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**THE ENERGETIC IMPORTANCE OF A GULF COAST BARRIER ISLAND  
FOR THE SPRING STOPOVER ECOLOGY OF LONG-DISTANCE  
MIGRATORY BIRDS**

**by**

**MARIAMAR GUTIERREZ RAMIREZ**

**A THESIS**

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## **Dedication**

I dedicate my graduate research to my husband, Stephen M. Ecrement, who encouraged me to pursue my love of birds and research, regardless of the sacrifices and difficulties he would have to face because of it. This would not have been possible without you.

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*¡Gracias totales!*

# **The Energetic Importance of a Gulf Coast Barrier Island for the Spring Stopover Ecology of Long-Distance Migratory Birds**

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## **Abstract**

The populations of many Nearctic-Neotropical migratory birds have experienced precipitous declines. A series of possible causes of these declines have been identified, including added anthropogenic stresses during the migratory period (e.g., loss or degradation of habitat due to sea level rise, collision with structures). While the quickest and most direct route, trans-Gulf migration is physiologically stressful and riskier for birds. For birds using a trans-Gulf migration strategy in the spring, barrier islands in the northern Gulf coast may provide critical stopover habitats to rest and replenish energy reserves. A complex of such barrier islands are found in the outer Apalachicola Bay, off the Florida panhandle. The aim of this study was to determine the energetic importance of St. George Island, Florida, during spring migration for short-distance and long-distance migratory birds. Songbirds were captured in April-May 2013 and 2014 to determine body condition and obtain blood samples for plasma metabolite assays. Plasma triglyceride concentrations were used as a qualitative measure of refueling rate during spring stopover.

I confirm the use of St. George Island, Florida, as a spring stopover site for both short-distance and long-distance Nearctic-Neotropical migratory birds. Long-distance migratory birds consistently arrive in poorer condition than short-distance migrants on St. George Island during the spring. However, refueling rates are consistently similar between the two migratory groups. Age-related effects did not drive the differences observed in arrival date, body condition, and refueling rate. Capture dates, age structure, and refueling rates were not consistent between years, reflecting the variability of spring migration along the Gulf coast and the influence of intrinsic and extrinsic factors. The predictor variables to best explain spring refueling rate of migratory birds on St. George Island during this study were year and fat content.

This study is the first to evaluate the use of barrier islands by spring migratory songbirds in the northeastern Gulf coast and the first to determine their energetic importance to short- and long-distance trans-Gulf migrants. Considering the consistently poor body condition upon arrival, the loss of this vulnerable coastal resource may represent an indirect source of mortality for long-distance migratory songbirds, many with already declining populations. St. George Island is energetically important to migratory songbirds because it provides refuge and opportunities to refuel. Therefore, the conservation, restoration, and management of these coastal resources should be considered an important aspect of the continental-scale needs of these migratory species.

## Table of Contents

<b>List of Tables .....</b>	<b>ix</b>
<b>List of Figures.....</b>	<b>xi</b>
<b>List of Abbreviations .....</b>	<b>xiii</b>
<b>CHAPTER 1: INTRODUCTION.....</b>	<b>1</b>
<b>CHAPTER 2: LITERATURE REVIEW .....</b>	<b>5</b>
<b>2.1. Gulf of Mexico barrier islands .....</b>	<b>5</b>
<b>2.2. Spring stopover habitat for trans-Gulf migrants .....</b>	<b>6</b>
<b>2.3. Energetic condition of migratory birds.....</b>	<b>11</b>
<b>2.4. Study species.....</b>	<b>12</b>
<b>CHAPTER 3: METHODOLOGY .....</b>	<b>14</b>
<b>3.1 Study site .....</b>	<b>14</b>
<b>3.2 Bird captures and blood collection.....</b>	<b>16</b>
3.2.1 Mist-net captures.....	16
3.2.2 Banding and data collection .....	17
3.2.3 Collection of blood samples .....	17
3.2.4 Post-collection blood processing .....	18
<b>3.3 Analysis of plasma metabolites.....</b>	<b>19</b>
<b>3.4 Molecular sex determination.....</b>	<b>21</b>
3.4.1 DNA extraction.....	21
3.4.2 Polymerase Chain Reaction to amplify CHD genes .....	23
3.4.3 Visualization of PCR products via gel electrophoresis .....	24
<b>3.5 Statistical Analysis.....</b>	<b>25</b>
3.5.1 Triglyceride (quality control).....	25
3.5.2 Size-corrected body mass calculations .....	26
3.5.3 Descriptive statistics .....	27
<b>CHAPTER 4: RESULTS .....</b>	<b>29</b>
<b>4.1 Capture dates and time.....</b>	<b>31</b>
<b>4.2 Recapture rate and stopover duration .....</b>	<b>34</b>
<b>4.3 Age structure .....</b>	<b>36</b>
<b>4.4 Sex structure .....</b>	<b>38</b>
<b>4.5 Body condition.....</b>	<b>38</b>
<b>4.6 Refueling rates.....</b>	<b>50</b>
<b>4.7 Models for plasma triglyceride concentration .....</b>	<b>55</b>

<b>CHAPTER 5: DISCUSSION .....</b>	<b>63</b>
<b>5.1 Refueling rates.....</b>	<b>63</b>
<b>5.2 Body condition.....</b>	<b>66</b>
<b>5.3 Capture date and time.....</b>	<b>68</b>
<b>5.4 Recapture rate and stopover duration.....</b>	<b>69</b>
<b>5.5 Age structure .....</b>	<b>70</b>
<b>5.6 Sex structure .....</b>	<b>72</b>
<b>5.7 Conservation implications.....</b>	<b>73</b>
<b>5.8 Conclusions .....</b>	<b>73</b>
<b>LITERATURE CITED .....</b>	<b>75</b>
<b>APPENDICES .....</b>	<b>89</b>
Appendix A: Gel electrophoresis for molecular sex determination.....	90
Appendix B: R code used for statistical analysis.....	91
<b>CURRICULUM VITA.....</b>	<b>92</b>

## List of Tables

TABLE 4.1	Total number of individual birds captured, recaptured, and percentages of different age classes for four species of migratory songbirds captured during spring migration on St. George Island, Florida, 2013-2014.....	28
TABLE 4.2	Estimated minimum stopover duration and change in body mass for individual birds recaptured >1 day after first capture on St. George Island, Florida, in spring 2013 and 2014.....	32
TABLE 4.3	Percentages of age classes for short- and long-distance migratory songbirds captured during spring migration on St. George Island, Florida, 2013-2014.....	34
TABLE 4.4	Mean $\pm$ one standard deviation (SD) fat and pectoral muscle scores for short- and long-distance migrants age classes captured during spring migration on St. George Island, Florida, 2013-2014.....	39
TABLE 4.5	Mean $\pm$ one standard deviation (SD) plasma triglyceride (TRIG) concentrations (mmol/L) of migratory landbirds during spring stopover on St. George Island, Florida, 2013-2014.....	49
TABLE 4.6	Generalized linear model (GLM) using plasma triglyceride (TRIG) of all migratory birds captured on St. George Island, Florida, as the predictor variable.....	53
TABLE 4.7	Generalized linear model (GLM) using plasma triglyceride (TRIG) of all migratory birds captured on St. George Island, Florida, in 2013 (Year 1 of the study) as the predictor variable.....	54

TABLE 4.8	Generalized linear model (GLM) using plasma triglyceride (TRIG) of all migratory birds captured on St. George Island, Florida, in 2014 (Year 2 of the study) as the predictor variable.....	55
TABLE 4.9	Generalized linear model (GLM) using plasma triglyceride (TRIG) of Gray Catbird captured on St. George Island, Florida, as the predictor variable.....	56
TABLE 4.10	Generalized linear model (GLM) using plasma triglyceride (TRIG) of Catharus thrushes captured on St. George Island, Florida, as the predictor variable.....	57

## List of Figures

FIGURE 3.1	Map of study site located in Apalachicola Bay, Florida.....	14
FIGURE 4.1	Capture dates for short-distance and long-distance migrants on St. George Island, Florida, during spring migration 2013-2014.....	30
FIGURE 4.2	Fat scores of short-distance and long-distance migratory birds captured on St. George Island, Florida, during spring migration 2013-2014.....	37
FIGURE 4.3	Pectoral muscle scores of short-distance and long-distance migratory birds captured on St. George Island, Florida, during spring migration 2013-2014.....	38
FIGURE 4.4	Size-corrected body mass (g) in Gray Catbirds captured on St. George Island, Florida, during spring migration 2013-2014.....	40
FIGURE 4.5	Daily change in size-corrected body mass of Gray Catbird captured during spring migration on St. George Island, Florida, in April-May 2013.....	42
FIGURE 4.6	Daily change in size-corrected body mass of Gray Catbird captured during spring migration on St. George Island, Florida, in April-May 2014.....	43
FIGURE 4.7	Hourly change in size-corrected body mass (g) in Gray Catbird captured on St. George Island, Florida, during spring migration April-May 2013.....	44
FIGURE 4.8	Hourly change in size-corrected body mass (g) in Gray Catbird captured on St. George Island, Florida, during spring migration April-May 2014.....	45
FIGURE 4.9	Size-corrected body mass (g) of long-distance migrants captured on St. George Island, Florida, during spring migration 2013-2014.....	46

FIGURE 4.10	Plasma triglyceride concentration (mmol/L) of short- and long-distance migratory birds captured on St. George Island, Florida, during spring migration 2013 and 2014.....	48
FIGURE 4.11	Plasma triglyceride concentration (mmol/L) of all migratory birds combined captured on St. George Island, Florida, during spring migration 2013 and 2014.....	51
FIGURE 4.12	Triglyceride concentration as a function of fat content in migratory birds captured on St. George Island, Florida, during spring migration in 2013 and 2014.....	58
FIGURE 4.13	Triglyceride concentration as a function of muscle score in migratory birds captured on St. George Island, Florida, during spring migration in 2013 and 2014.....	59

## List of Abbreviations

GCTH	Gray-cheeked Thrush, <i>Catharus minimus</i>
GRCA	Gray Catbird, <i>Dumetella carolinensis</i>
SWTH	Swainson's Thrush, <i>Catharus ustulatus</i>
VEER	Veery, <i>Catharus fuscescens</i>
HY	hatch year
SY	second year
AHY	after hatch year
ASY	after second year
NOAA	National Oceanic and Atmospheric Administration
ANERR	Apalachicola National Estuarine Research Reserve
μL	microliter
mg/dL	milligrams per deciliter
mmol/L	millimole per liter
CHD	chromo-helicase-DNA binding gene
TRIG	triglyceride concentration

## CHAPTER 1:

### INTRODUCTION

The increasing threat of land loss and modification due to sea-level rise and extreme storms on Gulf of Mexico barrier islands necessitates an understanding of their importance to vulnerable wildlife populations. Neotropical migratory birds, many with declining populations, may be particularly vulnerable to the loss of critical stopover habitat, including barrier islands.

Twice a year, millions of migratory birds fly non-stop across the Gulf of Mexico (800-1000 km) on their way to and from the wintering and breeding grounds. Migration is physiologically stressful, with mortality rates up to 50% higher than during stationary periods (Sillet and Holmes 2002). During this period, *en route* habitat is fundamental for the successful completion of migration (Moore and Woodrey 1993, Hurro 2000), and is particularly important for birds after they cross large ecological barriers (Mehlman et al. 2005, Bauchinger et al. 2011). The Gulf of Mexico represents the largest ecological barrier for Nearctic-Neotropical migrants, and as such, suitable habitat is necessary on both ends of the route. Coastal forests and barrier islands on the northern Gulf play an important role by providing migratory birds, an internationally shared resource, with

critical opportunities to rest and replenish energy reserves (Moore et al. 1990, Kuenzi et al. 1991, Lester et al. 2016).

While much study has been done in the western and central Gulf coast (e.g., Loria and Moore 1990, Moore et al. 1990, Kuenzi et al. 1991, Yong and Moore 1993, Yong and Moore 1997, Woodrey and Moore 1997, Simons et al. 2004, Owen and Moore 2008), this study is the first to evaluate the use of barrier islands by spring migrant songbirds in the northeastern Gulf coast. St. George Island is one of a complex of four barrier islands found in the outer Apalachicola Bay off the Florida panhandle and within the Apalachicola National Estuarine Research Reserve. Recent tracking studies have indicated that the Apalachicola River basin may be an important route for long-distance migratory thrushes during spring and fall migration (Heckscher et al. 2011, Stanley et al. 2012). My primary objective was to confirm the use of the island by northbound migratory passerines and to determine its energetic importance as a spring stopover site. My secondary objective was to provide data to inform conservation and management decisions to managers at Apalachicola National Estuarine Research Reserve.

For this study, I compared the refueling performance of long-distance and short-distance Neotropical migrants. Migratory thrushes in the genus *Catharus* are long-distance migrants that breed in the Northeast and boreal forests and winter in Central and South America. Gray Catbird (*Dumetella carolinensis*) is a shorter-distance migrant, breeding along the East coast and wintering in the southern U.S., Mexico, the Caribbean, and Central America. I expected that migration distance, including both wintering origin

and breeding destination, would have an important effect in body condition upon arrival and refueling rate on spring stopover. Given the differences in migration distance, I considered the following hypotheses: (1) during spring stopover on St. George Island, long-distance migratory birds will arrive in poorer body condition than short-distance migratory birds; (2) during spring stopover on St. George Island, long-distance migratory birds will have higher refueling rates than short-distance migratory birds; and (3) long-distance and short-distance migratory birds will present sex and age-related differences in arrival date, body condition, and refueling rate, with sub-adult birds arriving in later in the season, in poorer condition, and refueling at lower rates than adult birds, and males arriving earlier, in poorer condition, and refueling at higher rates than females.

In order to test these hypotheses, songbirds were captured during peak migratory periods in April and May 2013 and 2014 to determine body condition and obtain blood samples for plasma metabolite assays. Plasma triglyceride concentrations were used as a qualitative measure of refueling rate. I predicted that (1) long-distance migrants will arrive in poorer condition than short-distance migrants; (2) as a consequence, long-distance migrants will have a higher refueling rate than short-distance migrants; (3) in both long-distance and short-distance migrants, males will arrive in better condition, have higher refueling rates, and arrive earlier than females; and (4) in both long-distance and short-distance migrants, adult (after second year) birds will arrive in better condition, have higher refueling rates, and arrive earlier than sub-adult (second year) birds.

This study allows for an assessment of the ecological importance of Apalachicola Bay barrier islands to migratory songbirds during a vulnerable life stage of their annual cycle.

## **CHAPTER 2:**

### **LITERATURE REVIEW**

#### **2.1. Gulf of Mexico barrier islands**

Barrier islands are formations of sand, shell, and gravel that exist along coasts, and form a defense against winds and waves, while providing habitat for many wildlife species (NOS/NOAA 2008). Barrier islands are important coastal ecosystems that physically separate the ocean from inshore wetlands, bays, sounds, and estuaries, thus creating the structure that allows for rich coastal wetland habitats (Feagin et al. 2010).

