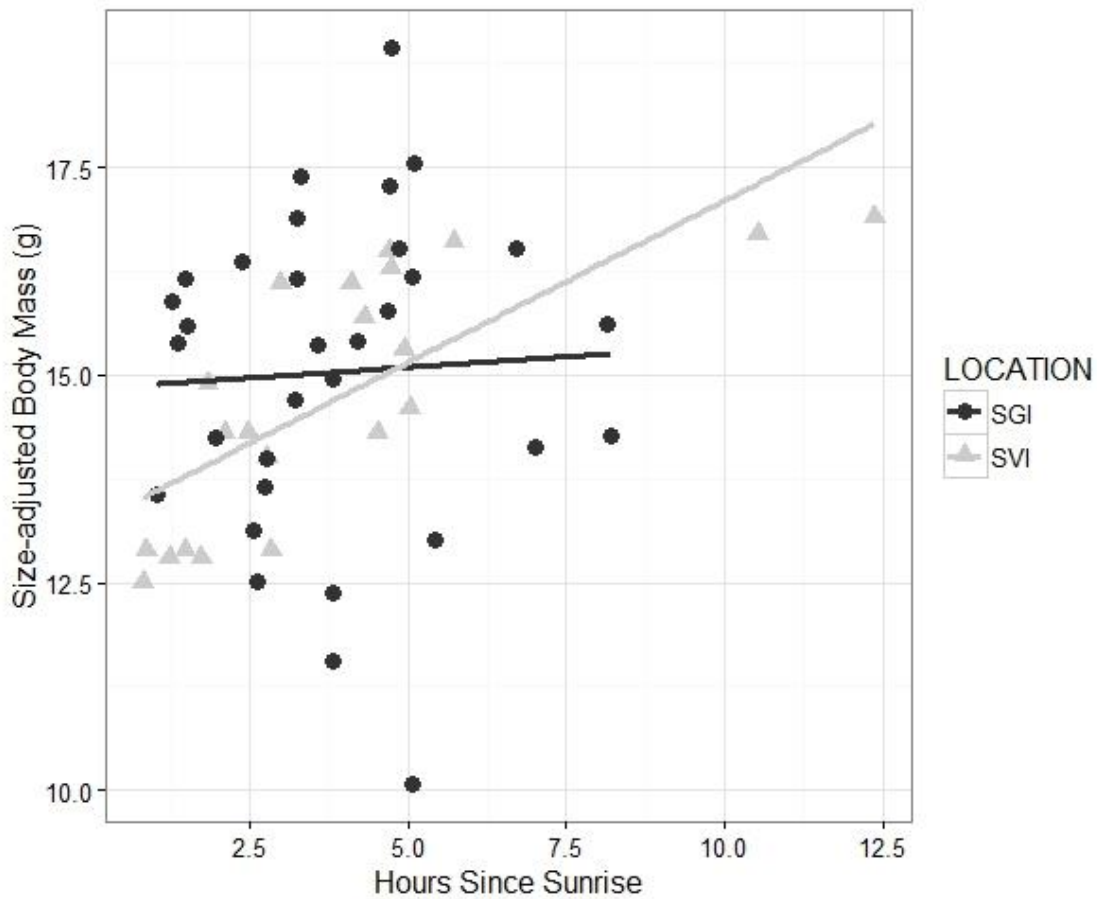
**Figure 2.18** Relationship between size-adjusted body mass (g) of Northern Waterthrushes and ordinal date on both St. George (SGI) and St. Vincent (SVI) islands, Apalachicola National Estuarine Research Reserve, Florida, in 2016. Neither island showed a significant relationship between body mass and ordinal date (St. George Island:  $P = 0.96$ ; St. Vincent Island:  $P = 0.07$ ), but the relationship on St. Vincent Island trended towards a positive correlation.



**Figure 2.19** Relationship between body mass (g) of Northern Waterthrushes and time of capture on St. George Island (SGI) and St. Vincent Island (SVI), Apalachicola National Estuarine Research Reserve, Florida, in 2015. Time of capture is expressed as hours passed since sunrise. Birds on both islands had a positive relationship between mass and capture time, but the correlations were not significant (SGI:  $P = 0.08$ ; SVI:  $P = 0.34$ ).



**Figure 2.20** Relationship between body mass (g) of Northern Waterthrushes and time of capture on St. George Island (SGI) and St. Vincent Island (SVI), Apalachicola National Estuarine Research Reserve, Florida, in 2016. Time of capture is expressed as hours passed since sunrise. Birds captured later in the day were significantly heavier on SVI ( $P < 0.001$ ), but results were not significant on SGI ( $P = 0.78$ ).



## 2.4 Discussion

### 2.4.1 Capture rates

My hypothesis that more birds would be captured per net hour on St. George Island was supported. St. Vincent Island is a larger barrier island and has been mostly undisturbed in recent history. Migratory passerines on St. Vincent Island experience a high diversity of habitat types which provide many of the resources they need to refuel and continue migration. However, St. George Island is mostly developed. A migratory passerine that lands on St. George Island may struggle to find fresh water and food. Unit 4 provides these resources for birds, but at only 86 acres, this means that migrants are found at greater densities. Therefore, the higher capture rate on St. George Island is likely a reflection of a greater density of birds per unit area. The greater density of migrants found on Unit 4 of St. George Island may increase competition among migrants during their stopover, which could negatively affect their ability to refuel and gain mass (Moore and Yong 1991). Therefore, I would expect to find reduced competition for food resources on St. Vincent Island. This hypothesis merits further study.

An interesting phenomenon experienced during the two seasons of spring migration banding was the high capture rates of Gray Catbirds as compared to other species. In the two years of the study, Gray Catbirds were the most common captured migrant (74 captured in 2015 and 126 captured in 2016). Even on days when no other migrants were encountered, I was able to capture and band several Gray Catbirds. I experienced low capture rates of other species throughout both seasons. I attribute the low encounters of most migrants to the reduced number of days with fallout conditions on the Florida Gulf coast during the two seasons. In general, more migrants are encountered during fallout conditions (Yaukey and Powell 2008; Kneidel 2016). Baird and Nisbet (1960) concluded that migrant Gray Catbirds are more common on

calmer days. The high capture rates of Gray Catbirds experienced during the current study can be attributed to the numerous days of calm weather experienced during the two seasons of banding.

#### 2.4.2 Physical condition of migrants

In 2015, I found a significant difference in fat and muscle scores of all Neotropical migrants between islands. Breeding species captured on St. Vincent Island such as White-eyed Vireo, Summer Tanager, and Prothonotary Warbler have no reason to store fat or catabolize muscle tissue. However, due to the difficulty in separating breeding from migratory individuals, all were included in analysis. Therefore, the lower fat scores and higher muscle scores found in Neotropical migrants on St. Vincent Island in 2015 is likely due to breeding individuals included in analysis. With increased netting effort in 2016 and more captured migrants, I found no significant difference between fat scores and muscle scores between islands. Due to the larger sample sizes, I believe that 2016 results more accurately reflect the equality in stopover habitat between islands.

Data on fat scores, muscle scores, and body mass in general do not support my hypothesis that St. Vincent Island provides more favorable stopover habitat. Specifically, Northern Waterthrushes are found in the same body condition on both islands. I found no significant difference in fat scores, muscle scores, or body mass between islands, suggesting that both islands provide sufficient resources for this species. Northern Waterthrushes thrive near freshwater wetlands or streams where they forage for invertebrate prey near the water's edge (Whitaker and Eaton 2014). While the small areas of freshwater wetlands on St. George Island are more disturbed and more fragmented, they appear to provide habitat for Northern Waterthrushes that is equal in quality compared to the wetlands on St. Vincent Island. Northern

Waterthrushes are known to quickly explore unfamiliar areas in order to locate preferred habitats during stopovers (Slager et al. 2015). Some will also hold and defend territories in ideal habitats during stopovers, and those that are able to defend a territory will gain more mass (Rappole and Warner 1976). Personal observations on both islands indicated territoriality and competition in this species, suggesting that Northern Waterthrushes were indeed able to find and defend high-quality habitats within the study sites. However, St. Vincent Island is much larger and would be able to support a higher number of Northern Waterthrushes as compared to St. George Island.

I found that Northern Waterthrush captured on St. George Island had significantly larger wing chords than those on St. Vincent Island in 2016. On St. George Island, approximately 90% of the Northern Waterthrush banded were captured before the middle of the season. On St. Vincent Island, only about 57% of those banded were captured before the middle of the season. Since more birds were captured early on St. George Island, more are likely to be either older birds or male birds. However, Northern Waterthrush are difficult to classify by age and sex (Pyle 1997). The larger sample size on St. George Island and the higher proportion of early captures resulted in the positive correlation between wing chord and mass for this subset of birds.

While my data in general does not support my hypothesis that St. Vincent Island provides higher quality stopover habitat for all migrants, it may provide better habitat for Gray Catbirds in some years. I found that Gray Catbirds had significantly higher size-corrected body mass on St. Vincent Island in 2015, but not in 2016. In 2015, Gray Catbirds were on average more than 2.5 g heavier on St. Vincent Island after adjusting for body size. St. Vincent Island may represent better foraging conditions for Gray Catbirds in some years, but more study is needed into the causes of this. During spring, Gray Catbirds mainly depend on insects for nutrition (Smith et al. 2011). Not only is St. Vincent Island undeveloped, but it also has not been treated with

insecticides like most developed areas of Florida (US Fish and Wildlife Service 2012). Therefore, St. Vincent Island may have a higher abundance and a greater variety of insects. More study on resources and resource use by Neotropical migrants is needed on these barrier islands. Data on fat and muscle scores, however, do not support my hypothesis. I found no significant difference between fat scores in either year and higher muscle scores on St. George Island in 2016 only, suggesting that fat and muscle scores may not always accurately reflect the size-corrected body mass of an individual.