Low-lying coastal areas, including barrier islands, are extremely vulnerable to sea-level rise and its related effects (i.e., salt water encroachment, fragmentation) and to landscape modification from human development of coastal areas (FitzGerald et al. 2008). The Gulf of Mexico and mid-Atlantic coasts have experienced the highest rates of relative sea-level rise in the United States (NABCI 2010). Global sea-level increased an average of 1.8 mm per year between 1961 and 1993, and an average of 3.1 mm between 1993 and 2003 (IPCC 2007). In fact, global mean sea level rise is predicted to rise at least 0.2 m and no more than 2 m by the year 2100 (Parris et al. 2012). Extreme weather

events, which are expected to increase in frequency (IPCC 2007), cause impacts on barrier island geography and stability. As observed after Hurricane Katrina in 2005, central Gulf Coast barrier islands experienced extreme changes, including landward migration and stripping of sand (Sallenger et al. 2005).

Erosion and land loss threaten barrier islands. The barrier islands associated with the Mississippi Delta have progressively diminished in size due to decreased sediment supply, increased rates of relative sea-level rise, and increased frequency of hurricanes and winter storms (Morton 2008, Stone and McBride 1998). In Louisiana, the Isles Derniers barrier island chain has eroded at a rate of 28.2 ha yr<sup>-1</sup> (Khalil and Lee 2006).

The loss of barrier islands would be detrimental because, in addition to coastal protection, barrier islands also provide critical habitat for wildlife. For instance, beaches on Apalachicola Bay barrier islands provide nesting habitats for various threatened bird species and Atlantic loggerhead turtle (*Caretta caretta*) (Edmiston 2008). Barrier islands along the Gulf of Mexico provide important stopover habitat for Nearctic-Neotropical migratory birds (Moore and Aborn 2000, Mehlman et al. 2005, Lester et al. 2016). Migratory birds are of important conservation concern as many populations have experienced precipitous declines (Rich et al. 2004).

## **2.2. Spring stopover habitat for trans-Gulf migrants**

During migration, most Nearctic-Neotropical migratory landbirds make frequent stops to rest and rebuild energy reserves before they are able to continue migration (Blem

1980, Mehlman et al. 2005). These “stopover” habitats are defined as the set of habitats that migratory landbirds use during the spring and fall migration seasons (Mehlman et al. 2005) and are important because they provide a place to rest, refuel, and avoid predators. Stopover habitats are especially important for birds completing or preparing for a trans-Gulf migration (Buler et al. 2007, Kuenzi et al. 1991). Considering that these birds spend a third of the year migrating (Mehlman et al. 2005), the stopover habitats required during this period of the life cycle are crucial for conservation and management of these species (Mehlman et al. 2005, Moore and Woodrey 1993, Moore et al. 1995, Donovan et al. 2002).

The coastal forests of the Gulf of Mexico, up to 100 km inland, are also crucial for migratory birds (Barrow et al. 2005, Petit 2000). Barrow et al. (2005) describe the geographic pattern of bird migration around the Gulf of Mexico and locations of remaining patches of extensive coastal forest. Barrier islands off the panhandle of Florida (including the barrier islands in Apalachicola Bay) are considered of sporadic common use; prevailing winds determine if the area will be used by a moderate number of migrants. The availability of these habitats are critical for migrating birds that may have been blown off their original course, thus requiring a safety net of habitat to stopover. Human development, agriculture, livestock grazing, pine plantations, exotic species, and climate change affect the coastal forests of the Gulf (Barrow et al. 2005, Lester et al. 2016).

The prioritization of conserving stopover habitats is difficult given that levels of migrant use and habitat quality at stopover sites vary both within and between seasons (Mehlman et al. 2005). Considering the variability in quality and migrant use of stopover sites, Mehlman et al. (2005) describe these sites based on their capacity to meet migrants' needs at a given point in time and space. Stopover sites can be described as "full-service hotel", "convenience stores", or "fire escapes". "Fire escape" stopover sites are typically small patches of isolated habitats surrounded by unusable habitat or adjacent to significant barriers, and are "utterly vital in emergency situations" (Mehlman et al. 2005). Barrier islands off the coast of the Gulf of Mexico are examples of fire escape stopover sites.

Many factors influence the selection and amount of time a migrant spends at particular stopover sites, including the habitat's resource availability and shelter from predators, as well as the weather conditions and the energetic condition of the bird (Moore et al. 1995). Lean birds with depleted energy reserves generally stay longer at stopover sites than birds with better energetic conditions (Loria and Moore 1990, Yong and Moore 1997, Bayly et al. 2012). On the other hand, birds with fat reserves will just take refuge in dense habitats to conserve energy and avoid predators while waiting for favorable conditions to continue migration (Moore and Aborn 2000). However, habitat suitability is a crucial consideration even for lean birds in need of quickly regaining mass (Kuenzi et al. 1991).

Migration is a physiologically stressful period in a bird's life cycle and mortality can be higher than during the stationary periods (Sillett and Holmes 2002). High energy demands are one of many challenges of migration (Loria and Moore 1990, Blem 1980). In order to meet the high energy demands required during migration, birds must balance foraging activity and predator avoidance (Loria and Moore 1990). Young birds have less experience foraging and avoiding predators, and are outcompeted by older, more experienced birds. Thus, younger birds are expected to have a harder time meeting the energetic demands of migration (Woodrey 2000, Woodrey and Moore 1997) and would reach their destinations after older birds (Gonzalez-Prieto et al. 2011). Yet, a recent study using plasma triglycerides found that juvenile and adult refueling rates did not differ during fall migration at a stopover site in New York in Swainson's Thrush (*Catharus ustulatus*), Common Yellowthroat (*Geothlypis trichas*), Yellow-rumped Warbler (*Setophaga coronata*), and White-throated Sparrow (*Zonotrichia albicollis*) (Seewagen et al. 2013). Heise and Moore (2003) found hatch-year Gray Catbirds (*Dumetella carolinensis*) were less efficient foragers than after hatch-year birds prior to fall migration; however, young birds continued to improve their efficiency and by the time migration started were just as efficient as the adults. Young birds started to deposit fat earlier, but did so more slowly than adults; while adults started to deposit fat later but more rapidly (Heise and Moore 2003).

There is also evidence of differential migration between sexes. Yong and Moore (1997) suggest differential migration between sexes of migratory thrushes based on wing

chord measurements, with males arriving earlier than females. Hermit Thrush (*Catharus guttatus*) males migrate around 20 days earlier than females (Stouffer and Dwyer 2003). During spring stopover, Seewagen et al. (2013) found that male Common Yellowthroats and Yellow-rumped Warblers had higher refueling rates than females.

Differences in speed of fat deposition may be critical during spring migration when birds are returning to breed. The challenges encountered during spring migration can have important carry over effects, such as influencing arrival at breeding sites and subsequent reproductive performance (Lozano et al. 1996, Smith and Moore 2003, Mehlman et al. 2005). For instance, female American Redstarts (*Setophaga ruticilla*) arriving on the breeding grounds with fat deposits experienced gains in reproductive performance measured by increased clutch size, egg volume, and nestling mass (Smith and Moore 2003).

Quantifying the duration of a migratory bird's stopover is key in assessing the importance of particular sites. This is done primarily by mark-recapture (e.g., Yong and Moore 1997, Bayly et al. 2012, Sillet and Holmes 2002). However, the length of stopover duration using mark-recapture methods may be underestimated since calculations only use the marked individuals and assume that birds were captured on date of arrival and that birds not recaptured have left the site (Schaub et al. 2001, Bachler and Schaub 2007).

### 2.3. Energetic condition of migratory birds

Birds use glucose and glycogen for muscular activity, but they rely on the accumulation and use of lipids for long-distance migrations (Blem 1980). Helms and Drury (1960) established a 6-scale description of the accumulated fat visible in the furcular or interclavicular region of birds. This description covers birds from “no accumulated fat” (class 0) to birds with fat stores overflowing the length of the furculum (class 5). This system is still used in many studies to date. Kaiser (1993) describes a 9-point scale, which provides a more detailed description of fat deposits in birds. Studies have used the change in fat deposits in recaptured birds to demonstrate refueling at stopover sites (Yong and Moore 1997, Yong and Moore 1993, Loria and Moore 1990, Heise and Moore 2003, Bayly et al. 2012). However, this method relies heavily on recapturing birds in order to determine a change, which is not always possible.

Plasma metabolite profiling offers a way to assess stopover refueling performance of migratory birds from a single capture (Guglielmo et al. 2002, Guglielmo et al. 2005). Plasma metabolite analysis uses residual plasma triglyceride levels to estimate rates of fattening or refueling. High levels of plasma triglyceride represent high fattening rates, because the amount of food ingested affects plasma triglyceride levels (Jenni and Jenni-Eiermann 1996). However, plasma metabolite profiling is a qualitative, or relative, measure (Guglielmo et al. 2005), and therefore it must be interpreted in reference to birds

refueling under different conditions (Seewagen et al. 2011, Seewagen 2013, Evans Ogden et al. 2013).

Understanding changes in lipid reserves is important information for assessing the quality of stopover habitat (Anteau and Afton 2008). For instance, Evans Ogden et al. (2013) used plasma triglyceride levels in birds throughout an elevation gradient to show that high elevations could be high-quality stopover habitats. Based on their results, they recommend that high elevation stopover sites should be considered for protection in songbird management and conservation plans. Migrating birds using an urban stopover site, both in fall and spring, refueled comparably to birds in less disturbed areas, indicating that urban parks provide adequate refueling opportunities for migrating birds (Seewagen et al. 2011). Acevedo Seaman et al. (2006) found a positive relationship between plasma triglyceride levels in migratory sandpipers and total macrofaunal prey abundance at their study sites.

#### **2.4. Study species**

Nearctic-Neotropical migratory thrushes (genus *Catharus*) were selected as the focal species of this study because most individuals that breed in Eastern North America undergo trans-Gulf migrations in the spring (Yong and Moore 1997), and therefore those populations may depend on quality coastal stopover habitats. The Swainson's Thrush (*C. ustulatus*, SWTH), Veery (*C. fuscescens*, VEER), and the Gray-cheeked Thrush (*C.*

*minimus*, GCTH) are forest birds that breed in North America and winter in Central and South America. There are information gaps for these species, as further research is needed on the habitat requirements during migration and the distribution of stopover sites along migration routes (Bevier et al. 2005, Mack and Yong 2000, Lowther et al. 2001). Recent geolocator technology has demonstrated that the Veery (*C. fuscescens*) performs one of the longest migrations by Neotropical migratory birds, which includes an intra-tropical migration to a second wintering site in South America (Heckscher et al. 2011, Heckscher et al. 2015)

In addition to the *Catharus* thrushes, I sampled Gray Catbird (*Dumetella carolinensis*, GRCA). The Gray Catbird is currently a common species in most areas with a stable population trend (IUCN 2013). The Gray Catbird has variable migratory patterns, wintering along the Gulf coast, Central America, and the Greater Antilles (Marsh 1983). The populations that winter in the Yucatan Peninsula and southward are trans-Gulf migrants that deposit significant stores of fat to complete the Gulf crossing (Smith et al. 2011). Marsh (1983) found that Gray Catbirds obtained maximum body mass during spring migration in inland Florida, indicating refueling. While Gray Catbirds are in the Mimidae family, they are closely related and have a comparable size to *Catharus* thrushes (mass 23–56 g), which allows for comparison. But while *Catharus* thrushes are insectivorous, Catbirds have a more generalist diet (e.g., Lowther et al. 2001, Mack and Yong 2000, Smith et al. 2011, Bevier et al. 2005). In addition, the wintering distribution of *Catharus* thrushes reaches farther south than Gray Catbirds.

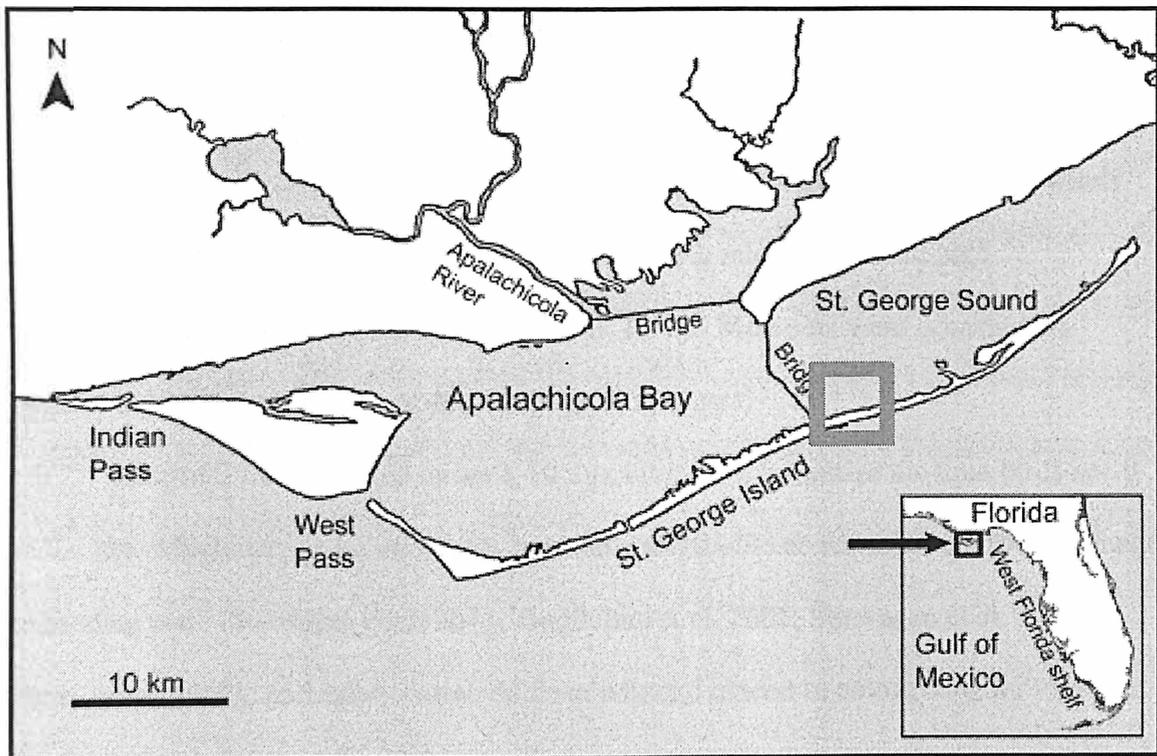
## **CHAPTER 3:**

### **METHODOLOGY**

#### **3.1 Study site**

This study was conducted during April and May of 2013 and 2014 at St. George Island, Florida, a barrier island along the Gulf of Mexico. The narrow 33 km long island is approximately 7 km from the mainland and is one of four barrier islands at the southern edge of Apalachicola Bay (Figure 3.1). The vegetation on St. George Island consists of a narrow band of beaches and low-lying sand dunes that grade into mixed woodland grass, palmetto, and bayside marshes (Edmiston 2008). St. George Island is connected to the mainland via the Bryant Patton Bridge and is rapidly being developed (Florida Department of Environmental Protection 1998). Fieldwork was conducted on property owned and managed by the Apalachicola National Estuarine Research Reserve (ANERR) - a patch of coastal pine forest on the sound side of the island (lat 29.667172, long - 84.853591).

Figure 3.1. Map of study site located in Apalachicola Bay, Florida. Red box indicates location of constant effort mist-net station operated during spring migration from April-May 2013 and 2014.



## 3.2 Bird captures and blood collection

### 3.2.1 Mist-net captures

A constant-effort mist-net station was operated from 21 April – 10 May 2013 and 14 April – 9 May 2014 to coincide with peak thrush and catbird migration in the Northern coast of the Gulf of Mexico (Yong and Moore 1997, Moore pers. comm. 2012). A three-person crew operated 120 m of mist-nets (two 6 m, four 9 m and six 12 m x 2.6 m; 36 mm-mesh size). Mist-nets were opened daily after sunrise, unless rain or steady winds (>10 mph) prevented safe operation, and closed during midday to avoid high temperatures and decreased shade (Ralph et al. 1993). Mist-nets were occasionally opened in the afternoon and operated until prior to sunset.

Mist-nets were checked on an 8-10 min interval to minimize the time birds spent in the net, which may cause variations in plasma metabolite concentrations from a change in feeding state (Seewagen et al. 2011, Guglielmo et al. 2005, Seewagen et al. 2013). Birds were quickly and safely extracted from nets and placed in cotton bags for transport to a banding table. Net lanes could be checked in under 3 minutes, and target species were given priority processing when possible (e.g., communication via radio so a bander was ready to process, birds were sent back to banding table before net run was completed).

### 3.2.2 Banding and data collection

Upon removal from mist-nets, every individual was identified to species and fitted with a uniquely numbered United States Geological Survey (USGS) aluminum band. Once a bird was banded and identified as a focal species, a blood sample and feather/claw samples (for use in a separate concurrent project) were immediately collected. Every individual was aged as second year (SY) or after second year (ASY) when possible (following Pyle 1997), and was scored on an 8-point scale to determine fat deposits (Kaiser 1993), scored on a 4-point scale to determine pectoral muscle (Redfern and Clark 2001), measured (wing chord, to 1 mm), weighed to 0.1g on a digital scale, and released. Additional morphometric data were collected from *Catharus* thrushes: bill width, exposed culmen, nares to tip, p10-primary coverts, tail, and tarsus to allow for flexibility in future morphometric analyses that might be desired.

### 3.2.3 Collection of blood samples

Blood samples were collected from *Catharus* thrushes and Gray Catbird, taking a maximum of 10% body mass (roughly equivalent to ~1% blood volume; Owen 2011, Fair et al. 2010). For thrushes and catbirds, permissible samples sizes would have been between 200-300  $\mu\text{L}$ ; however, I rarely collected more than 150  $\mu\text{L}$  of whole blood to account for additional blood loss from hematomas or bleeding into extravascular space (Owen 2011), as well as the difficulty of obtaining sufficient blood flow from dehydrated birds.

Blood samples were taken by brachial venipuncture using a 26-gauge needle (BD Medical PrecisionGlide; Owen and Moore 2008, Seewagen et al. 2011, Seewagen et al. 2013) and collected in one-step heparinized capillary/centrifuge tubes (Sarstedt microvette CB300). After collection, each tube was marked in the field with the date and the bird's corresponding USGS band number using an ultra-fine permanent pen and stored in a cooler until the end of the banding session (8-10 hrs). To ensure proper clotting, pressure was applied to the bird's puncture area and cornstarch was applied when necessary (Owen 2011). The time of capture, as well as the time of blood collection, were recorded in order to calculate bleed time which could not exceed 20 minutes (Seewagen et al. 2011, Seewagen pers. comm. 2013).

#### 3.2.4 Post-collection blood processing

After collection in the field, blood samples were processed each afternoon in temporary lab space in the field housing. Blood samples were centrifuged for 10 minutes at 2000xG (Fisherbrand Mini Centrifuge S67601A), making sure samples were well balanced within the centrifuge. Plasma was then carefully separated using a variable micropipet (0.5-10  $\mu$ L), ensuring tips were changed between samples, and transferred into a 1mL cryogenic vial. Each cryogenic vial was marked with the species four-letter alpha code, band number, and date. In addition, the color of the plasma was noted on the control sheet, as recommended by Owen (2011) in order to assess if assay outliers are due to possible effects of hemolysis. Separated plasma was stored on either dry ice (2013) or liquid nitrogen (2014) until transferred to a -80°C freezer (Florida A&M University in

Tallahassee and/or Delaware State University). The remaining residual blood cells were stored in 1 mL of Queen's lysis buffer (Seutin et al. 1991) and stored at room temperature until transferred to a -20°C freezer (Owen 2011).

### **3.3 Analysis of plasma metabolites**

Analysis of plasma metabolites was conducted in the Department of Biological Sciences, Delaware State University. Triglyceride concentration was measured by endpoint colorimetric assay (Cayman Chemical Company, Item No. 100010303) and determined by enzymatic hydrolysis of triglycerides to glycerol and free fatty acids followed by colorimetric measurement of the glycerol released (Cayman Chemical Company 2013). Due to the limited amount of plasma for each sample, only triglyceride concentration was measured.

As per manufacturer's instructions, the 4 mL Standard Diluent (CCC Item No. 700732) was diluted using 16 mL of HPLC-grade water. The diluted Standard Diluent, a 5X salt solution, was used to prepare the triglyceride standards and to dilute plasma samples threefold in order to increase their volume. In order to run plasma samples in duplicate, and because each reaction required 10  $\mu$ L of sample, plasma was diluted threefold (e.g., to 10  $\mu$ L of plasma, add 20  $\mu$ L diluent to make 30  $\mu$ L of diluted sample which could be used to run in duplicate/triplicate; Seewagen et al. 2011, Seewagen pers. comm. 2013).

Triglyceride standards were used to create a standard curve. They were prepared by following the manufacturer's instructions to serially dilute 8 samples, starting with

400  $\mu\text{L}$  of diluted Standard Diluent and 100  $\mu\text{L}$  of Triglyceride Standard (CCC Item No. 10010509) in the first tube. Each of the remaining seven tubes had 200  $\mu\text{L}$  each of diluted Standard Diluent. 200  $\mu\text{L}$  were removed from the first tube and added to the second tube and mixed well. Next, 200  $\mu\text{L}$  were removed from the second tube and added to the third tube. This process was repeated for the remaining tubes, leaving the last tube untouched, with only diluted Standard Diluent as a blank.

In a 400  $\mu\text{L}$  flat-bottom 96-well microplate, 10  $\mu\text{L}$  of each diluted triglyceride standard was added per well. Each standard was run in duplicate, changing pipette tips between standards. In the remaining 80 wells, 10  $\mu\text{L}$  of diluted plasma samples were added. Each sample was run in duplicate, and again, pipette tips were changed between samples.

The assay reaction was initiated by adding 150  $\mu\text{L}$  of triglyceride enzyme mixture (CCC Item No. 10010511), which was reconstituted with a diluted sodium phosphate assay buffer (CCC Item No. 700003). The triglyceride enzyme mixture was carefully added to each well, ensuring the tip did not come into contact with the contents of the well. The final volume of the assay was 160  $\mu\text{L}$  in each well (e.g., 150  $\mu\text{L}$  triglyceride enzyme mixture + 10  $\mu\text{L}$  plasma sample). The plate was allowed to incubate at room temperature for 15 minutes on a lab stirrer. The absorbance was read at 530-550 nm using a microplate spectrophotometer (Molecular Devices SpectraMax M5).

The corrected absorbance of each standard and sample was calculated by subtracting the average absorbance value of the blank standard (tube 8) from itself and all

the other values. The corrected absorbance values of the standards were graphed as a function of the final triglyceride concentration (mg/dL). The equation obtained from the linear regression of the standard curve was utilized to calculate the triglyceride concentrations in the samples by substituting the corrected absorbance value of each sample into the following equation:

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

The results of the equation were multiplied by the appropriate dilution factor (e.g., by 3, diluted threefold) and by the International System of Units (SI Units; e.g., 0.0113) to convert from mg/dL to mmol/L.

### **3.4 Molecular sex determination**

*Catharus* thrushes and Gray Catbird are sexually monomorphic species (Pyle 1997) that cannot be reliably sexed outside of the breeding season. DNA-based sex identification allows a way to determine sex during the non-breeding seasons.

#### **3.4.1 DNA extraction**

DNA was extracted from residual blood cells (remaining after plasma extraction for metabolite assays) by one of either two methods: a spin protocol using silica-membrane-based spin columns (Qiagen QIAamp DNA Mini Kit Cat. No. 51304) or a rapid extraction enzymatic system (prepGEM Blood ZyGEM No. PBL0050).

#### *3.4.1.1 Silica-membrane spin protocol*

The DNA purification from blood or body fluids (spin protocol) detailed in the product handbook (QIAGEN 2012) was followed for the silica-membrane spin protocol. Briefly, the protocol steps were: adding 20  $\mu\text{L}$  of proteinase K to a 1.5 mL microcentrifuge tube and adding to it 200  $\mu\text{L}$  of blood sample and 200  $\mu\text{L}$  of Buffer AE, and incubating at 56°C for 10 minutes. After incubating, 200  $\mu\text{L}$  of 100% ethanol (EtOH) was added to the tube, pulse-vortexed for 10-15 seconds, and carefully transferred to a 2mL spin column. Each spin column was centrifuged at 6000xG for 60-90 seconds. Afterwards, the filtrate was discarded and 500  $\mu\text{L}$  of Buffer AW1 was added and centrifuged again at 6000xG for 60-90 seconds. Filtrate is again discarded, and 500  $\mu\text{L}$  of Buffer AW2 was added and the tube was centrifuged again at 20,000xG for 3 minutes. The filtrate was again discarded and the spin column was placed in a clean 2 mL microcentrifuge tube, 200 $\mu\text{L}$  distilled water was added, and allowed to incubate for 1 minute at room temperature. The tube was centrifuged a final time at 6000xG for 1 minute, and the spin column discarded. The eluate remaining in the microcentrifuge tube contained the extracted DNA. DNA concentrations for each sample were immediately measured with a spectrophotometer (NanoDrop Lite Spectrophotometer) and samples were stored in a -20°C freezer.

#### *3.4.1.2 Rapid enzymatic extraction protocol*

DNA extraction was completed following the recommended methods outlined in the product guide (ZyGEM Quick-Start Guide DNA Extraction Using prepGEM Blood).

Briefly, the steps were: add 86.5  $\mu$ L nuclease-free water, 10  $\mu$ L of the included 10x buffer, 2.5  $\mu$ L of blood sample, and 1  $\mu$ L of prepGEM into a 0.2 mL thin-walled PCR tube. The tube was incubated in a thermal cycler at 75°C for 15 minutes followed by 95°C for 5 minutes. After incubation, tubes were centrifuged at 20,000xG for 5 minutes. A pipette was used to carefully transfer the supernatant into a new tube without disturbing the pellet. The new tube contained the single-stranded extracted DNA. DNA concentrations for each sample were immediately measured with a spectrophotometer (NanoDrop Lite Spectrophotometer) and samples were stored in a -20°C freezer.

#### 3.4.2 Polymerase Chain Reaction to amplify CHD genes

After DNA extraction, the homologous sections of sex chromosome-based chromo-helicase-DNA binding (CHD) genes were amplified by PCR. Griffiths et al. (1998) describe a test based on two conserved CHD (chromo-helicase-DNA-binding) genes that are located on the avian sex chromosomes of all birds (except ratites). Only females possess the W chromosome, where the CHD-W gene is located. The CHD-Z chromosome is located in the Z chromosome, present in both sexes. The set of P2/P8 primers (Griffiths et al. 1998) were custom-ordered from Invitrogen Life Technologies (P2: 5'-TCT GCA TCG CTA AAT CCT TT-3'; and P8: 5'-CTC CCA AGG ATG AGR AAY TG-3'). These primers have been successfully used for sex determination of *Catharus* thrushes and Gray Catbird (*C. bicknelli*: Townsend et al. 2011, Townsend et al. 2010; *C. guttatus*: Stouffer and Dwyer 2003; *C. ustulatus*: Wilson et al. 2008; *D. carolinensis*: Owen et al. 2010).

A 50  $\mu\text{L}$  reaction mixture was prepared by adding 28.5  $\mu\text{L}$  of nuclease-free water, 2  $\mu\text{L}$  of 10 $\mu\text{M}$  primer P2, 2  $\mu\text{L}$  of 10 $\mu\text{M}$  primer P8, 1.5  $\mu\text{L}$  of 10 $\mu\text{M}$  dNTPs, 5  $\mu\text{L}$  of 10x buffer, 10  $\mu\text{L}$  template DNA, and 1  $\mu\text{L}$  of Taq polymerase (short). Up to 24 PCR tubes were set in a thermocycler at once with the following settings: 94°C for 60 seconds, 30 cycles of 30 seconds at 94°C, 30 seconds at 50°C, 60 seconds at 72 °C, followed by a final extension at 72°C for 10 minutes, and held at 4°C.

### 3.4.3 Visualization of PCR products via gel electrophoresis

PCR products were visualized on a simple 1.5 or 2% agarose gel. When observed under UV-light, male (one band) are distinguishable from female (two bands) samples (Appendix B). To prepare the 2% agarose gel, 2 grams of agarose powder was dissolved in an Erlenmeyer flask with 100 mL of 1x Tris-acetate-EDTA (TAE) buffer and heated in the microwave for 2 minutes until the liquid was clear. The liquid was allowed to cool for a few minutes, and 0.3  $\mu\text{L}$  of Ethidium bromide (EtBr) was carefully added. The liquid was poured into casting trays prepared with combs. After 20-30 minutes, the gel was removed from casting tray and the comb removed.

Gels were placed in an electrophoresis chamber and covered with 1% TAE buffer. The first depression was loaded with 8  $\mu\text{L}$  of a dyed 100-basepair DNA ladder. The other seven depressions were loaded with 5  $\mu\text{L}$  of PCR product, each mixed with 3  $\mu\text{L}$  of blue loading dye. A current of 90-volt was delivered for 45-60 minutes. Gels were visualized in a UV camera hood (VersaDoc 5000 MP Gel Photo Documentation System). Samples with a single band were determined as males, and those with two bands as

females. Sex determinations were validated with four samples from known-sex specimens (1 male, 1 female each for Gray Catbird and Veery) collected during the breeding season in White Clay Creek State Park, Delaware.