Because spring migration and stopover on the Gulf coast can vary drastically from year to year (Lafleur et al. 2016), a long-term study would be more beneficial to fully understand differences in stopover habitat quality between these two islands. For example, Gray Catbirds were heavier on St. Vincent Island in 2015 only, and they had higher muscle scores on St. George Island in 2016 only. Gutierrez Ramirez (2016) found that muscle scores for Gray Catbirds on St. George Island differed significantly between years. Weather patterns can differ drastically from one migration season to the next. Differing weather patterns result in differences in stopover habitat use (Lafleur et al. 2016) and likely differences in the ability to refuel and gain fat. Additionally, global climate patterns such as El Niño can also affect spring migration. Paxton et al. (2014) found that Neotropical migrants that winter in South America were more likely to utilize stopover sites near the Gulf coast and were more likely to arrive on coastal stopover sites with reduced fuel reserves. This is likely due to the drier conditions present in the tropics during El Niño years, which creates poor refueling conditions throughout South and Central America (Paxton et al. 2014). Both winters prior to spring migration in the current study were classified as El Niño conditions, but the winter of 2015/2016 was more extreme (National Weather Service 2017). A long-term study would provide data on barrier

island use by Neotropical migrant songbirds during differing weather and differing global climate patterns.

I expected to find migrants in poorer condition on St. George Island. However, my results show that for many species, the resources available on Unit 4 are nearly equal in quality to those on St. Vincent Island. While St. George Island may provide less suitable habitat for Gray Catbirds, it is still important for other migratory species, like Northern Waterthrushes. This study highlights the need to conserve small stopover sites such as those present on St. George Island. Even though Unit 4 is more disturbed, more fragmented, and smaller than St. Vincent Island, migrants are able to find adequate resources, enabling them to refuel and continue northward. My results support the idea that it is better to focus conservation efforts on high-priority stopover sites rather than habitat types for priority species (Mehlman et al. 2005). Indeed, the locations of both islands as stopover sites near a large ecological barrier justify their importance for Neotropical migrants.

#### 2.4.3 Temporal effects on size-corrected body mass

I found no significant correlation between ordinal date and size-corrected body mass for Gray Catbirds on either island or either year. These findings support my initial hypothesis. However, the correlation approached significance for St. Vincent Island in both years ( $P = 0.07$ ). Interestingly, this was a negative correlation in 2015 and a positive correlation in 2016. In 2016, I found evidence that some Gray Catbirds wintering on St. Vincent Island may not initiate migration until after the sampling period began. A single Gray Catbird was recaptured in 2016 that had been captured and banded the previous season. This finding suggests wintering site fidelity, but it also suggests that some captured Gray Catbirds are wintering residents and not transients. Therefore, the increase in size-corrected body mass in 2016 may be due to wintering

birds increasing mass before the onset of migration. On the breeding grounds, after hatching-year (AHY) Gray Catbirds are known to increase fat load quickly prior to fall migration (Heise and Moore 2003). However, this does not explain the negative correlation with ordinal date in 2015. This study did not attempt to identify or examine extrinsic factors that may affect migration in this region. Varying weather patterns or the abundance of food throughout the season likely play a large role in determining the ability of a migrant to refuel and gain mass. These variables were not examined in the current study, but are, nevertheless, important aspects of stopover ecology.

I found no significant relationship between size-adjusted body mass and time of capture in Gray Catbirds in either island or year. I hypothesized that heavier birds would be captured later in the day due to the need to refuel throughout the day to account for overnight fasting. While my results trended towards a positive correlation, no significant effects were found. Again, this may be due to the smaller sample sizes, especially for St. Vincent Island, where only 19 Gray Catbirds were captured in 2015 and 33 in 2016. Results may have also been affected by Gray Catbirds wintering on the islands that we captured prior to the onset of their migration northward. These individuals may not have the increased need to store fat. Other studies involving Gray Catbirds have found differing results. For example, Gray Catbirds were found to gain mass throughout the day in southern Mexico during fall migration (Winker 1995). However, Winker et al. (1992) found mass loss in the Gray Catbird in Minnesota during fall migration after accounting for overnight mass loss.

My findings support the hypothesis that the majority of Gray Catbirds are circum-Gulf rather than trans-Gulf migrants. After completing a trans-Gulf crossing, one would expect to find fat-depleted birds (Kuenzi et al. 1991) that increase mass throughout their stopovers. Gray

Catbirds were rarely found with depleted fat reserves or catabolized muscles, and they did not significantly increase mass throughout the day. During fall migration, studies have reported that very few Gray Catbirds have sufficient fat reserves to successfully complete a Gulf crossing (Marsh 1983; Eddins and Rogers 1992; Woodrey and Moore 1997). Eddins and Rogers (1992) found that the majority of Gray Catbirds were moving east in the fall during a study on coastal Alabama. They hypothesized that Gray Catbirds are likely travelling through southern Florida before making either a trans-Gulf crossing to the Yucatan Peninsula or a trans-Caribbean flight to the Caribbean islands or Central America. They found no such correlation in the spring. If Gray Catbirds do not rely on a trans-Gulf pathway, they may be less vulnerable to habitat changes occurring on barrier islands on the Gulf coast. Indeed, Gray Catbirds throughout the United States are not experiencing significant population declines (Sauer et al. 2004).

Due to the lack of correlation between wing chord and body mass, I only adjusted body mass for Northern Waterthrushes captured on St. George Island in 2016. It is likely that the larger sample size ( $N = 32$ ) and the higher percentage of captures occurring early in the season on St. George Island in 2016 contributed to the stronger relationship between wing chord and body mass. I found no significant relationship between body mass or size-adjusted body mass and ordinal date on St. George Island in either year. On St. Vincent Island, there was a strong negative correlation ( $P < 0.001$ ) in 2015. This strong relationship contradicts my hypothesis. Furthermore, it is unlikely that this relationship is due to smaller birds, likely females, migrating later in the season, as would be expected. In many species, males arrive on the Gulf coast before females (Moore et al. 1990). However, since it is difficult to distinguish male Northern Waterthrushes from females (Pyle 1997), a difference in timing of arrival on the Gulf coast for this species has not been documented. In Louisiana Waterthrush (*Parkesia motacilla*), a closely

related species, males are known to arrive on breeding grounds in New York earlier than females (Eaton 1958). Therefore, it is likely that male Northern Waterthrush also arrive at breeding territories earlier than females and, thus, either begin migration sooner or migrate at a faster rate. I did not collect blood samples from Northern Waterthrushes for molecular sex determination. However, Covino (2015) recently showed that wing chord measurements can be used to determine sex with 95% confidence in 58% of Northern Waterthrushes. Following Covino's methods, I cannot confirm that males arrived on St. Vincent Island before females, as many Northern Waterthrushes that were captured were in the overlap zone for wing measurements, and some definite females were captured early in the season. If male Northern Waterthrushes do arrive on the breeding grounds before females, my data supports that it is due to males migrating at a faster rate after crossing the Gulf. More study at other stopover areas throughout the continent is needed to test this hypothesis. As with the Gray Catbirds, it is likely that the relationship between ordinal date and size-corrected body mass is related to extrinsic factors such as resource availability or weather patterns.

I found a significant positive correlation between body mass and time of capture for the Northern Waterthrush on St. Vincent Island in 2016 only. The lack of significance in the other regressions could be due to the small sample sizes. Previous studies have suggested that this type of analysis only be completed with sample sizes above 30 (Seewagen et al. 2011), 40 (Carlisle et al. 2005), or even 120 individuals (Dunn 2001). Therefore, these results must be interpreted with caution. These results are similar to other studies in different areas of the continent. For example, in New York City parks, Northern Waterthrush gain mass throughout the day during spring migration (Seewagen and Slayton 2008). Data from fall migration banding stations across Canada have shown the ability of Northern Waterthrush to gain mass during their

stopovers as well (Dunn 2002). However, this correlation could also show that in some years, St. Vincent Island offers better stopover habitat than St. George Island for this species. This would not be the case for all years. In 2015, the positive correlation between body mass and time of capture approached significance on St. George Island, but not St. Vincent. Extrinsic factors likely contribute to differences in this correlation between years. Besides weather and climate patterns, differences in land management may also affect the ability of migrants to gain mass on their stopovers. Because St. Vincent Island and Unit 4 on St. George Island are managed by different governmental agencies, their management strategies differ, which may result in habitat and resource differences between islands for the Northern Waterthrush.

Apalachicola Bay barrier islands are important for migrant Northern Waterthrush because of the availability of freshwater wetlands, a requirement for refueling in this species. While barrier islands across the Gulf of Mexico are used by a variety of Neotropical migrant songbirds, few studies report high numbers of Northern Waterthrush. Simons et al. (2004) reported 6.75 captured Northern Waterthrush per 1,000 net hours on East Ship Island in Mississippi. During the current study, an average of  $17.37 \pm 2.38$  Northern Waterthrush were captured per 1,000 net hours. Only a single Northern Waterthrush was encountered during point count surveys on Horn Island in Mississippi during a spring migration season (Moore and Woodrey 1993). This species may be more common on barrier islands in the northeastern portion of the Gulf of Mexico due to the large amounts of freshwater wetlands available, but more research is needed regarding this hypothesis since stopover patterns can vary from year to year. St. Vincent Island contains approximately 3 km<sup>2</sup> of freshwater wetlands and two deep freshwater ponds with goals to increase freshwater acreage in the future (US Fish and Wildlife Service 2012). Management efforts should focus on protecting freshwater wetlands on these islands, especially as sea-level

facilitates salt water intrusion and erosion. Protecting freshwater wetlands will not only benefit the Northern Waterthrush, but it may also benefit other Neotropical songbirds, native fish species, amphibians, reptiles, mammals, and invertebrates.

#### 2.4.4 Differences between sex and age classes

I hypothesized that male Gray Catbirds would have higher fat scores, muscle scores, and size-adjusted body mass due to the increased time sensitivity of males arriving on the breeding territories before females. However, I found no significant effects of sex in this species. While I was unable to determine the sex of all samples, I found that no analyses on the effects of sex on fat, muscle, or body mass approached significance in either island or year. I also found no differences between the arrival timing of male and females. These findings suggest that males do not initiate migration before females as is known to occur in other species. Hatch and Smith (2009) found no difference between arrival dates of male and female Gray Catbirds on their breeding grounds in northeastern Pennsylvania. Therefore, it seems that male Gray Catbirds do not experience the same time sensitivity when migrating to the breeding grounds as compared to males of other passerine species.

During fall migration, more HY Gray Catbirds are captured than AHY on the Gulf coast (Eddins and Rogers 1992; Woodrey and Moore 1997). However, I found nearly equal ratios of SY and ASY Gray Catbirds. This suggests that most HY Gray Catbirds do not survive their first migration attempts or winter, adding support to the idea that Neotropical migrants are limited by their wintering habitat (Norris et al. 2004; Keller and Yahner 2006). These findings point to the need for more long-term studies of stopover ecology on coastal barrier islands. While I found nearly equal ratios of age classes, Gutierrez-Ramirez (2016) found more SY Gray Catbirds in 2013 and fewer SY birds in 2014 on St. George Island.

I found a significant difference in the average capture date between SY and ASY Gray Catbirds for St. George Island, but not for St. Vincent Island. However, Gray Catbirds on St. Vincent also followed this trend. Smaller sample sizes on St. Vincent Island likely led to non-significant results. On St. George Island, SY birds arrived before ASY in 2015, but after in 2016. Woodrey and Moore (1997) found that HY Gray Catbirds were captured on the Gulf coast earlier in two out of three seasons of fall migration banding. Timing of migration can vary substantially depending on the year and location (Woodrey and Chandler 1997). Little can be understood based solely on the migration timing in a specific location during only two seasons. My results point to the need for further research in the timing of departure from the wintering grounds as well as the timing of arrival at other stopover sites along the migratory route for this species. It is unclear whether Gray Catbirds exhibit differing rates of migration between the age classes or differing departure times (Chandler and Mulvihill 1990).

I did not expect to find a significant difference in fat, muscle, or body mass between SY and ASY birds. While I found no differences in fat or muscle scores, SY birds were significantly heavier after adjusting for body size in 2016 ( $P = 0.002$ ). During a study of the post-breeding and pre-migratory phases of Gray Catbirds in Rhode Island, Heise and Moore (2003) found that HY Gray Catbirds were less successful at handling food, but they gained efficiency as the season progressed towards migration. During the pre-migratory phase, there was no significant difference between the fat scores of HY and AHY Gray Catbirds. Their study suggests that Gray Catbirds learn quickly. By the time a juvenile bird begins migration, it is just as efficient as an adult. Woodrey and Moore (1997) also found no significant difference between fat scores of HY and AHY Gray Catbirds during fall migration on the Gulf coast. They did, however, find differences in Swainson's Thrush (*Catharus ustulatus*), White-eyed Vireo, and American

Redstart (*Setophaga ruticilla*), with AHY birds carrying more fat than HY birds. Woodrey and Moore (1997) found that HY Gray Catbirds and Magnolia Warblers (*Setophaga magnolia*) were heavier than adults, even though HY and AHY carried similar fat stores. I attribute the higher size-adjusted body mass of SY birds in my study to the difference in wing chord measurements. In the fall HY Gray Catbirds have smaller wing chords than AHY birds (Marsh 1983). SY Gray Catbirds in my study also had shorter wing chords than ASY. Therefore, SY birds would be expected to have higher size-corrected body mass, even if the body masses were similar.

With only a few seasons of migration research on St. George and St. Vincent Islands, little can be concluded about stopover ecology in general. It is clear that birds use these coastal islands as stopover habitat, but the extent and variability of that use is less understood. A long-term study would be beneficial in order to understand variation among years. Global climate patterns likely play a role in the physical condition and dates of arrival to the Gulf coast (Gonzalez-Prieto and Hobson 2013; Paxton et al. 2014). Local weather patterns and temperatures experienced on the Gulf coast during spring migration influence the rate of capture and condition of migrants (Simons et al. 2004; Kneidel 2016). Our research within ANERR has brought more awareness to stopover ecology on the northeastern Gulf coast, but it has also raised more questions about the use of these islands that are beyond the scope of my project. In the following chapter, I investigate the refueling performance of Gray Catbirds captured on St. George and St. Vincent Islands in order to determine which variables most affect the ability of Gray Catbirds to refuel on the islands.

## CHAPTER III

### REFUELING PERFORMANCE OF MIGRANT GRAY CATBIRDS (*DUMETELLA CAROLINENSIS*) ON SPRING PASSAGE AT TWO FLORIDA GULF COAST BARRIER ISLANDS

#### 3.1 Introduction

##### 3.1.1 Measuring stopover habitat quality

Conserving stopover habitats is a research priority for the continued survival and prevention of future population declines of Neotropical migrants (Donovan et al. 2002; Mehlman et al. 2005). However, conservationists are faced with the challenge of quantifying the importance of a stopover habitat in order to prioritize sites for protection. This challenge is further complicated because the ecological quality of a stopover site for migratory songbirds can vary seasonally or even daily depending on exogenous factors such as weather.

Conventional techniques of measuring the quality of a stopover habitat involve quantifying the body condition of captured birds. This is usually accomplished with measurements of body fat, muscle content, and mass (Moore and Kerlinger 1987; Kuenzi et al. 1991; Yong and Moore 1997). Recaptured birds often provide valuable data about stopover site use, such as the ability of an individual bird to gain mass and the stopover duration of individuals. For example, Bayly et al. (2012) used data from recaptured birds to calculate the percent change in lean body mass over the duration of each individual's stopover and the potential flight ranges for those birds. However, often during migration studies, a migrating bird will only be captured a single time, limiting the amount of data that can be gathered from these types of studies. For example, Kuenzi et al. (1991) found that only about 3% of birds captured on a barrier island were recaptured on the days following their first capture. In those cases, other

techniques are useful. Some studies have examined the relationship between body mass and time of day in which the bird is captured (Winker et al. 1992; Dunn 2002; Seewagen and Slayton 2008). These studies only require a single capture event for each individual bird and examine the daily trends of mass changes within a species. However, the main drawback of these types of studies is the need for a large sample size (Dunn 2000).

### 3.1.2 Using plasma metabolites to measure stopover site quality

Recent advances in the study of stopover ecology have led to the use of plasma metabolites to provide information about a bird's physiological condition, rate of fattening, and rate of fat loss. In actively migrating birds, lipids provide the main source of fuel due to their low mass and high energy content (Jenni and Jenni-Eiermann 1998). During fat consumption and deposition, plasma triglyceride levels will be high, whereas during a period of fasting,  $\beta$ -hydroxybutyrate levels will be elevated (Jenni-Eiermann and Jenni 1991). Therefore, a large amount of information about a bird's fattening rate or rate of fat loss can be determined from a blood sample taken from a single capture event.

Studies of captive birds have validated the use of plasma metabolites in understanding the physiology of migration. Using wild birds kept in a controlled setting, Jenni-Eiermann and Jenni (1994) found that body mass changes in a passerine can be predicted by concentrations of triglycerides and  $\beta$ -hydroxybutyrate in the plasma. Williams et al. (1999) found that the rate of mass change was positively related to plasma triglyceride concentrations and negatively related to  $\beta$ -hydroxybutyrate concentrations in captive Western Sandpipers (*Calidris mauri*). Another study mimicked high and low quality stopover sites by altering the feeding rate of captive Wilson's Warblers (*Wilsonia pusilla*), showing that changes in the feeding rates could be

observed through changes in plasma triglyceride and  $\beta$ -hydroxybutyrate levels after as little as 10 and 20 minutes, respectively (Zajac et al. 2006).

Studies of these kinds have also been tested in free-living birds to determine differences in stopover habitats. Schaub and Jenni (2001) compared metabolite levels of migrating passerines at fourteen different sites throughout Europe and Africa, suggesting that this method could be used to investigate differences between sites. Guglielmo et al. (2005) validated this theory on two separate stopover sites of previously known quality. The relationship between plasma metabolites and abundance of food at stopover sites has also been investigated in shorebirds (Seaman et al. 2006) and passerines (Seewagen et al. 2011; Smith et al. 2015).

Plasma metabolite profiling enables researchers to compare the quality of different stopover sites without the use of unlikely recaptures. Seewagen et al. (2011) used plasma metabolite analysis to determine that the refueling rates of migratory birds stopping over in urban parks in New York City were comparable to birds using stopover locations outside of the city. More recently, Evans Ogden et al. (2013) employed plasma metabolite analysis to determine that frugivorous bird species had higher refueling rates at high-elevation sites, but insectivorous species had higher refueling rates at low-elevation sites. Understanding the use of stopover sites by migratory songbirds on a physiological level will aid in the conservation of high-quality stopover sites.