### **3.5 Statistical Analysis**

#### **3.5.1 Triglyceride (quality control)**

Triglyceride assays were run in duplicate (and in few instances, in triplicate); therefore, the intra-assay coefficient of variation (CV) was calculated. The intra-assay CV for each sample was calculated as: the sample standard deviation divided by the mean, and multiplied by 100 to give a percentage. Samples with CV > 15% were discarded from subsequent analysis (e.g., Seewagen et al. 2011 and Seewagen 2013 used CV < 15%; Smith et al. 2007 repeated measurements until reaching a CV < 10%). In some instances, it was obvious that the sample would have to be discarded (a CV of 117% caused by a pipetting error). The average inter-assay CV in my samples is 4.13% (Guglielmo et al. 2002 averaged CV 4.1% for TRIG).

Linear regression was used to investigate the relationship between triglyceride concentration and the maximum time elapsed since capture (i.e., two net runs prior to capture and time blood sample is collected, to be as conservative as possible) to ensure triglyceride concentration was not rapidly decreasing with handling time. Numerous studies have shown that triglyceride concentrations rapidly decrease after capture, and

that samples taken after 20 minutes should not be considered. No significant relationship was found in my data for all samples (species/years) combined, confirming the careful field protocol for timing blood collection (2013:  $R^2 = 0.02$ ,  $F_{1,55} = 1.07$ ,  $p = 0.31$ ; 2014:  $R^2 = 0.002$ ,  $F_{1,67} = 0.14$ ,  $p = 0.71$ ).

### 3.5.2 Size-corrected body mass calculations

I used a simple linear regression to test the relationship of mass and wing chord to determine if body mass required adjustment to body size. A significant linear regression ( $p \leq 0.05$ ) would require calculation of adjusted body mass. I followed Peig & Green (2009) methodology to calculate a scaled-mass index:

$$\hat{M}_i = M_i \left[ \frac{L_o}{L_i} \right]^{bSMA}, \text{ where:}$$

$\hat{M}_i$ : is the predicted mass of an individual with length  $L_o$

$M_i$  = body mass (in this case, body mass in grams)

$L_i$  = linear body measurement (in this case, wing chord in mm)

$L_o$  = arbitrary value of L (in this case, I am using population mean of  $L_i$ )

$bSMA$  = the scaling exponent taken from the standardized major axis (SMA) regression of M on L

$bSMA$  and the standardized major axis (SMA) was calculated using automated online software (Bohonak and van der Linde 2004).

Size-corrected body mass was calculated for each focal species using their initial capture body mass and mean wing length of the study population. Resulting size-corrected body mass equations are: 2013 GRCA n=74: ( $M_i [(87.2027)/L_i]^{3.375}$ ); 2014 GRCA n=110: ( $M_i [(88.6455)/L_i]^{2.932}$ ); SWTH 2013, n=32: ( $M_i [(96.84)/L_i]^{2.206}$ ); GCTH 2013 n=10: ( $M_i [(98)/L_i]^{3.78}$ ); VEER 2013, n=10: ( $M_i [(96.9)/L_i]^{2.694}$ ). Simple linear regression of body mass on wing chord was non-significant for GCTH ( $F_{1,2} = 0.07$ ,  $p = 0.82$ ,  $R^2 = -0.45$ ), VEER ( $F_{1,1} = 5.56$ ,  $p = 0.26$ ,  $R^2 = 0.69$ ), and SWTH ( $F_{1,2} = 0.50$ ,  $p = 0.55$ ,  $R^2 = -0.19$ ) in 2014, which meant adjusting for size was not necessary.

### 3.5.3 Descriptive statistics

Variables were tested for normality distribution (Shapiro-Wilks test) and all data were tested for homoscedasticity (Fisher's F-test). All statistical analyses were done using R version 2.8.1 (R Development Core Team 2009). Values are presented as means  $\pm$  one standard deviation (SD), and results were interpreted as significant when  $p \leq 0.05$ . Values for all variables are from the first capture of individuals. A one-way analysis of variance (ANOVA) was calculated to determine if there were differences in the size-corrected body mass in *Catharus* thrushes prior to pooling data into a single group for continued analysis. That allowed grouping data into short-distance migrants (Gray Catbird) and long-distance migrants (*Catharus* thrushes). Recapture data consists of birds caught at least one day after first capture. Pearson's Chi-square test and contingency tables were used to determine differences in the recapture frequency among species, age groups, and study years. For the Pearson's Chi-square tests, the categorical values were

species (GRCA, SWTH, VEER, or GCTH and/or short-distance or long-distance), age (second year, SY or after-second year, ASY), and study year (2013 or 2014). To determine differences in triglyceride concentrations in each species between years, two-sample Student's t-tests were calculated. Differences in refueling performance and body condition variables were examined between short-distance and long-distance migrants using a one-way ANOVA. The non-parametric Mann-Whitney U-test was used to compare fat and muscle scores between short-distance and long-distance migrants.

Linear regression was used to determine the relationship between size-adjusted body mass and capture time, and to obtain the hourly rate of mass change from the  $\beta$  coefficient (Seewagen et al. 2013). Capture time was standardized as total hours elapsed from daily sunrise (using NOAA Solar Calculator) to time of capture. Linear regressions were used to examine the relationship between ordinal date and body condition variables to determine the influence on arrival dates.

Generalized linear models (GLM) with backward elimination ((step) command in R) procedures and Akaike's information criterion (AIC) values were used to identify variables that contributed to differences in plasma triglyceride concentrations between migratory strategy and years. The variables included in the models were muscle score, size-corrected body mass, ordinal date, fat score, age, time elapsed since sunrise, year, genera, and bleed time.

## CHAPTER 4:

### RESULTS

Sampling efforts totaled 1,395 net hours in 2013 and 1,204 net hours in 2014. In 2013, banding efforts on St. George Island resulted in 0.31 birds/net-hour; while in 2014, there were 0.53 birds/net-hour. A total of 249 focal species individuals were captured during the study period (Table 4.1). Gray Catbird was the most abundant species during the study period ( $n = 186$ ). Swainson's Thrush was the most abundant thrush species ( $n = 36$ ), followed by Gray-cheeked Thrush ( $n = 14$ ), and Veery ( $n = 13$ ). Due to the small sample sizes, and after confirming no significant differences in size-corrected body mass ( $F_{2,27} = 1.68$ ,  $p = 0.21$ ), *Catharus* thrush data were pooled. Pooling data allowed for more robust testing between short-distance (i.e., Gray Catbird) and long-distance (*Catharus* spp.) migratory songbirds during spring stopover in St. George Island.

Table 4.1 Total number of individual birds captured, recaptured, and percentages of different age classes (SY = second year, ASY = after second year) for four species of migratory songbirds captured during spring migration on St. George Island, Florida, 2013-2014.

	Gray-cheeked Thrush	Swainson's Thrush	Veery	Gray Catbird
<b>2013</b>				
Captures	10	32	10	76
Recaptures	1 (10%)	0 (0%)	2 (20%)	2 (2.6%)
Age SY	50%	65.60%	70%	60.80%
Age ASY	50%	34.40%	30%	39.20%
<b>2014</b>				
Captures	4	4	3	110
Recaptures	1 (25%)	0 (0%)	0 (0%)	4 (3.6%)
Age SY	75%	25%	33.30%	29.40%
Age ASY	25%	75%	66.70%	70.60%
<b>Both years</b>				
Captures	14	36	13	186
Recaptures	2 (14%)	0 (0%)	2 (0%)	6 (3.6%)
Age SY	57.10%	61.10%	61.50%	42.10%
Age ASY	42.90%	38.90%	38.50%	57.90%

#### 4.1 Capture dates and time

Median capture dates for long-distance migrants differed significantly between years ( $W = 541.5$ ,  $n_1 = 52$ ,  $n_2 = 11$ ,  $p < 0.001$ ), from day  $126.5 \pm 2.8$  in 2013 to day  $113 \pm 6.5$  in 2014. In 2013, Veery was the earliest to occur (day 111), followed by Swainson's Thrush (day 122). The earliest long-distance migrant in 2014 was Swainson's Thrush (day 109), followed by Gray-cheeked Thrush (day 110).

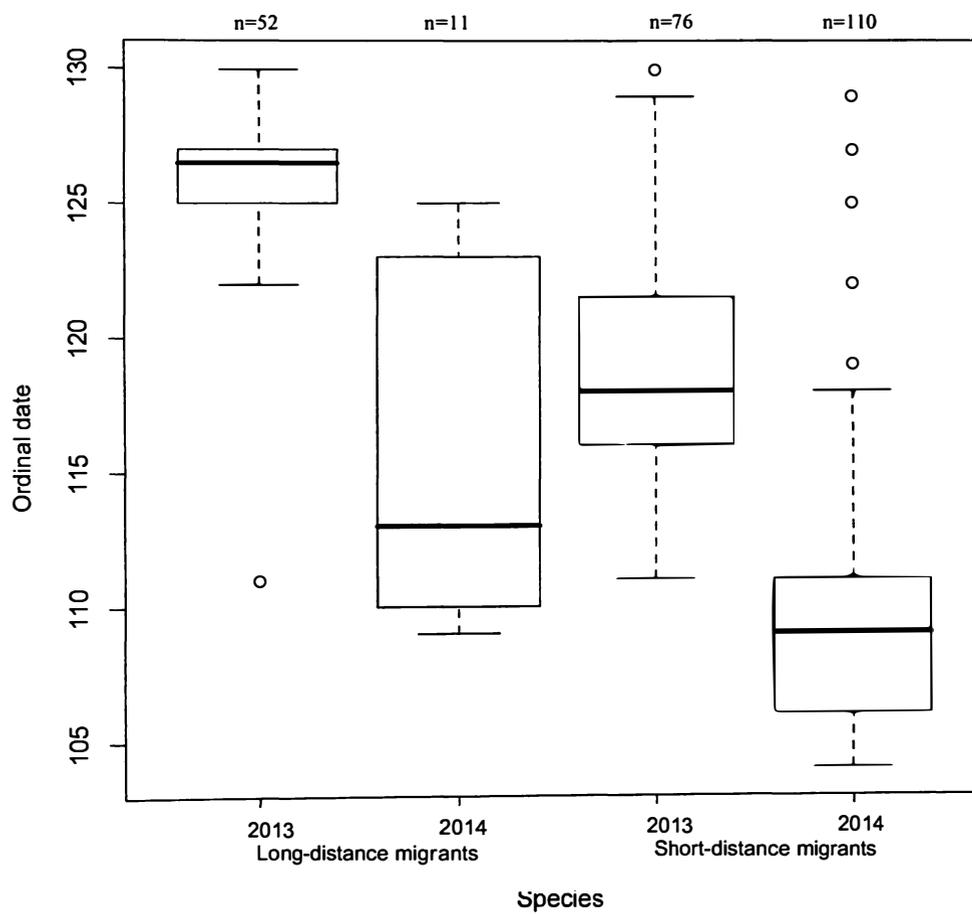
Median capture dates of short-distance migrants differed significantly between years ( $W = 7423.5$ ,  $n_1 = 76$ ,  $n_2 = 110$ ,  $p < 0.001$ ), from day  $118 \pm 4.5$  in 2013 to day  $109 \pm 5.2$  in 2014. The earliest capture dates for short-distance migrants corresponded with the first day of the sampling period, day 111 in 2013 and day 104 in 2014.

Median capture dates for short-distance (day  $114 \pm 6.4$ ) migrants was significantly earlier than for long-distance (day  $126 \pm 5.4$ ) migrants ( $W = 10360$ ,  $n_1 = 186$ ,  $n_2 = 63$ ,  $p < 0.001$ ) (Figure 4.1). Age effects in arrival date were not detected for short-distance (2013:  $W = 710$ ,  $n_1 = 45$ ,  $n_2 = 29$ ,  $p = 0.53$ ; 2014:  $W = 1498.5$ ,  $n_1 = 32$ ,  $n_2 = 77$ ,  $p = 0.07$ ) and long-distance migrants (2013:  $W = 366$ ,  $n_1 = 33$ ,  $n_2 = 19$ ,  $p = 0.31$ ; 2014:  $W = 12$ ,  $n_1 = 5$ ,  $n_2 = 6$ ,  $p = 0.64$ ) in either year.

Time of capture between short-distance (3.3 hours from sunrise) and long-distance (3.7 hours from sunrise) migrants did not differ significantly ( $W = 6374.5$ ,  $n_1 =$

186,  $n_2 = 63$ ,  $p = 0.3$ ). Capture time of short-distance migrants did not differ between years ( $W = 4866$ ,  $n_1 = 76$ ,  $n_2 = 110$ ,  $p = 0.06$ ). Capture time of long-distance migrants did not differ significantly between 2013 (3.1 hours from sunrise) and 2014 (5.0 hours from sunrise) ( $W = 209$ ,  $n_1 = 52$ ,  $n_2 = 11$ ,  $p = 0.17$ ).

Figure 4.1 Capture dates for short-distance and long-distance migrants on St. George Island, Florida, during spring migration 2013-2014. The median is represented by the bold line, the box represents the interquartile range, and whiskers stand for minimum and maximum observations. Outliers are represented by open circle.



## 4.2 Recapture rate and stopover duration

The proportion of individuals recaptured after >1 day of original capture was higher in long distance (6.3%) than in short distance (3.2%) migrants. However, there was no statistical significance in recapture frequency ( $\chi^2 = 0.45$ ,  $df = 1$ ,  $p = 0.5$ ).

Detailed analysis of stopover duration and mass change was hindered by small sample size. There was no significant relationship between stopover duration and mass change in either year (2013:  $R^2 = 0.19$ ,  $F_{1,3} = 0.7$ ,  $p = 0.46$ ; 2014:  $R^2 = 0.013$ ,  $F_{1,3} = 0.04$ ,  $p = 0.86$ ). Overall, the mean stopover duration of recaptured birds in 2013 was  $3 \pm 2.12$  days with a mean daily mass change of  $0.14 \pm 0.97$  g day<sup>-1</sup>. In 2014, mean stopover duration was  $4.2 \pm 3.15$  days, with mean daily body mass change of  $-0.28 \pm 0.82$  g day<sup>-1</sup>. There was no significant difference in expected daily mass change between years ( $t = 0.74$ ,  $df = 7.79$ ,  $p = 0.484$ ). No significant difference was found in stopover duration of recaptured individuals between years ( $t = 0.71$ ,  $df = 8$ ,  $p = 5$ ).

While there were no significant differences between years for daily mass change, it appears that recaptured birds lost mass at a greater daily rate in 2014 ( $-0.28$  g day<sup>-1</sup>) than in 2013 ( $0.14$  g day<sup>-1</sup>). In 2013, four of five individuals gained mass during stopover, while only two of five individuals gained mass during stopover in 2014.

Table 4.2 Estimated minimum stopover duration and change in body mass for individual birds recaptured >1 day after first capture on St. George Island, Florida, in spring 2013 and 2014.