### 3.1.3 Study species

I sampled triglyceride concentrations from Gray Catbirds (*Dumetella carolinensis*) to determine refueling rates of individuals. Gray Catbirds were chosen for this study in part due to their abundance in the Apalachicola region in the spring (Gutierrez Ramirez 2016). The species is classified as one of least concern due to its stable population trend (IUCN 2017). Gray

Catbirds breed throughout most of temperate North America and winter in Central America, the Caribbean, coastal Gulf of Mexico, and much of the mid and south-Atlantic coasts of the United States (Smith et al. 2011). During the fall, birds from the Midwestern population migrate into Central America, while eastern populations migrate to the Gulf coast, southern Florida, or the Caribbean (Ryder et al. 2011). Marsh (1983) found that in the fall, some Gray Catbirds captured in Florida had sufficient fat reserves to complete the crossing over the Gulf of Mexico to the Yucatan Peninsula. Some of Marsh's sampled Gray Catbirds showed hypertrophy of the breast muscles, suggesting that they were preparing for the trans-Gulf crossing. However, Woodrey and Moore (1997) captured very few Gray Catbirds in coastal Alabama with sufficient fat reserves to complete a Gulf crossing. Assuming that some Gray Catbirds make this crossing during the fall migration, a certain number will likely complete a trans-Gulf migration northward in the spring, stopping to rest and refuel on Apalachicola Bay barrier islands. Gray Catbirds were found to be able to adjust their migration timing with spring temperatures, suggesting that their populations are less likely than other species to experience declines with a warming climate (Miller-Rushing et al. 2008).

The large number of Gray Catbirds available to study in comparison to other migrants made them an appealing study subject. Gray Catbirds range in mass from 27.4 g - 54.5 g with an average of  $39.9 \text{ g} \pm 3.15$  (Raynor 1979). Their large size allowed me to take a blood sample that was adequate for plasma metabolite profiling.

This chapter focuses on identifying the most relevant variables that influence the refueling rate (measured as triglyceride concentration) of Gray Catbirds on both islands. I considered the following hypotheses:

- 1.) Gray Catbirds will have higher refueling rates on St. Vincent Island. I expect that Gray Catbirds will find better foraging opportunities on St. Vincent Island due to its larger size and larger diversity of habitat types. Unit 4 on St. George Island is smaller and more fragmented. Therefore, I expect Gray Catbirds on St. George Island to have a lower refueling performance due to the difficulties in finding adequate resources.
- 2.) Male Gray Catbirds will refuel at a higher rate than females. In general, males are under a higher time-sensitivity to arrive on the breeding grounds (Kokko et al. 2006). Therefore, I expect to find increased refueling rates in males because of the higher time constraints.
- 3.) ASY Gray Catbirds will have higher refueling rates than SY. ASY Gray Catbirds arrive on the breeding grounds before SY birds (Hatch and Smith 2009). Therefore, I expect to find higher refueling rates in ASY as they are under increased pressure to arrive on the breeding grounds.
- 4.) Gray Catbirds with higher fat scores, higher muscle scores, and higher size-corrected body mass will have higher refueling rates. Studies have found that body mass is positively correlated with triglyceride concentrations in migratory birds (Smith and McWilliams 2010; Seewagen 2013). Because birds with higher fat and muscle scores have a higher size-corrected body mass, I expect to find increased refueling rates in those individuals.
- 5.) Date, capture time, and bleed time may also affect refueling rates. Capture time has proven to be an influential variable in some studies (Seaman 2006; Smith and McWilliams 2010; Seewagen 2013). However, seasonal effects are difficult to measure when sampling takes place only for a limited amount of time during peak migration

(Schaub and Jenni 2001). Bleed time in general is negatively correlated with triglyceride concentrations (Guglielmo et al. 2002; Seaman et al. 2006; Smith et al. 2015), but careful protocols were in place to limit the effect of bleed time in the current study.

## 3.2 Methods

### 3.2.1 Preparation of reagents, standards, and plasma samples

I used the Triglyceride Colorimetric Assay Kit (Cayman Chemical Company, Item Number 10010303) for the analysis of plasma triglycerides. The assay produces glycerol and free fatty acids through enzymatic hydrolysis of triglycerides and is followed by a colorimetric measurement of released glycerol (Cayman Chemical Company 2014).

Before the assay was initiated, I prepared reagents following the protocol (Cayman Chemical Company 2014). I prepared the standard diluent by combining 48mL of HPLC-grade water with 12 mL of the standard diluent assay reagent (5x) (Item Number 700732). The product was stored in a 100 mL vial, labeled with its name and date prepared, and stored at room temperature until needed. Next, I prepared the assay buffer solution, which is used to prepare the triglyceride enzyme solution. 4 mL of sodium phosphate buffer (Item Number 700003) was combined with 16 mL of HPLC-grade water and placed in a 100 mL vial labeled with the name and date prepared. Both the diluent and buffer solutions were stable at room temperature for at least six months. I prepared the triglyceride enzyme mixture by first reconstituting the lyophilized enzyme mixture (Item Number 10010511) with 1 mL of HPLC-grade water. I poured the reconstituted mixture into a 15 mL vial and wrapped the vial with aluminum foil. To this mixture, I added 14 mL of the diluted assay buffer solution and mixed thoroughly.

Due to the small samples of plasma collected, I diluted the samples threefold using the diluted standard diluent to increase the volume (Seewagen et al. 2011). I took care to change pipette tips between samples and ensure that all vials were labeled with their corresponding lab numbers.

I prepared standards using the diluted standard diluent and triglyceride standard (Item Number 10010509). I added 200  $\mu\text{L}$  of diluted standard diluent to eight vials, labeled 1-8. To the first vial, I added an additional 200  $\mu\text{L}$  of standard diluent and 100  $\mu\text{L}$  of triglyceride standard and mixed thoroughly. I serially diluted the first vial by removing 200  $\mu\text{L}$  from vial 1 and adding it to vial 2. From vial 2, I removed 200  $\mu\text{L}$  and added to vial 3. I repeated this process until vial 7. Vial 8 contained only standard diluent to be used as a blank. I prepared standards no more than two hours before performing the assay.

### 3.2.2 Analysis of plasma triglycerides

I performed triglyceride assays using a flat-bottom 96-well microplate (Item Number 400014), following the protocol for the suggested arrangement of standards and samples (Cayman Chemical Company 2014). First, I transferred 10  $\mu\text{L}$  from each of the prepared eight standards into two separate, but neighboring wells. Then I added 10  $\mu\text{L}$  of each diluted sample to two wells. If enough diluted plasma was available, I added a third well for the sample so that each sample was run in duplicate or triplicate. I initiated the reaction by adding 150  $\mu\text{L}$  of prepared enzyme buffer solution to each well, making the total reaction 160  $\mu\text{L}$ . The microplate was covered and incubated for 15 minutes at room temperature on a microplate shaker (Fisher Scientific). After 15 minutes, I placed the microplate in a microplate spectrophotometer (Epoch Biotek) and read the absorbance at 540 nm.

After the completion of the spectrophotometer reading, I averaged the absorbance from each sample and standard and subtracted the average absorbance of the blank standard 8 from itself and all other averages, resulting in a corrected absorbance for each sample and standard. The corrected absorbance from each standard was used to create a standard curve. The equation

of the linear regression of this curve was used to transform average absorbance to triglyceride concentration using the following equation:

$$\text{Triglycerides } \left( \frac{\text{mg}}{\text{dL}} \right) = \left[ \frac{(\text{corrected absorbance}) - (y\text{-intercept})}{\text{Slope}} \right]$$

The triglyceride concentration from the equation was multiplied by three to account for dilutions and by 0.0113 to convert each sample to the Standard International Unit of mmol/L.

### 3.2.3 Statistical analysis

All statistical analyses were completed using R version 3.2.3 (R Core Team 2015), and I determined results to be significant when  $P \leq 0.05$ . I tested all values for normality using the Shapiro-Wilk test. All data presented are taken from the first capture of each individual bird.

Because triglyceride assays were run in duplicate or triplicate, I calculated a coefficient of variation (CV) between all replicate absorbances for each individual bird. The CV was calculated by dividing the standard deviation of replicated absorbances by the mean of those values and multiplying by 100 to get a percentage. If I did not reach a CV of  $< 10\%$ , I repeated the assays if sufficient plasma remained. Samples with a CV  $> 10\%$  with no additional plasma were eliminated from subsequent analysis (Smith et al. 2007). Seewagen et al. (2011) used a CV of 15%, but I found that triglyceride concentrations for samples with a CV  $> 10\%$  were often extremely high. After eliminating samples with a CV  $> 10\%$ , the average CV for all samples was 2.89%.

I confirmed the blood collection protocol using a simple linear regression to assess the relationship between triglyceride concentrations (TRIG) and bleed time (calculated as the maximum amount of minutes between capture and blood collection). Combining all samples, I found no significant effects of bleed time ( $F_{1,140} = 1.07$ ,  $R^2 = 0.01$ ,  $P = 0.30$ ).

I compared differences in TRIG between islands, age classes, and sexes using a t-test. I used backwards stepwise regressions to determine which variables or combinations of variables best describe variation in TRIG. This analysis was performed separately for the two islands in 2016 because a different assay kit was used for determining triglyceride concentrations for birds captured by the University of Massachusetts crew working on St. George Island. Variables entered into the model in 2015 include location, date (expressed as ordinal date), capture time (expressed as hours after sunrise), bleed time (maximum amount of minutes between capture and blood sampling), age, fat score, muscle score, and size-corrected body mass. In both 2016 models, I ran the models using the same variables, with the exception of location.