Species	Date first capture	Date last capture	$\Delta$ day	Mass first capture (g)	Mass last capture (g)	$\Delta$ mass (g)	$\text{g day}^{-1}$
<b>2013</b>							
Gray Catbird	111	117	6	33	34	1	0.17
Gray Catbird	113	117	4	33	34	1	0.25
Veery	123	126	3	24.5	25.7	1.2	0.4
Veery	125	126	1	24.1	25.4	1.3	1.3
Gray-cheeked Thrush	128	129	1	20.7	19.3	-1.4	-1.4
<b>2014</b>							
Gray Catbird	106	115	9	36.8	36.2	-0.6	-0.07
Gray Catbird	106	110	4	29.4	28.8	-0.6	-0.15
Gray Catbird	107	109	2	39.9	40.7	0.8	0.4
Gray Catbird	109	110	1	33.9	32.2	-1.7	-1.7
Gray-cheeked Thrush	110	115	5	25.7	26.4	0.7	0.14

### 4.3 Age structure

There were no significant differences in age structure between short-distance and long-distance migrants in 2013 ( $\chi^2 = 0.01$ ,  $df = 1$ ,  $p = 0.9$ ) or 2014 ( $\chi^2 = 0.58$ ,  $df = 1$ ,  $p = 0.5$ ). However, age structure varied significantly by year for short-distance migrants ( $\chi^2 = 16.6$ ,  $df = 1$ ,  $p < 0.001$ ). A higher proportion (60.8%) of catbirds were second-year (SY) in 2013, while a higher proportion (70.6%) of catbirds were after second-year (ASY) in 2014 (Table 4.3). There were no differences in age structure proportion between years for long-distance migrants ( $\chi^2 = 0.6$ ,  $df = 1$ ,  $p = 0.44$ ) (Table 4.3).

Table 4.3 Percentages of age classes (SY = second year, ASY = after second year) for short- and long-distance migratory songbirds captured during spring migration on St. George Island, Florida, 2013-2014.

Year	Age	Migratory strategy		All
		Short-distance	Long-distance	
2013	SY	60.8%	63.5%	62%
	ASY	39.2%	36.5%	38%
2014	SY	29.4%	45.5%	31%
	ASY	70.6%	54.5%	69%
Both years	SY	42%	60.3%	47%
	ASY	58%	39.7%	53%

#### 4.4 Sex structure

Eighteen percent of the 118 samples attempted for molecular sexing were successful. Fifteen individuals were successfully sexed: Gray Catbird (8 female, 4 male), Swainson's Thrush (2 male), and Gray-cheeked Thrush (1 male).

#### 4.5 Body condition

Fat score ( $W = 796$ ,  $n_1 = 186$ ,  $n_2 = 63$ ,  $p < 0.001$ ; Figure 4.2) and pectoral muscle score ( $W = 576$ ,  $n_1 = 186$ ,  $n_2 = 63$ ,  $p < 0.001$ ; Figure 4.3) differed dependent on migratory strategy. Fat score did not differ significantly between years for either migratory group (short-distance:  $W = 4548.5$ ,  $n_1 = 76$ ,  $n_2 = 110$ ,  $p = 0.3$ ; long-distance:  $W = 272.5$ ,  $n_1 = 52$ ,  $n_2 = 11$ ,  $p = 0.8$ ).

Short-distance migratory birds presented significantly lower pectoral muscle scores in 2013, and higher pectoral muscle scores in 2014 ( $W = 2193.5$ ,  $n_1 = 76$ ,  $n_2 = 110$ ,  $p < 0.001$ ). Pectoral muscle scores were not significantly different between years in long-distance migratory birds ( $W = 252.5$ ,  $n_1 = 52$ ,  $n_2 = 11$ ,  $p = 0.5$ ). Median fat and pectoral muscle scores are presented in Table 4.4.

There were no significant age effects in pectoral muscle score (2013:  $\chi^2 = 2.7$ ,  $df = 2$ ,  $p = 0.26$ ; 2014:  $\chi^2 = 4.9$ ,  $df = 2$ ,  $p = 0.08$ ) or fat score (2013  $\chi^2 = 3.6$ ,  $df = 3$ ,  $p = 0.3$ ; 2014:  $\chi^2 = 3.9$ ,  $df = 3$ ,  $p = 0.27$ ) for long-distance migrants. Likewise, no significant age

effects were detected in short-distance migrants for pectoral muscle score (2013  $\chi^2 = 1.76$ ,  $df = 2$ ,  $p = 0.4$ ; 2014:  $\chi^2 = 5.1$ ,  $df = 3$ ,  $p = 0.7$ ) and fat score (2013  $\chi^2 = 1.9$ ,  $df = 5$ ,  $p = 0.9$ ; 2014:  $\chi^2 = 2.8$ ,  $df = 6$ ,  $p = 0.8$ ). Fat and pectoral muscle scores for different age classes of spring migrants are presented in Table 4.4.

The size-corrected body mass for short-distance migrants was  $34.92 \pm 3.86$  g in 2013 and  $35.66 \pm 4.61$  g in 2014. These differences in size-corrected body mass between years were not significant ( $t_{173,5} = -1.17$ ,  $p = 0.2$ ) (Figure 4.4).

Figure 4.2 Fat scores of short-distance and long-distance migratory birds captured on St. George Island, Florida, during spring migration 2013-2014

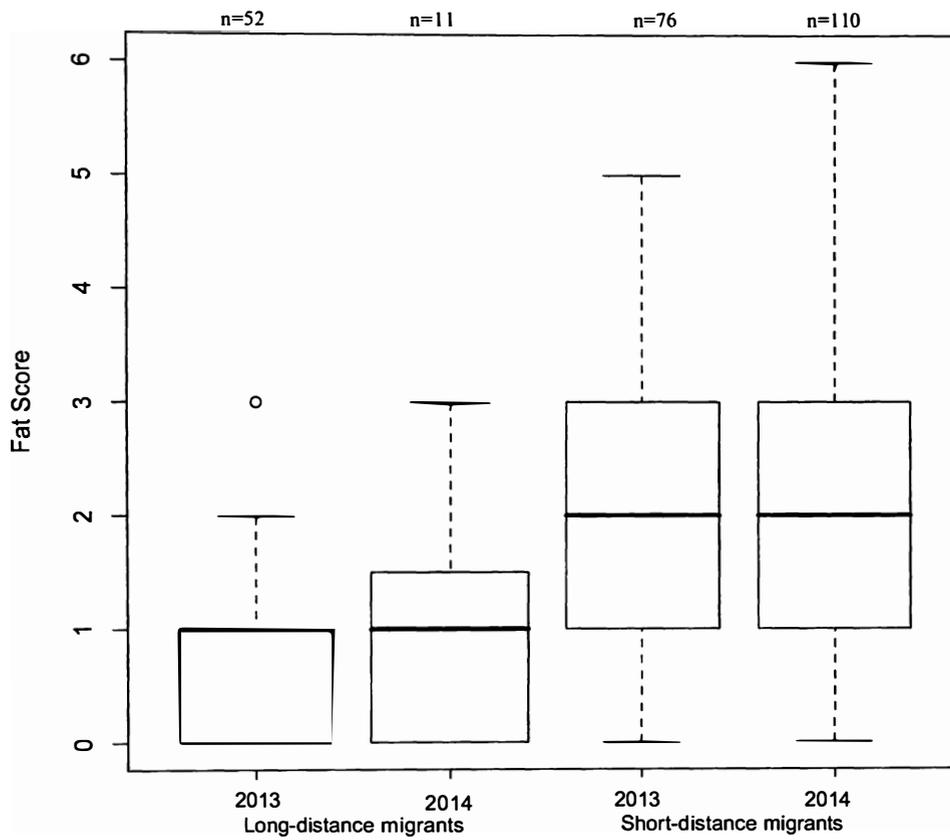


Figure 4.3 Pectoral muscle scores of short-distance and long-distance migratory birds captured on St. George Island, Florida, during spring migration 2013-2014

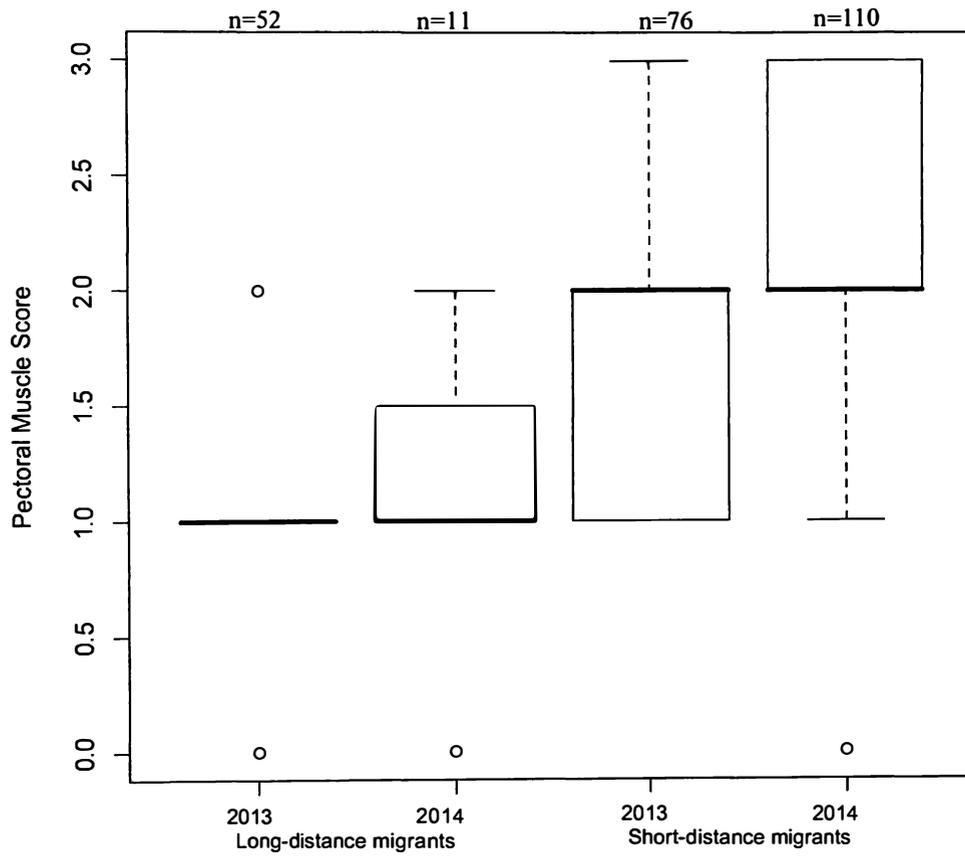
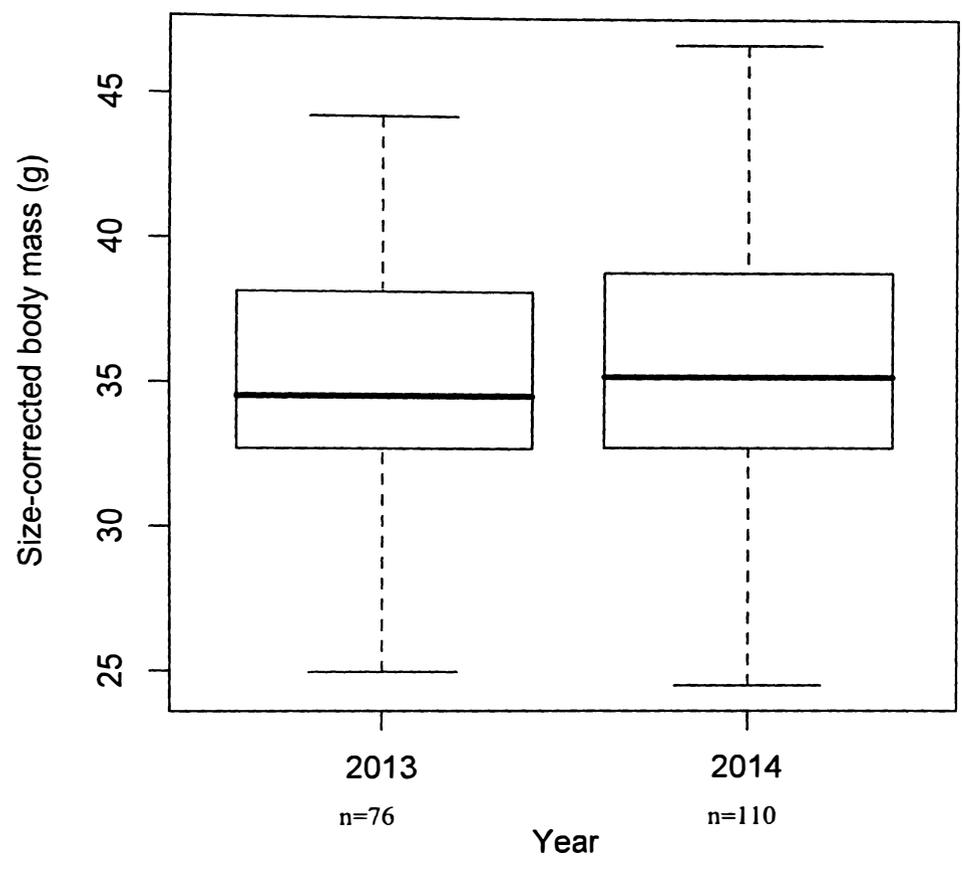


Table 4.4 Mean  $\pm$  one standard deviation (SD) fat and pectoral muscle scores for short- and long-distance migrants age classes captured during spring migration on St. George Island, Florida, 2013-2014. Medians presented in parenthesis.

	Age Class	Muscle Score	Fat Score	Size-corrected mass
<b>2013</b>				
Short distance	SY	1.56 $\pm$ 0.5 (2)	2.2 $\pm$ 1.5 (2)	35.7 $\pm$ 3.9 (35.3)
	ASY	1.7 $\pm$ 0.6 (2)	2.3 $\pm$ 1.4 (2)	33.5 $\pm$ 3.4 (34.1)
	All	1.6 $\pm$ 0.5 (2)	2.23 $\pm$ 1.5 (2)	34.9 $\pm$ 3.9 (34.5)
Long distance	SY	0.85 $\pm$ 0.7 (1)	0.73 $\pm$ 0.7 (1)	25.0 $\pm$ 2 (25.2)
	ASY	1.1 $\pm$ 0.6 (1)	1.1 $\pm$ 0.8 (1)	25.2 $\pm$ 2.4 (24.4)
	All	0.9 $\pm$ 0.64 (1)	0.87 $\pm$ 0.7 (1)	25.0 $\pm$ 2.1 (25.1)
<b>2014</b>				
Short distance	SY	2.41 $\pm$ 0.56 (2)	1.8 $\pm$ 1.23 (2)	37.7 $\pm$ 4.6 (37.9)
	ASY	2.1 $\pm$ 0.67 (2)	2.1 $\pm$ 1.3 (2)	34.9 $\pm$ 4.4 (34.5)
	All	2.2 $\pm$ 0.65 (2)	2 $\pm$ 1.3 (2)	35.7 $\pm$ 4.6 (35.2)
Long distance	SY	0.6 $\pm$ 0.55 (1)	0.6 $\pm$ 0.55 (1)	25.8 $\pm$ 1.7 (25.4)
	ASY	1.5 $\pm$ 0.55 (1.5)	1.3 $\pm$ 1.2 (1.5)	27.2 $\pm$ 1.8 (27.5)
	All	1.1 $\pm$ 0.7 (1)	1 $\pm$ 1 (1)	26.1 $\pm$ 1.8 (26.1)

Figure 4.4 Size-corrected body mass (g) in Gray Catbirds captured on St. George Island, Florida, during spring migration 2013-2014.



There was a significant relationship between ordinal date and size-corrected body mass in short-distance migrants in 2014 ( $R^2 = 0.05$ ,  $F_{1,108} = 5.61$ ,  $p = 0.02$ ; Fig. 4.6). Heavier birds arrived later in the season in 2014. No such relationship was determined for short-distance migrants in 2013 ( $R^2 = 0.03$ ,  $F_{1,72} = 1.84$ ,  $p = 0.18$ ; Fig. 4.5). There was no relationship in time of day and size-corrected body mass for short-distance migrants in 2013 ( $R^2 = 0.04$ ,  $F_{1,72} = 3.02$ ,  $p = 0.09$ ) or 2014 ( $R^2 = 0.006$ ,  $F_{1,108} = 0.5$ ,  $p = 0.5$ ).

Figure 4.5 Daily change in size-corrected body mass of Gray Catbird (n = 76) captured during spring migration on St. George Island, Florida, in April-May 2013.

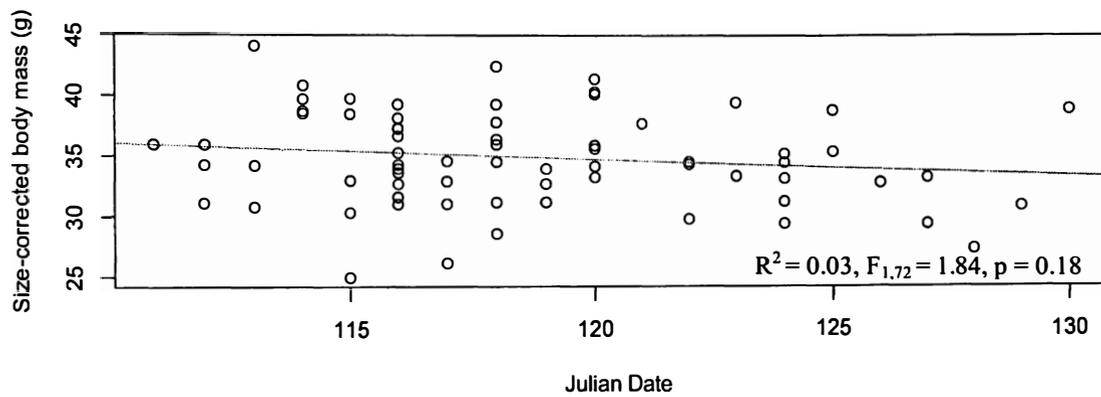


Figure 4.6 Daily change in size-corrected body mass of Gray Catbird (n = 110) captured during spring migration on St. George Island, Florida, in April-May 2014.

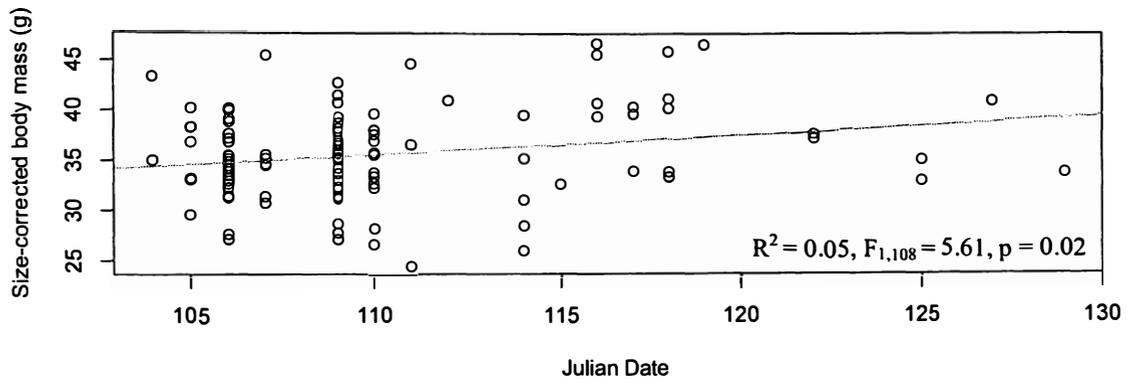


Figure 4.7 Hourly change in size-corrected body mass (g) in Gray Catbird (n = 76) captured on St. George Island, Florida, during spring migration April-May 2013.

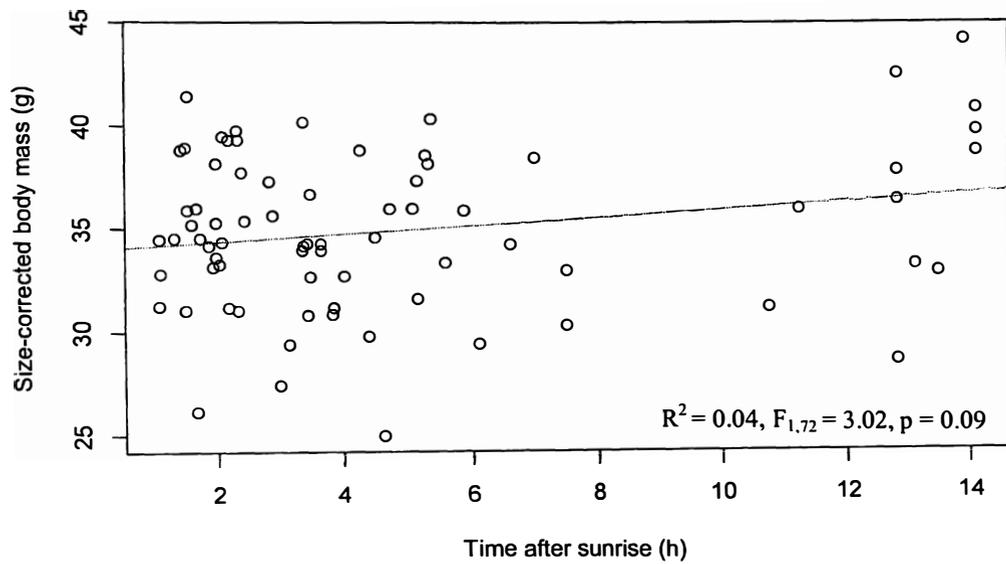


Figure 4.8 Hourly change in size-corrected body mass (g) in Gray Catbird (n = 110) captured on St. George Island, Florida, during spring migration April-May 2014.

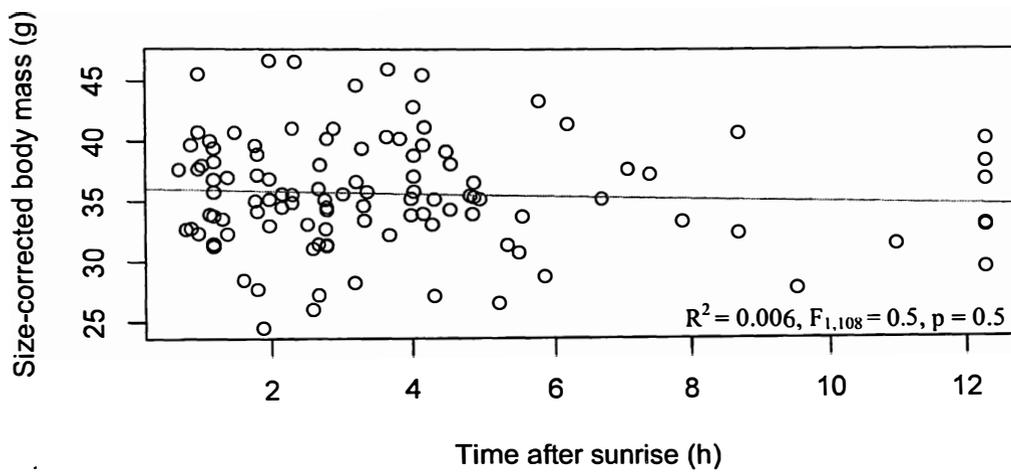
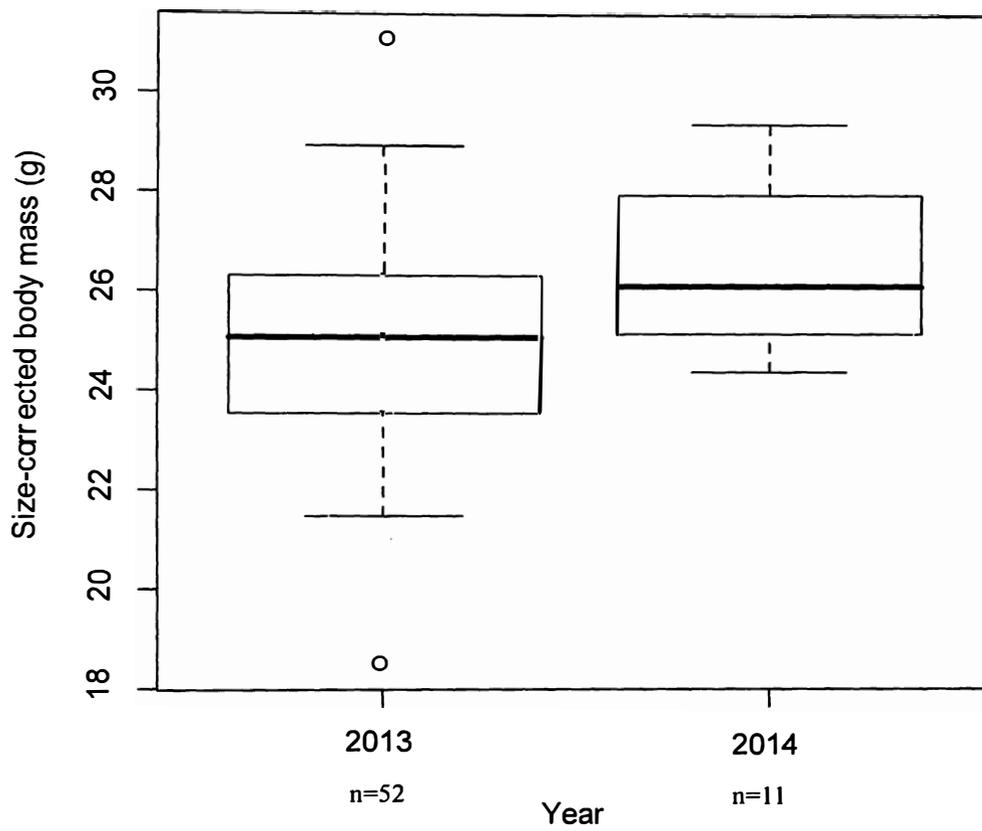


Figure 4.9 Size-corrected body mass (g) of long-distance migrants captured on St. George Island, Florida, during spring migration 2013-2014.



For long distance migrants, mean size-corrected body mass differed between years ( $t_{16.4} = -2.5$ ,  $p = 0.02$ ) from  $25 \pm 2.1$  g in 2013, to  $26.6 \pm 1.8$  g in 2014. There was no relationship between size-corrected body mass and ordinal date (2013:  $R^2 = 0.001$ ,  $F_{1,50} = 0.05$ ,  $p = 0.83$ ; 2014:  $R^2 = 0.03$ ,  $F_{1,9} = 0.31$ ,  $p = 0.6$ ) or capture time (2013:  $R^2 = 0.01$ ,  $F_{1,50} = 0.55$ ,  $p = 0.46$ ; 2014:  $R^2 = 0.13$ ,  $F_{1,9} = 1.34$ ,  $p = 0.28$ ) in long-distance migrants.

#### 4.6 Refueling rates

Plasma triglyceride samples were successfully obtained for Gray Catbird (2013,  $n = 27$ ; 2014,  $n = 60$ ), Gray-cheeked Thrush (2013,  $n = 5$ ; 2014 = 4), Swainson's Thrush (2013,  $n = 19$ ; 2014,  $n = 4$ ), and Veery (2013,  $n = 6$ ; 2014,  $n = 2$ ). Handling time was efficient, as there was no relationship between bleed time and triglyceride concentration ( $R^2 = 0.006$ ,  $F_{1,124} = 0.74$ ,  $p = 0.39$ ).

Plasma triglyceride concentrations were significantly different between years for Gray Catbird, with higher concentrations in 2013 ( $t = 2.36$ ,  $df = 85$ ,  $p = 0.02$ ). Triglyceride concentrations in long-distance migrants also differed between years. In Gray-cheeked Thrush, triglyceride concentrations were significantly higher in 2013 ( $t = 4.08$ ,  $df = 7$ ,  $p = 0.01$ ), while Swainson's Thrush ( $t = 1.49$ ,  $df = 21$ ,  $p = 0.17$ ) and Veery ( $t = 1.25$ ,  $df = 6$ ,  $p = 0.26$ ) did not present significant differences between years. Plasma triglyceride concentrations of all migratory birds combined were significantly different between years ( $t = 3.75$ ,  $df = 122.5$ ,  $p < 0.001$ ), with lower concentrations in 2014. Mean metabolite values and sample sizes for all species sampled are shown in Table 4.5.

Figure 4.10. Plasma triglyceride concentration (mmol/L) of short- and long-distance migratory birds captured on St. George Island, Florida, during spring migration 2013 and 2014.