Backwards stepwise regressions first run all possible variables in the model and drop the least relevant variable with each succeeding step. The final model with the lowest AIC value represents variables that best describe variations in TRIG. Each step in each model is shown in the tables below. I provide figures depicting the relationship between TRIG and variables included in the best ranked model. Weights ( $W_i$ ) for each model were calculated using the following formula, where  $\Delta_i$  is the difference in AIC value between model  $i$  and the best model:

$$W_i = \frac{\exp\left[-\frac{1}{2}\Delta_i\right]}{\sum \exp\left[-\frac{1}{2}\Delta_i\right]}$$

Stepwise regressions are controversial in ecological studies (Wittingham et al. 2006). However, I used this technique more as an exploratory analysis because I expected multiple variables to contribute to differences in refueling rates. A similar analysis was used in a recent study to determine which variables most influenced differences in levels of several plasma metabolites in migratory sandpipers stopping over in North American prairies (Thomas and Swanson 2013).

### 3.3 Results

#### 3.3.1 Refueling rates

I successfully determined TRIG for 142 Gray Catbirds during the 2015 and 2016 spring migration seasons on St. George and St. Vincent Islands. A summary is provided in Table 3.1 and Figure 3.1.

In 2015, there was no significant difference in TRIG between the two islands ( $t = 1.05$ ,  $df = 16.99$ ,  $P = 0.31$ ). Combining both islands, I found no differences in TRIG between sex ( $t = 1.33$ ,  $df = 36.84$ ,  $P = 0.19$ ) or age classes ( $t = -1.49$ ,  $df = 51.83$ ,  $P = 0.14$ ).

In 2016, I found that Gray Catbirds on St. Vincent Island had higher TRIG than those captured on St. George Island ( $t = -2.24$ ,  $df = 70.00$ ,  $P = 0.03$ ). I found no significant effect of age on TRIG on St. George Island ( $t = 0.97$ ,  $df = 50.84$ ,  $P = 0.34$ ) or St. Vincent Island ( $t = -1.82$ ,  $df = 29.61$ ,  $P = 0.08$ ). Sex had no effect on TRIG for Gray Catbirds on St. Vincent Island ( $t = -0.23$ ,  $df = 10.88$ ,  $P = 0.82$ ).

#### 3.3.2 Modeling plasma triglyceride concentrations

Year and fat score were the most relevant variables in explaining variations in TRIG for all Gray Catbirds captured during the study (Table 3.2; Figure 3.2). In 2015, I found that location and date described most of the variation in TRIG (Table 3.3; Figure 3.3). In 2016 on St. George Island, bleed time and muscle scores best explained the variation (Table 3.4, Figure 3.4), and on St. Vincent Island, age was most relevant to TRIG (Table 3.5, Figure 3.5).

### 3.3.3 Tables and Figures

**Table 3.1** Summary of triglyceride concentrations of Gray Catbirds captured on St. George (SGI) and St. Vincent Islands (SVI), Apalachicola National Estuarine Research Reserve, Florida, in 2015 and 2016. TRIG is expressed as mmol/L.

Year	Location	N	TRIG $\pm$ SE	TRIG range
2015	SGI	41	1.42 $\pm$ 0.06	0.68 – 2.43
	SVI	13	1.25 $\pm$ 0.15	0.68 – 2.45
2016	SGI	56	1.17 $\pm$ 0.05	0.36 – 2.36
	SVI	32	1.37 $\pm$ 0.07	0.60 – 2.04
TOTAL		142	1.30 $\pm$ 0.04	0.36 – 2.45

**Table 3.2** Backwards stepwise regression models explaining variation in triglyceride concentrations for Gray Catbirds captured on St. George and St. Vincent Islands, Apalachicola National Estuarine Research Reserve, Florida, in 2015 and 2016. N = 136. Variables include location, year, date (expressed as ordinal date), time (expressed as hours since sunrise), bleed time (the maximum amount of minutes between capture and blood sampling), age, fat scores, muscle scores, and SCBM (size-corrected body mass).  $W_i$  represents Akaike weights for each model.

Step	Model	AIC	$\Delta$ AIC	$W_i$
1	Location + Year + Date + Time + Bleed Time + Age + Fat + Muscle + SCBM	-222.50	9.65	0.003
2	Location + Year + Date + Time + Bleed Time + Age + Fat + Muscle	-224.49	7.66	0.009
3	Location + Year + Date + Time + Age + Fat + Muscle	-226.44	5.71	0.023
4	Year + Date + Time + Age + Fat + Muscle	-227.87	4.28	0.046
5	Year + Date + Time + Age + Fat	-229.13	3.02	0.087
6	Year + Date + Time + Fat	-230.27	1.88	0.154
7	Year + Date + Fat	-231.49	0.66	0.284
8	Year + Fat	-232.15	0	0.394

**Table 3.3** Backwards stepwise regression model explaining variation in triglyceride concentrations for Gray Catbirds captured on St. George and St. Vincent Islands, Apalachicola National Estuarine Research Reserve, Florida, in 2015. N = 52. Variables include location, date (expressed as ordinal date), time (expressed as hours since sunrise), bleed time (the maximum amount of minutes between capture and blood sampling), age, fat scores, muscle scores, and SCBM (size-corrected body mass).  $W_i$  represents Akaike weights for each model.

Step	Model	AIC	$\Delta$ AIC	$W_i$
1	Location + Date + Time + Bleed Time + Age + Fat + Muscle + SCBM	-82.40	8.88	0.005
2	Location + Date + Time + Bleed Time + Age + Fat + SCBM	-84.40	6.88	0.014
3	Location + Date + Time + Bleed Time + Age + Fat	-86.39	4.89	0.037
4	Location + Date + Time + Age + Fat	-87.97	3.31	0.082
5	Location + Date + Time + Age	-89.43	1.85	0.170
6	Location + Date + Time	-90.31	0.97	0.264
7	Location + Date	-91.28	0	0.428

**Table 3.4** Backwards stepwise regression models explaining variation in triglyceride concentrations for Gray Catbirds captured on St. George Island, Apalachicola National Estuarine Research Reserve, Florida, in 2016. N = 52. Variables include date (expressed as ordinal date), time (expressed as hours since sunrise), bleed time (the maximum amount of minutes between capture and blood sampling), age, fat scores, muscle scores, and SCBM (size-corrected body mass).  $W_i$  represents Akaike weights for each model.

Step	Model	AIC	$\Delta$ AIC	$W_i$
1	Date + Time + Bleed Time + Age + Fat + Muscle + SCBM	-79.49	6.71	0.014
2	Time + Bleed Time + Age + Fat + Muscle + SCBM	-81.22	4.98	0.033
3	Time + Bleed Time + Fat + Muscle + SCBM	-82.99	3.21	0.080
4	Time + Bleed Time + Fat + Muscle	-84.44	1.76	0.165
5	Time + Bleed Time + Muscle	-85.72	0.48	0.312
6	Bleed Time + Muscle	-86.20	0	0.397

Table 3.5 Backwards stepwise regression models explaining variation in triglyceride concentrations for Gray Catbirds captured on St. Vincent Island, Apalachicola National Estuarine Research Reserve, Florida, in 2016. N = 32. Variables include date (expressed as ordinal date), time (expressed as hours since sunrise), bleed time (the maximum amount of minutes between capture and blood sampling), age, fat scores, muscle scores, and SCBM (size-corrected body mass).  $W_i$  represents Akaike weights for each model.

Step	Model	AIC	$\Delta$ AIC	$W_i$
1	Date + Time + Bleed Time + Age + Fat + Muscle + SCBM	-53.40	8.39	0.006
2	Date + Time + Age + Fat + Muscle + SCBM	-55.39	6.40	0.015
3	Date + Time + Age + Fat + SCBM	-57.15	4.64	0.037
4	Time + Age + Fat + SCBM	-58.88	2.91	0.089
5	Time + Age + SCBM	-60.51	1.28	0.200
6	Time + Age	-61.13	0.66	0.273
7	Age	-61.79	0	0.380

Figure 3.1 Triglyceride concentrations for Gray Catbirds captured on St. George and St. Vincent Islands, Apalachicola National Estuarine Research Reserve, Florida, in 2015 and 2016. TRIG is expressed as mmol/L.

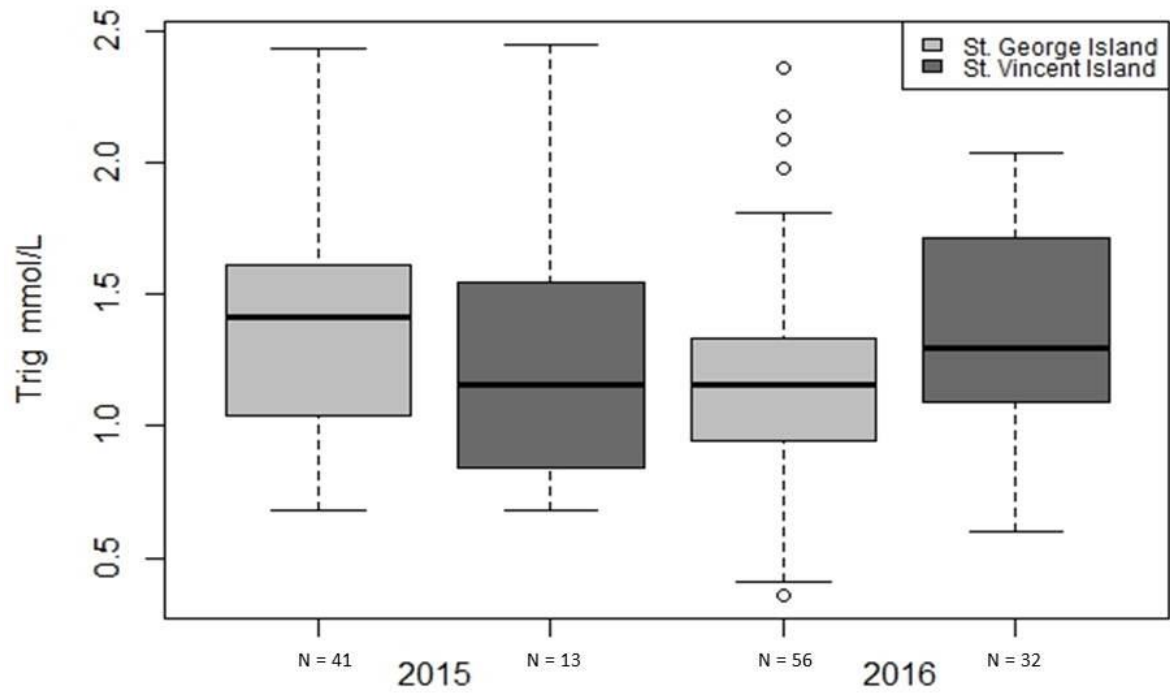
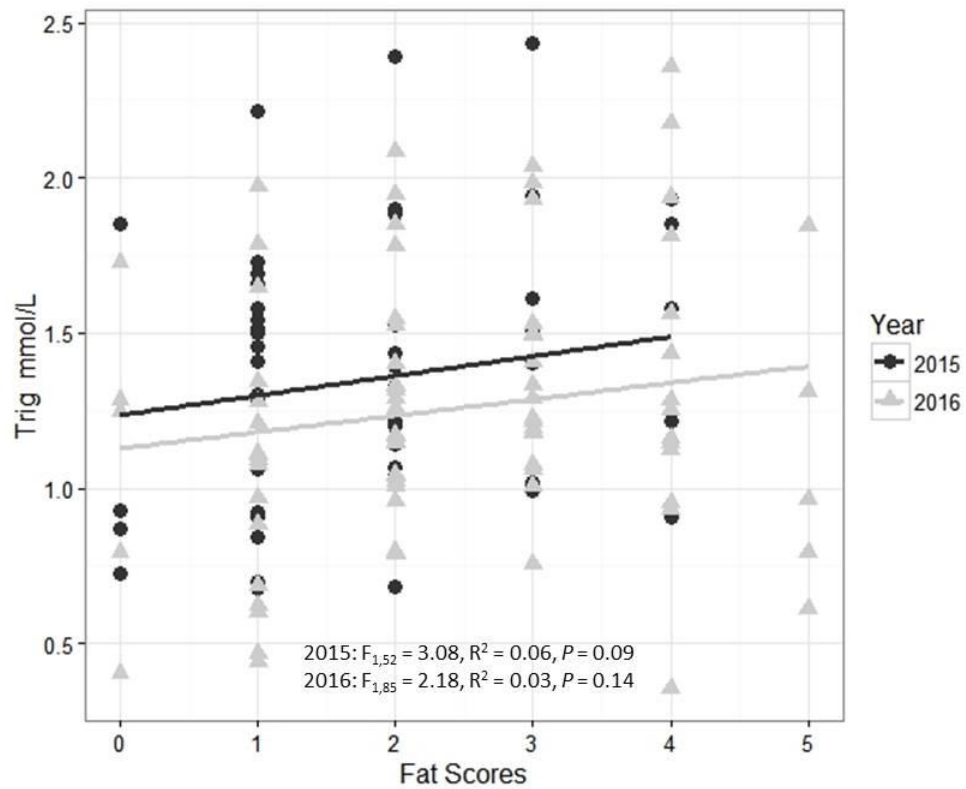
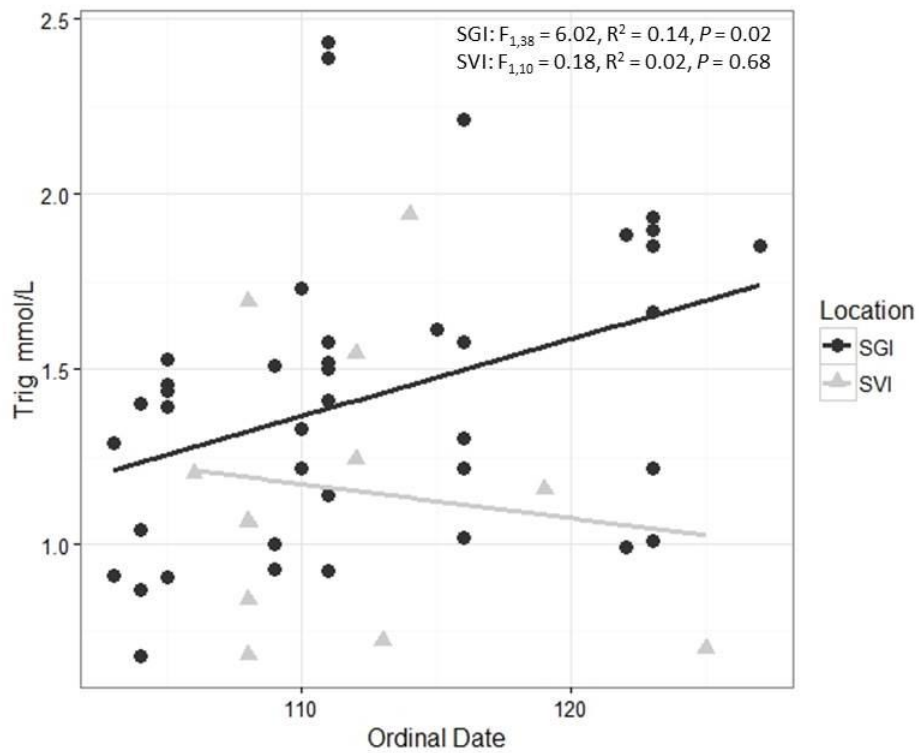


Figure 3.2 Triglyceride concentrations (mmol/L) as a function of fat scores for Gray Catbirds captured on St. George and St. Vincent Islands, Apalachicola National Estuarine Research Reserve, Florida, in 2015 and 2016.



**Figure 3.3** Triglyceride concentrations (mmol/L) as a function of ordinal date for Gray Catbirds captured on St. George Island (SGI) and St. Vincent Island (SVI), Apalachicola National Estuarine Research Reserve, Florida, in 2015.



**Figure 3.4** Triglyceride concentrations (mmol/L) as a function of bleed time (maximum amount of minutes between capture time and blood sampling) for Gray Catbirds with high (2-3) and low (0-1) muscle scores captured on St. George Island, Apalachicola National Estuarine Research Reserve, Florida in 2016.

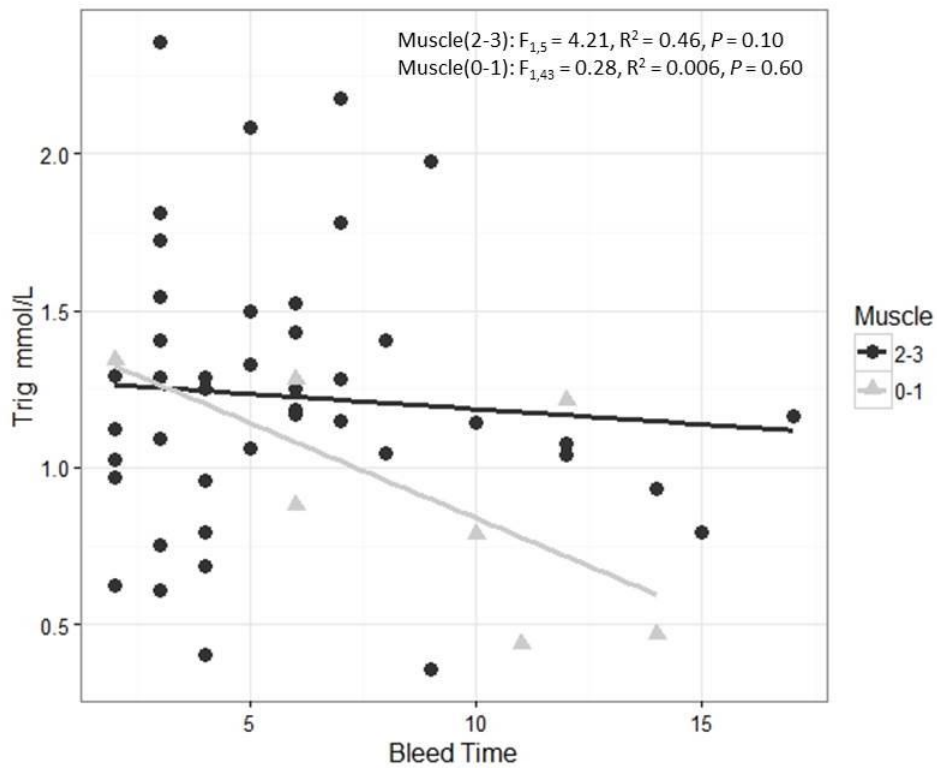
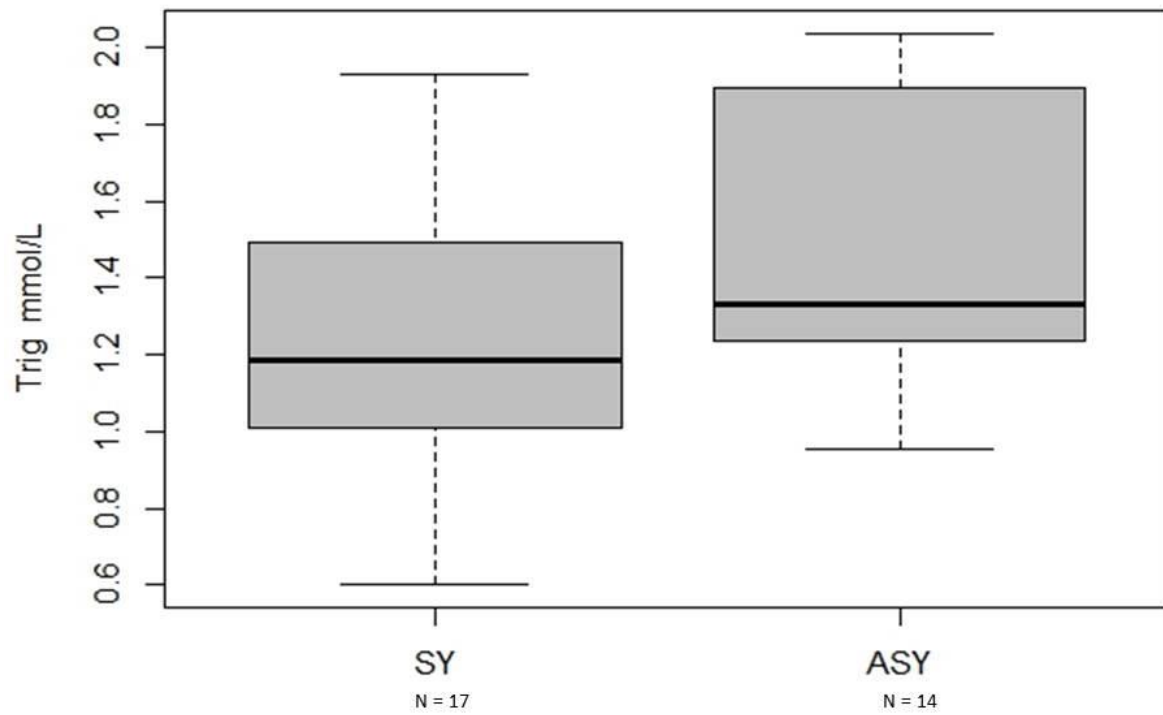