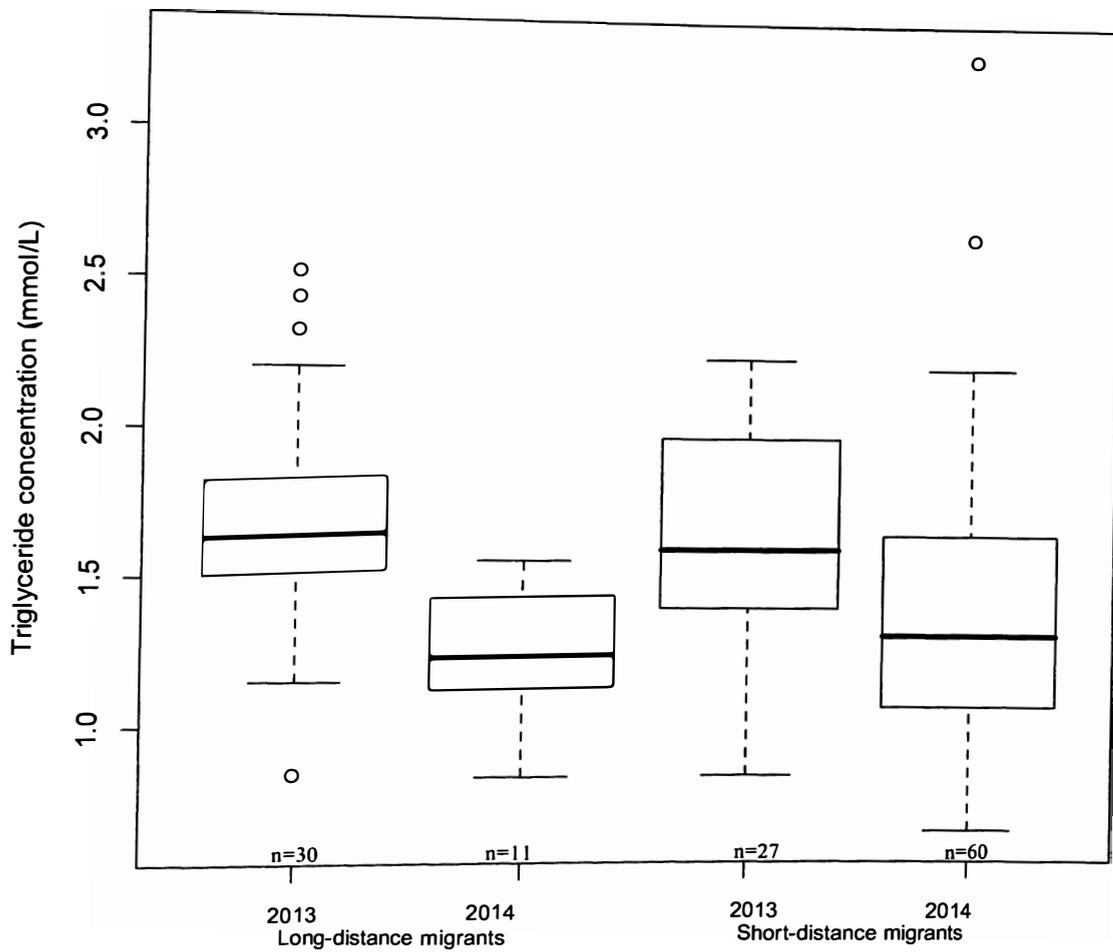


TABLE 4.5 Mean  $\pm$  one standard deviation (SD) plasma triglyceride (TRIG) concentrations (mmol/L) by species during spring stopover on St. George Island, Florida, 2013-2014.

Species	2013 (n)	2014 (n)
Gray-cheeked Thrush <sup>1</sup>	1.67 $\pm$ 0.21 (5)	1.11 $\pm$ 0.2 (4)
Swainson's Thrush <sup>1</sup>	1.66 $\pm$ 0.43 (19)	1.35 $\pm$ 0.22 (4)
Veery <sup>1</sup>	1.78 $\pm$ 0.38 (6)	1.43 $\pm$ 0.02 (2)
Gray Catbird <sup>2</sup>	1.65 $\pm$ 0.38 (27)	1.40 $\pm$ 0.49 (60)

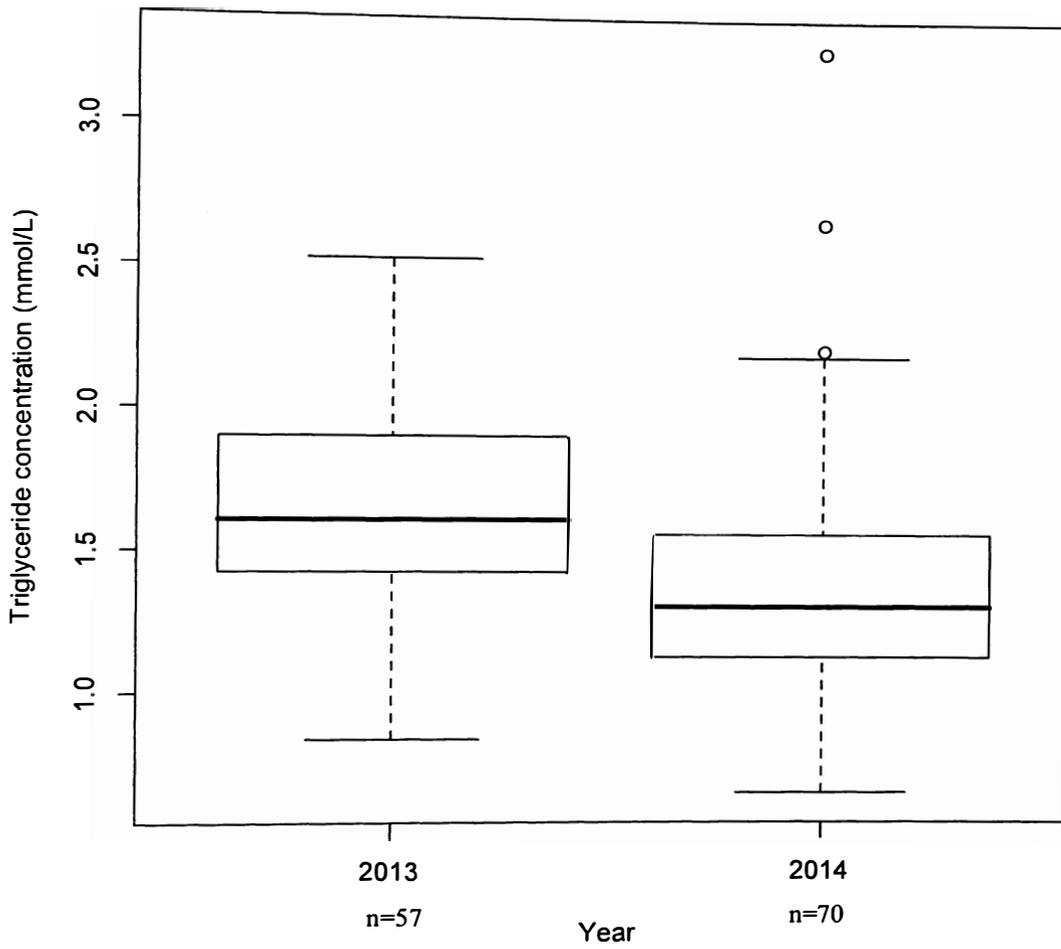
<sup>1</sup>Long-distance migrant  
<sup>2</sup>Short-distance migrant

There were no significant differences in plasma triglyceride concentrations between short-distance and long-distance migrants in 2013 ( $F_{1,55} = 0.096$ ,  $p = 0.76$ ), in 2014 ( $F_{1,68} = 0.75$ ,  $p = 0.39$ ), or in both years combined ( $F_{1,125} = 1.36$ ,  $p = 0.25$ ).

Plasma triglyceride concentrations in long-distance migrants were significantly higher in 2013 ( $1.69 \pm 0.38$  mmol/L) than in 2014 ( $1.27 \pm 0.22$  mmol/L) ( $F_{1,38} = 10.56$ ,  $p = 0.002$ ). This pattern was also found in short-distance migrants ( $t = 2.36$ ,  $df = 85$ ,  $p = 0.02$ ).

Plasma triglyceride concentrations were not affected by age in either migratory group (short-distance 2013:  $t = -1.0443$ ,  $df = 23.13$ ,  $p\text{-value} = 0.31$ ; short-distance 2014:  $t = -1.05$ ,  $df = 49.1$ ,  $p = 0.3$ ; long-distance 2013:  $t = -0.14$ ,  $df = 22.13$ ,  $p = 0.89$ ; long-distance 2014:  $t = 0.65$ ,  $df = 6.78$ ,  $p = 0.537$ ).

Figure 4.11 Plasma triglyceride concentration (mmol/L) of all migratory birds combined captured on St. George Island, Florida, during spring migration 2013 and 2014.



There was no significant relationship found between triglyceride concentration and time of day (hours since sunrise) in either year (2013:  $R^2 = 0.002$ ,  $F_{1,53} = 0.09$ ,  $p = 0.77$ ; 2014:  $R^2 = 0.027$ ,  $F_{1,67} = 1.84$ ,  $p = 0.18$ ).

#### **4.7 Models for plasma triglyceride concentration**

For migratory birds using St. George Island as a spring stopover site, plasma triglyceride concentration is best explained primarily by the year and the birds' fat content (Table 4.6). In the first year of this study (2013), triglyceride concentration in migratory birds is best explained by the birds' fat content, bleed time, and genus (Table 4.7). In the second year of this study (2014), triglyceride concentration was best explained by a single explanatory variable: the birds' pectoral muscle score (Table 4.8).

In short-distance migratory birds on St. George Island, triglyceride concentration was best explained by the birds' pectoral muscle score, size-corrected body mass, birds' age, and the year of study (Table 4.9).

For long-distance migratory birds using St. George Island as spring stopover habitat, triglyceride concentration was best explained by the birds' fat score and the year (Table 4.10).

Fat score and pectoral muscle scores are important predictors of triglyceride concentration in migratory birds utilizing St. George Island as spring stopover habitat (Fig 4.12 and Fig 4.13).

Table 4.6 Generalized linear model (GLM) using plasma triglyceride (TRIG) of all migratory birds captured on St. George Island, Florida, as the predictor variable.

Variables included in the GLM model are: **P** = pectoral muscle, **M** = size-corrected mass, **S** = hours since sunrise, **D** = ordinal date, **F** = fat, **A** = age, **G** = genus, **Y** = year, **B** = bleed time.

<b>MODEL</b>	<b>AIC</b>
P+M+S+D+F+A+G+Y+B	149.57
P+M+D+F+A+G+Y+B	147.78
P+M+D+F+A+G+Y	146.1
P+M+F+A+G+Y	144.62
P+M+F+G+Y	143.58
P+F+G+Y	142.64
F+G+Y	142.08
F+Y	141.08

Table 4.7 Generalized linear model (GLM) using plasma triglyceride (TRIG) of all migratory birds captured on St. George Island, Florida, in 2013 (Year 1 of the study) as the predictor variable. Variables included in the GLM model are: **P** = pectoral muscle, **S** = hours since sunrise, **D** = ordinal date, **F** = fat, **A** = age, **G** = genus, **B** = bleed time.

<b>MODEL</b>	<b>AIC</b>
P+D+S+B+F+A+G	47.32
P+D+S+B+F+G	45.37
P+S+B+F+G	43.46
P+B+F+G	41.71
B+F+G	41.26

Table 4.8 Generalized linear model (GLM) using plasma triglyceride (TRIG) of all migratory birds captured on St. George Island, Florida, in 2014 (Year 2 of the study) as the predictor variable. Variables included in the GLM model are: **P** = pectoral muscle, **S** = hours since sunrise, **D** = ordinal date, **F** = fat, **A** = age, **G** = genus, **B** = bleed time.

<b>MODEL</b>	<b>AIC</b>
P+D+S+B+F+A+G	98.85
P+D+S+B+F+A	97.01
P+D+S+B+A	95.18
P+S+B+A	93.43
P+S+B	92.31
P+S	91.22
P	90.27

Table 4.9 Generalized linear model (GLM) using plasma triglyceride (TRIG) of Gray Catbird captured on St. George Island, Florida, as the predictor variable. Variables included in the GLM model are: **P** = pectoral muscle, **M** = size-corrected mass, **S** = hours since sunrise, **D** = ordinal date, **F** = fat, **A** = age, **Y** = year, **B** = bleed time.

<b>MODEL</b>	<b>AIC</b>
P+M+S+D+F+A+Y+B	115.37
P+M+D+F+A+Y+B	113.45
P+M+F+A+Y+B	111.64
P+M+A+Y+B	109.97
P+M+A+Y	108.35

Table 4.10 Generalized linear model (GLM) using plasma triglyceride (TRIG) of *Catharus* thrushes captured on St. George Island, Florida, as the predictor variable.

Variables included in the GLM model are: **P** = pectoral muscle, **M** = size-corrected mass, **S** = hours since sunrise, **D** = ordinal date, **F** = fat, **A** = age, **Y** = year, **B** = bleed time.

<b>MODEL</b>	<b>AIC</b>
P+M+S+D+F+A+Y+B	41.71
P+M+S+D+F+A+Y	39.71
M+S+D+F+A+Y	37.79
M+S+F+A+Y	36.0
M+S+F+Y	34.24
M+F+Y	33.71
F+Y	32.78

Figure 4.12 Triglyceride concentration as a function of fat content in migratory birds captured on St. George Island, Florida, during spring migration in 2013 and 2014.

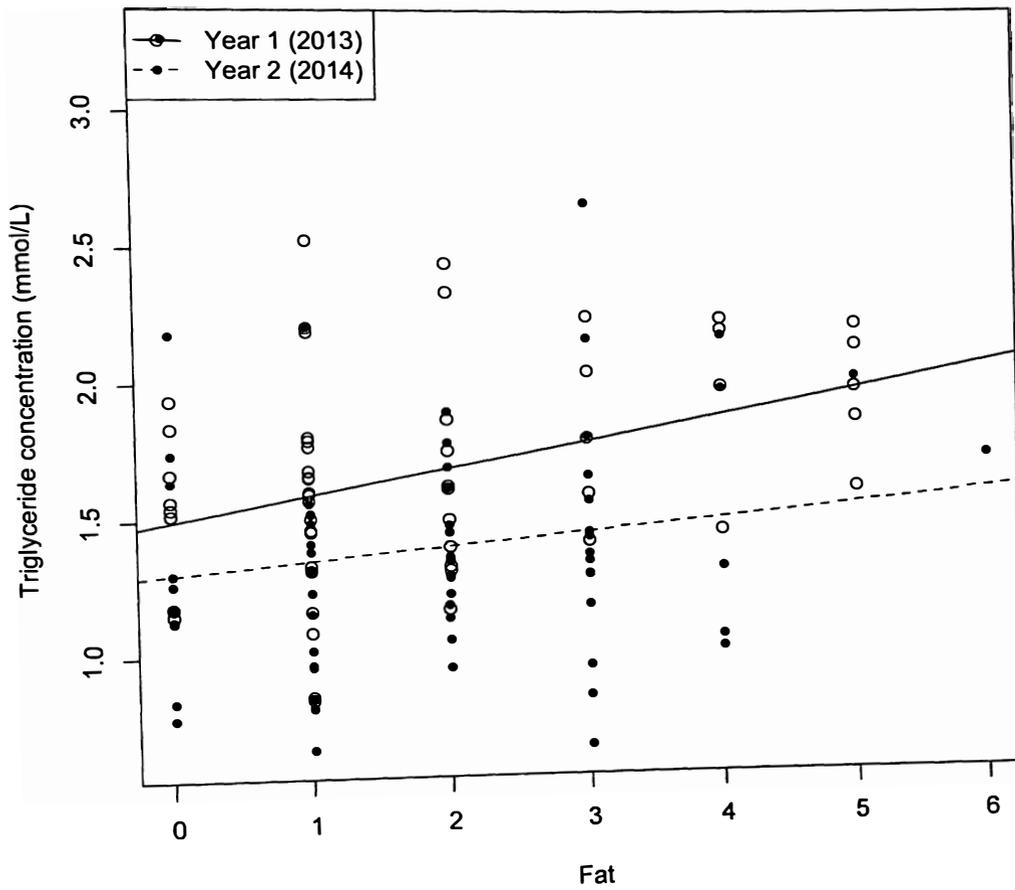
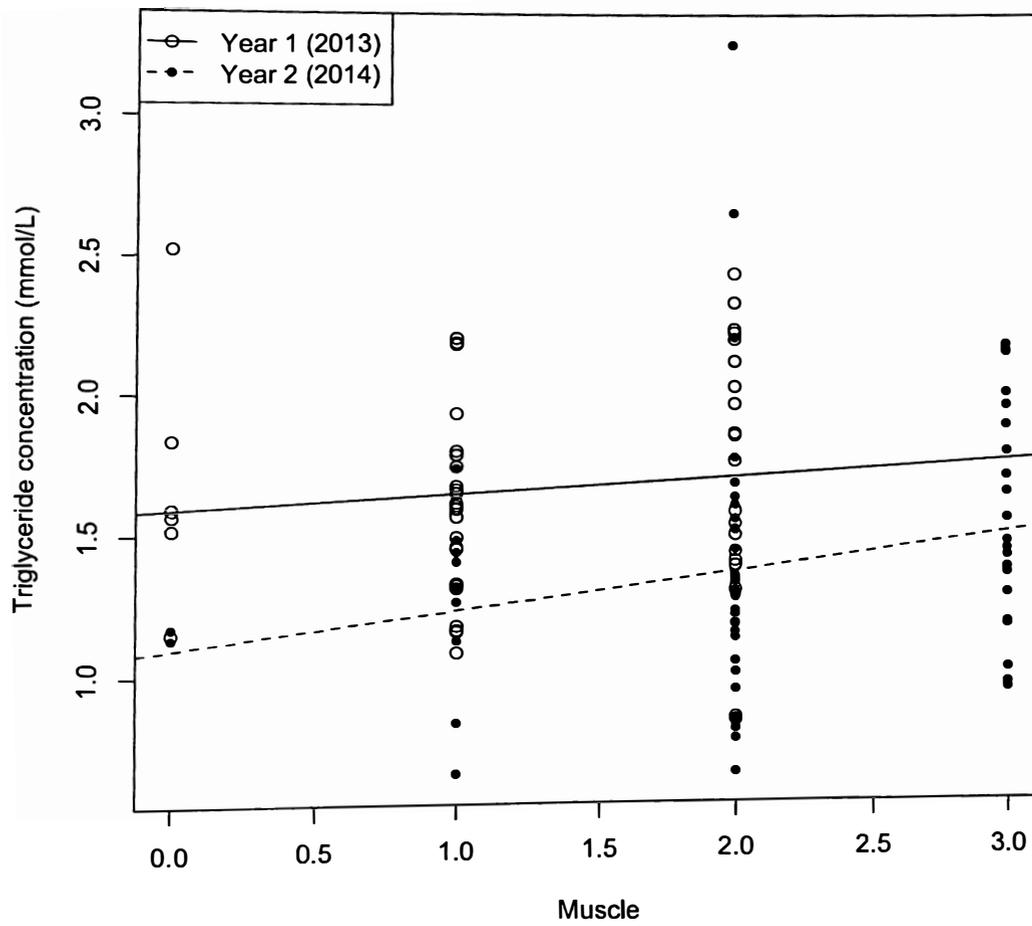