Figure 3.5 Triglyceride concentrations (mmol/L) of second-year (SY) and after second-year (ASY) Gray Catbirds captured on St. Vincent Island, Apalachicola National Estuarine Research Reserve, Florida in 2016. Differences between the age classes approached significance ( $P = 0.08$ ).



### 3.4 Discussion

My first hypothesis, that St. Vincent Island would have higher refueling rates than St. George Island was incorrect for the first year of the study. This suggests that Gray Catbirds are refueling at equal rates on both islands. This does not support my hypothesis that St. Vincent Island provides higher quality stopover habitat. However, for 2016, Gray Catbirds on St. Vincent Island were found to have significantly higher TRIG than those on St. George Island. In 2016, triglyceride assays were performed in two separate labs using two separate assay kits. While both assay kits used similar methods, I cannot rule out the effects of different assay kits, different equipment, or different researchers. More study is needed in comparing the precision of different assay kits.

I expected to find higher TRIG in males due to the higher time-sensitivity of spring migration, but my study did not support this hypothesis. Seewagen et al. (2013) found higher TRIG in male Yellow-rumped Warblers (*Setophaga coronata*) and Common Yellowthroats (*Geothlypis trichas*), but failed to find similar results in the Swainson's Thrush, Northern Waterthrush, Ovenbird (*Seiurus aurocapilla*), and White-throated Sparrow (*Zonotrichia albicollis*). Furthermore, Hatch and Smith (2009) found no differences in arrival timing of Gray Catbirds to the breeding grounds in northeastern Pennsylvania. Therefore, it is possible that male Gray Catbirds do not experience the same time sensitivity of migration as compared to males of other species.

I found no significant effects of age on TRIG during either year of the study. While not statistically significant, age had more support in the model for St. Vincent Island in 2016 than any other variable or combination of variables. In general, age is thought to be more influential during fall migration, when many young birds are experiencing their first migration attempt. HY

birds are often found with lower fat scores than AHY birds in the fall, but Gray Catbirds do not appear to follow this trend (Woodrey and Moore 1997; Heise and Moore 2003). Many studies have found no difference in the efficiency of older and younger birds during their stopovers (Carlisle et al. 2005; Smith and McWilliams 2010). During fall migration, Seewagen et al. (2013) found no effect of age on TRIG for any species in their study. It seems that age is not as important in determining refueling rates for Gray Catbirds.

Combining all birds from both years and both sites, I found that year and fat were the most relevant variables related to TRIG. In both years, there was a positive correlation between TRIG and fat scores, indicating that birds with higher fat reserves were refueling at faster rates. This supports my hypothesis that birds storing more fat would have higher refueling rates. However, I found little evidence supporting the hypothesis that higher muscle scores would be indicative of higher refueling rates. Muscle scores were only ranked in the top model for St. George Island in 2016. Size-corrected body mass is related to both fat scores and muscle scores. While some studies have found a correlation between body mass and TRIG (Guglielmo et al. 2002; Smith and McWilliams 2010; Seewagen 2013), the current study found no such relationship.

Other studies have found relationships between TRIG and capture time during fall migration (Smith and McWilliams 2010; Seewagen 2013) with TRIG increasing later in the day. While capture time was not included in any of the top models in the current study, it was included in models with high weights. Whittingham et al. (2006) stress the issue of reliance on a single best fit model when using stepwise regression. Multiple models may explain variations nearly equally well. Therefore, it is likely that capture time is also an important variable even though it is not included in any best fit model. For example, in the analysis for St. Vincent

Island in 2016, the last three models all have high weights, suggesting that not only age, but also capture time and body mass may play a role in explaining variations in TRIG. Therefore, it is important to examine the weights of all model presented to determine the relevancy of models ranked lower than the top model.

Date was a relevant variable, along with location, in the top model for the 2015 season only. However, a significant relationship between date and TRIG was found only for St. George Island, with TRIG increasing as the season progressed. This significant relationship could be due to the higher sample size on St. George Island in 2015. This finding suggests that Gray Catbirds are refueling at higher rates later in the season. However, it is unclear whether this is due to a higher availability of food, an increased pressure to make up for lost time in later migrants, or another extrinsic factor such as weather conditions. These hypotheses merit further study in migrating songbirds.

Bleed time has proven to affect TRIG in numerous studies, with TRIG decreasing with an increase in bleed time (Guglielmo et al. 2002; Seaman et al. 2006; Smith et al. 2015). Careful protocols were in place in the current study so that blood sampling did not occur after twenty minutes had passed since capture. However, bleed time was in the top model for St. George Island in 2016. In 2016, the University of Massachusetts crew had a more efficient method of checking nets, recording times, returning birds for processing, and bleeding birds. This resulted in blood sampling for most birds taking place within ten minutes of capture. In contrast, the Delaware State University crew bled most birds after ten minutes of capture. These differences in protocol may have led to bleed time having more of an effect on St. George Island in 2016.

Stepwise regressions are controversial in ecological studies, but their use is still widespread (Whittingham et al. 2006). In the current study, no two analyses included the same

variable in its best fit model, which is a problem discussed in depth in Whittingham et al. (2006). While this could be due to smaller sample sizes, another explanation is that other factors that may also influence TRIG were not measured or accounted for in the modeling process. For example, extrinsic factors such as weather conditions, abundance of food, or even global climate patterns such as El Niño likely influence the refueling performance of the Gray Catbird. Food abundance or quality can impact refueling performance in other species (Seaman et al. 2006; Smith et al. 2007; Smith et al. 2015). Weather patterns likely play a role, especially on barrier islands. Birds in all physiological conditions are captured on fallout days or days with inclement weather (Simons et al. 2004; Kneidel 2016). In general, most birds bypass barrier islands if they are physically able (Buler and Moore 2011). In the current study, I was unable to test how those weather conditions affected refueling performance. Weather conditions greatly influence the density of migrants on the Florida Gulf coast (Lafleur et al. 2016), and they may influence refueling performance as well. El Niño can also affect the energetic condition of migrants as well as stopover habitat use (Paxton et al. 2014).

The amount of time between a bird's arrival to the island and its capture can potentially impact results as well. For example, if a bird is caught as soon as it arrives on the island, it may have lower TRIG because it has likely experienced an extended period of fasting. Stopover duration for birds that are not recaptured is impossible to measure using traditional mist-netting techniques. Therefore, it was not a variable that I measured or included in analysis. Recaptures on barrier islands are generally rare (Kuenzi et al. 1991), suggesting that many birds depart soon after arriving. However, those that are recaptured generally carry reduced fat stores at the time of their first capture (Kuenzi et al. 1991; Simons et al. 2004; Kneidel 2016). Because TRIG was positively correlated with fat scores, and lower fat scores mean that a bird is more likely to

remain at a stopover site, I would expect that stopover duration would be negatively correlated with TRIG. This means that inefficient foragers must remain at stopover sites longer in order to increase fuel reserves. However, lean migrants have been found to forage at a faster rate than fat migrants (Yong and Moore 2005). Furthermore, because the Gray Catbird is likely a circum-Gulf migrant and arrives on barrier island stopover sites in better condition than trans-Gulf migrants (Gutierrez Ramirez 2016), it may not experience the same pressure to quickly refuel as other species. More study is needed to test the effects of stopover duration on the refueling performance of migratory birds as well as which variables affect stopover duration.

The results reported here using stepwise regressions point to the need for more research in this area. No single variable had support in all best fit models, and best fit models differed drastically between years and between islands in 2016. Larger sample sizes in the future may allow a clearer picture of factors that influence refueling rates. Furthermore, more research is needed into variables that were out of scope for this project. Future research should focus on the effects of changing climatic patterns, local weather conditions, food availability, and stopover duration, as they are important considerations for barrier islands that may be threatened by sea-level rise.

## CHAPTER IV

### CONCULSION

Most studies of stopover ecology on the northern coast of the Gulf of Mexico have focused on the western or central coasts and have indicated that those areas are of high conservation importance (Moore et al. 1995). This study, along with two previous studies (Gutierrez Ramirez 2016; Kneidel 2016), were the first to examine stopover ecology along the northeastern coasts. My results support findings by Gutierrez Ramirez (2016) and Kneidel (2016) that barrier islands on the eastern Gulf are important stopover habitats for migratory songbirds. However, these islands face extreme threats due to sea-level rise. The majority of songbirds using St. George Island as a stopover site are found in forested habitats, areas which will decrease in area as sea-level rise progresses (Lester et al. 2016).

While St. Vincent Island is larger and less disturbed, my data did not support the hypothesis that St. Vincent Island is a better-quality stopover site. Nevertheless, more birds likely use St. Vincent Island because of its larger size and larger variety of habitats. These findings indicate that St. George Island, although smaller, more developed, and under more anthropogenic threats, is equally as important as St. Vincent Island for migrant songbirds. Therefore, I conclude that both islands are important for Neotropical migrants, and conservation actions should be taken to prevent catastrophic land loss of these islands in the future.

For example, my data suggests that Apalachicola Bay barrier islands are especially important for migrant Northern Waterthrush. Freshwater wetlands on both islands support high numbers of this species. While I found no differences in the physical condition of Northern Waterthrush between islands, those on St. Vincent Island significantly increased mass

throughout the day in 2016, suggesting that individuals were able to find adequate resources during their stopover. My data shows that resource use and stopover ecology can differ from year to year, possibly due to extrinsic factors. However, freshwater wetlands on these islands will be especially threatened in the future as sea-level rise progresses. Management efforts should focus on protecting these fragile ecosystems so that they can continue to benefit species that depend on them, such as the Northern Waterthrush.

My results support hypotheses from other studies that the majority of Gray Catbirds are circum-Gulf migrants. Most Gray Catbirds were found in good physical condition, and they did not, in general, increase mass throughout the day. While most Neotropical migrants are declining in population sizes, Gray Catbirds have shown steady population trends over the years (Sauer et al. 2004). My results indicate that Gray Catbirds are not likely to be affected as severely as other Neotropical migrants by barrier island land loss. Furthermore, I found no evidence that male Gray Catbirds are under increased pressure to migrate earlier, migrate faster, or refuel faster than females. These results were not expected and are different than results from other Neotropical species.

Many Neotropical migrant species are undergoing population declines throughout their ranges, and it has been suggested that more effort be put toward identifying high-quality stopover sites rather than species-specific stopover sites (Mehlman et al. 2005). With this objective in mind, I conclude that Apalachicola Bay barrier islands are, indeed, high-quality stopover sites for Neotropical migrants. They could prove to be especially important during specific weather circumstances, when migrants are blown off course from their migratory pathway (Lafleur et al. 2016). Conservation efforts should focus on preserving barrier islands

and other coastal habitats throughout the northern Gulf of Mexico, as the entire northern coastline is important for Neotropical migrants.

Finally, the current study has pointed out multiple research areas that require further investigation. Specifically, how weather patterns and food abundance influence refueling performance and the ability to gain mass on barrier islands and how those variables will change with a changing climate. My research found that Apalachicola Bay barrier islands are important for Northern Waterthrush, but I suggest that more study is needed involving their resource use, territoriality, and refueling performance on these islands. More research is also needed on other Neotropical songbirds using Florida Gulf coast barrier islands, as my sample sizes only allowed me to examine two species in depth. Due to increasing sea-levels, it is imperative to understand the full extent of the importance of these barrier islands for Neotropical migrant songbirds and how conservation actions can best support their preservation.

## Appendix A

Blood collection and molecular sex determination was not successful in all samples. While many factors are involved, I attempt here to explain which protocols were the most successful and suggestions that can help improve success rate.

If using Queen's lysis buffer solution for storage, the final solution should be autoclaved. While I did not autoclave microcentrifuge tubes that were used for blood storage and pipette tips used for removal of the plasma, I suggest this be incorporated into future protocols to prevent contamination of samples. I found that blood collection was easier using capillary tubes and then transferring into a microcentrifuge tube after collection. I was generally able to collect more blood using this technique. However, one-step capillary tubes have been used successfully for blood collection in the past. One-step tubes should not be used for long-term storage. Heparinized capillary tubes should be used to prevent hemolysis. Blood should be stored either in Queen's lysis buffer (Seutin et al. 1991) or 70 - 96% ethanol (Owen 2011). See Owen (2011) for exact blood collection technique using brachial venipuncture.

In 2015, I stored blood samples in 1 mL Queen's lysis buffer and kept them at room temperature until return to Delaware when they were placed in a  $-20^{\circ}\text{C}$  freezer. In 2016, I reduced the amount of buffer to 200  $\mu\text{L}$  and kept samples in a weak refrigerator for the remainder of the season before transferring them to the  $-20^{\circ}\text{C}$  freezer in Delaware. I had better success for samples collected in 2015, which I attribute to the larger amount of buffer solution. Some samples in 2016 seemed to be dryer and more difficult to work with. Best results will be obtained from samples that are frozen shortly after removing plasma, but if freezer access is unreliable in the field, it is best to keep them at  $4^{\circ}\text{C}$  or at room temperature because freeze-thaw cycles may cause cells to lyse (Owen 2011).

I had little success with the Zygem prepGEM blood DNA extraction kit and do not recommend it for future DNA extractions. Using the QIAGEN QIAamp DNA mini kit (cat. no. 51304), I had much greater success. The extracted DNA was cleaner and contained fewer proteins, resulting in more accurate readings of DNA concentrations.

I used two methods for PCR using either One*Taq* 2x Master Mix with Standard Buffer (New England BioLabs Inc. M0482) or LongAmp *Taq* DNA Polymerase (New England Biolabs Inc. M0323S). I had much greater success using LongAmp and recommend it for future studies.

After gel electrophoresis, I found that some samples revealed very faint bands. For many samples, simply re-running the gels using more PCR product made the bands more visible. Therefore, I recommend using ~10  $\mu$ L of PCR product in wells.

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## **CURRICULUM VITAE**

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### **EDUCATION**

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### **EMPLOYMENT**

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8/2014-10/2014      **Bird Bander**  
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6/2014-8/2014      **Research Technician**  
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Fort Hood, Texas

8/2014-10/2014      **Bird Bander**  
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Scottsbluff, Nebraska

3/2013-7/2013      **Field Crew Leader**  
USGS and Idaho State University  
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6/2012-8/2012      **Seasonal Field Biologist**  
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4/2010-3/2011	<b>Biology Intern</b> San Francisco Bay National Wildlife Refuge Complex Newark, California
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## **AWARDS**

2015-2016	NOAA Environmental Cooperative Science Center graduate fellowship at Delaware State University
2007	University of New Orleans Ambassador's Scholarship
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2006-2009	Taylor Opportunity Program for Students – Honors Award
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## **TRAINING AND WORKSHOPS**

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Ground Truthing Campaign. NOAA Environmental Cooperative Science Center. May 17-23, 2015, Grand Bay National Estuarine Research Reserve, Mississippi

Shortgrass Range School. Kansas Grazing Lands Coalition. August 5-7, 2014. The Nature Conservancy's Smokey Valley Ranch, Oakley, Kansas

## **ORAL PRESENTATIONS**

**Mancuso, M.L.**, A.H. Kneidel, A.A. Aispuro, L.A. Lester, and C.M. Heckscher. 2016. "Examining the Energetic Importance of Two Barrier Islands for Transient Birds during Spring Migration." NOAA EPP/MSI 8<sup>th</sup> Biannual Education and Science Forum. August 30, 2016. New York, NY.

**Mancuso, M.L.**, A.A. Aispuro, D.C. Mendez, and C.M. Heckscher. 2016. "The Energetic Importance of Apalachicola Bay Barrier Islands for Transient Birds during Spring Migration." North American Ornithological Conference VI. August 17, 2016. Washington, DC.

**Mancuso, M.L.** and A.A. Aispuro. (2016) "Spring Migration on St. George and St. Vincent Islands: Assessing Coastal Habitat Use through the Use of Avian Triglyceride Levels and Stable Isotope Analysis." Apalachicola National Estuarine Research Reserve Lecture Series. May 4, 2016. Eastpoint, FL.

**Mancuso, M.L.** 2016. "Examining the Energetic Importance of Two Barrier Islands for Transient Birds during Spring Migration." Delaware State University Graduate Symposium. April 8, 2016. Dover, DE.

**Mancuso, M.L.** & A.A. Aispuro. 2015 "Stopover Ecology of Migratory Songbirds on St. George and St. Vincent Islands." Apalachicola National Estuarine Research Reserve Lecture Series. May 6, 2015. Eastpoint, FL.