Figure 4.13 Triglyceride concentration as a function of muscle score in migratory birds captured on St. George Island, Florida, during spring migration in 2013 and 2014.



## CHAPTER 5:

### DISCUSSION

#### 5.1 Refueling rates

My results consistently indicated no differences in refueling rates between Gray Catbirds and *Catharus* thrushes. These data do not lend support to my hypothesis of differing refueling rates between short-distance and long-distance migratory birds using St. George Island during spring stopover. This is an interesting finding considering that *Catharus* thrushes arrive in consistently poorer body condition than Gray Catbirds; apparently, both are refueling at the same rate.

I did not find age-related differences in plasma triglyceride concentration in either short-distance or long-distance migrants. Therefore, I reject my hypothesis of age-related differences in refueling rates for migratory birds using St. George Island during spring stopover. During autumn migration, Seewagen et al. (2013) found no differences in refueling rates between juvenile and adults in New York. Age-dependent difference may be more apparent prior to crossing an ecological barrier. There are age-dependent differences documented for Gray Catbird refueling during autumn

migration along the Gulf coast. Juvenile (hatch year, HY) birds were found to be less efficient foragers than adult (after hatch year, AHY) birds (Heise and Moore 2003). However, these differences in refueling rates and foraging efficiency have been documented during the autumn migration, not spring, when age differences are expected to be most evident (Seewagen et al. 2013). Birds embarking on their first spring migration back to the breeding grounds have already acquired experience foraging and navigating unfamiliar environments, since they have survived autumn migration and the overwintering period.

Plasma triglyceride concentrations differed significantly between years. In both short-distance and long-distance migrants, triglyceride concentrations were higher in 2013 than in 2014. This difference in refueling rates between years highlights the variability of migration along the Gulf coast and the importance of a network of coastal stopover sites that are able to meet migrants' needs at a given point in time and space (Mehlman et al. 2005, Paxton et al. 2014).

A bird's ability to refuel is dependent on many factors, including its own body condition. In 2013, when refueling rates were higher, I also documented significantly lower pectoral muscle scores for short distance migrants. While not statistically significant, fat scores for all migrants and muscle scores for long-distance migrants, were lower in 2013. Higher fat and muscle scores and lower refueling rates in 2014 may indicate birds used St. George Island as a refuge from weather or to rest, but not to

replenish reserves. Alternatively, because birds were in poorer condition in 2013 (lower fat and muscle scores), they used their time on St. George Island to forage more and replenish reserves, reflecting the relatively higher plasma triglyceride concentrations.

The higher capture rates in 2014 are reflective of a difference in species abundance, which may have resulted in higher intra-specific competition and, thus, lower refueling rates (Moore and Yong 1991). However, while there was higher overall species abundance in 2014, there were higher numbers of *Catharus* thrushes the first year which correspond with the higher refueling rates. In 2013, *Catharus* thrushes were frequently observed actively foraging throughout the island, including residential lawns and along trails (A. Kneidel and M. Gutierrez Ramirez, unpublished data). My study did not consider variation in resource availability or weather conditions, factors that would directly influence refueling rate and subsequently stopover duration (Smith and McWilliams 2010, Seewagen et al. 2011, Smith and McWilliams 2014). We should expect spring migrants in poor body condition would feed at higher trophic levels to rebuild protein or become hyperphagic (McWilliams et al. 2004); abundance and diversity of potential food resources on St. George Island are unknown, and represent an interesting research question.

Previous studies have found a consistent variation between triglyceride concentration and time of day, with the lowest triglyceride levels found during the first hours since sunrise (Acevedo Seaman et al. 2006, Jenni and Jenni-Eiermann 1996, Evans

Ogden et al. 2013). I found no such relationship in either study year for either migratory group. This finding, in conjunction with the low recapture rate, may be indicative of a generally short stopover length on St. George Island. Plasma triglyceride concentrations of Swainson's Thrush in this study are lower than those found in various urban stopover sites in New York, which ranged between  $2.07 \pm 0.14 \text{ mmol}^{-1}$  and  $3.01 \pm 0.52 \text{ mmol}^{-1}$  (Seewagen et al. 2011). It is worth reiterating, however, that plasma triglyceride concentrations are a qualitative measure of refueling rate (Guglielmo et al. 2005), and therefore, are useful only in comparing groups at a given site or timeframe.

## 5.2 Body condition

My results indicate that long-distance migrants consistently arrive on St. George Island in poorer condition than short-distance migrants. *Catharus* thrushes had significantly lower fat, muscle, and percent lean body mass than Gray Catbirds. These data support my first hypothesis that body condition upon arrival will be different between long- and short-distance migrants. *Catharus* thrushes migrate from Central and South America, having longer migrations than Gray Catbirds. *Catharus* thrushes with protruding keels have been documented on East Ship Island, a barrier island farther west of St. George (Kuenzi et al. 1991). Moreover, *Catharus* thrushes were among the most frequently encountered species found dead during beach surveys on Horn Island (Moore et al. 1990). However, 62% of *Catharus* thrushes captured on Johnson's Bayou, Louisiana immediately after trans-Gulf migration were found to have sufficient fat scores

( $\geq 2$ ) to continue migration (Owen and Moore 2008). In addition, pectoralis muscle scores in these *Catharus* thrushes were higher than on St. George Island, ranging from a mean of  $2.02 \pm 0.39$  for Swainson's Thrush to  $1.48 \pm 0.6$  for Veery (Owen and Moore 2008).

I did not find support for my hypothesis of age-related differences in body condition upon arrival for either *Catharus* thrushes or Gray Catbird. In line with the finding from Deppe et al. (2015), other factors independent of age, such as body condition, play a greater role in crossing an ecological barrier such as the Gulf of Mexico.

My results for body condition are consistent with observations from other stopover sites along large ecological barriers, such as the one presented by the Gulf of Mexico (Karasov and Pinshow 1998, Jenni-Eiermann et al. 2011). In migrating Blackcaps (*Sylvia atricapilla*), after crossing the ecological barrier presented by the Sahara, reductions in lean mass were primarily from liver, stomach, and small intestine (Karasov and Pinshow 1998). In addition to fat, migratory birds utilize lean tissue during migration. During stopover, birds must rebuild atrophied digestive organs before being able to adequately rebuild fat reserves. The rate of food intake and assimilation during stopover is, thus, dependent on the state of the bird's digestive tract (McWilliams et al. 2004). This may be a very important consideration for migrant birds with low muscle scores utilizing St. George Island as a stopover site. As such, the lack of relationship between mass and time of day and the lack of mass increase in recaptured individuals on St. George Island should be interpreted with caution (Delingat et al. 2009).

This study did not examine the extrinsic factors that may affect body condition, refueling rate, or stopover duration; however, these should be considered in future research, especially in the context of climate change. During El Niño years, after crossing the Gulf of Mexico in spring, birds that winter in South America arrive in reduced energetic condition and are more likely to stopover in small forest patches found immediately along the coast instead of in forests farther inland (Paxton et al. 2014). Therefore, it should be expected that coastal stopover sites, like St. George Island, may be especially important for a suite of long-distance migrants during these recurring climate patterns.

### 5.3 Capture date and time

Differences in capture dates between years for Gray Catbirds were due primarily to more captures earlier in the season in 2014. However, this difference may be an artifact of the longer and earlier field season conducted the second year. The same cannot be said about the differences in capture date for *Catharus* thrushes. Thrushes were captured later in the season in 2014; yet, sampling effort overlapped with the previous year's capture days for thrushes. The difference in capture date between short-distance and long-distance migrants is consistent with theoretical predications and previous studies. Yong and Moore (1997) describe *Catharus* thrush migration along the Gulf coast as later in the season and more synchronous than that of the shorter-distance

migrant, the Wood Thrush (*Hyalocichla mustelina*). It is recognized that birds travelling greater distances to their breeding grounds will depart the wintering grounds later in the season, but will travel more quickly (Alerstam and Lindström 1990). Migratory songbirds with longer journeys exhibit faster migration speeds, which is more pronounced during spring migration, and travel speed may differ along the migration route, with birds slowing down as they approach the breeding grounds (La Sorte et al. 2013). As this study did not consider any extrinsic factors, and given the relatively short study period and temporal and spatial variability inherent in migration, my data on capture rates should be interpreted with caution.

#### **5.4 Recapture rate and stopover duration**

Recapture rates for *Catharus* thrushes and Gray Catbirds on St. George Island was extremely low when compared to other barrier islands along the northern Gulf of Mexico that typically report rates between 8% and 20.7%, more than twice those reported in my study (Simons et al. 2004, Kuenzi et al. 1991). For example, the chenier forest of Johnson's Bayou along the coast of Louisiana have consistently reported recapture rates for thrushes between 14% and 20% (Owen and Moore 2008, Yong and Moore 1997). Stopover duration for *Catharus* thrushes in the chenier forests ranged from 2 to 9 days (Owen and Moore 2008). Spring stopover duration on East Ship Island, along the Mississippi coast, was  $4.2 \pm 4$  for Gray Catbird,  $1.5 \pm 1.1$  for Swainson's Thrush, and 2.6

$\pm 2.4$  for Gray-cheeked Thrush (Kuenzi et al. 1991). On St. George Island, the few recaptured *Catharus* thrushes stayed for one day, except for one individual Gray-cheeked Thrush (5 days). In comparison, recaptured Gray Catbirds stayed for  $4.3 \pm 2.9$  days. In this respect, St. George Island is most similar to East Ship Island, another Gulf coast barrier island, in regard to stopover use by migratory thrushes and Gray Catbird. The majority of individuals captured on St. George Island during spring migration were never recaptured, which I presume means they departed shortly after arrival. This is a similar conclusion reached by Kuenzi et al. (1991) for spring migrants utilizing East Ship Island. East Ship Island is more than twice the distance from the mainland than St. George Island. However, determining stopover duration is complicated by spatial and temporal scales, where relocation movements within a stopover site can be misinterpreted as continuation of migration (Taylor et al. 2011). It is possible birds not recaptured during this study relocated either on St. George Island or the mainland, or may have continued their migration.

### **5.5 Age structure**

The results did not support my hypothesis for age-related differences in arrival date in either short- or long-distance migrants. Age-effects in migration strategies have been previously documented in departures from wintering grounds (Stanley et al. 2012,

McKinnon et al. 2014) and arrival times to breeding grounds (Lozano et al. 1996, Stanley et al. 2012). Geolocator tracking in Wood Thrush (*Hylocichla mustelina*) found that birds on their first spring migration arrived on average  $12.48 \pm 2.24$  days later on the breeding grounds than birds that had migrated at least once before and was correlated with departure date from Central America (Stanley et al. 2012).

However, in directly navigating across the Gulf of Mexico, Deppe et al. (2015) found no relationship between age and migratory decisions in autumn. Rather, other factors such as fat reserves and favorable weather conditions determined whether birds crossed the Gulf. More pertinent to this discussion, they found no age effects in crossing time and arrival in the Yucatan Peninsula during autumn migration.

While there was no relationship between ordinal date and age in either migratory group in either year, there were other age structure findings. Most interestingly, age structure was not consistent from year to year. During the first year of the study (2013), there was a higher proportion of second year (SY) birds. While in 2014 there was a higher proportion of after second year (ASY) birds. The difference in age structure was significant in Gray Catbird. This finding suggests an important role of St. George Island as a “fire escape”, as it may provide habitat for a different suite of migrants during various conditions. In April 2014, the Florida panhandle experienced record-breaking rainfall with 5 times the normal monthly rainfall accumulated over a couple of days. The average rainfall recorded at the Panama City station in April 2013 was 3.79 inches,

compared to 13.95 inches in April 2014 (NOAA Climate Data). Whether these extraordinary conditions were only survived by experienced (ASY) birds and resulted in increased mortality of SY birds or created conditions where ASY birds were obliged to stopover, is unknown and beyond the scope of this project. However, it highlights the importance of long-term migration studies along the Gulf coast coupled with the tracking of environmental and weather conditions.

### 5.6 Sex structure

Yong and Moore (1997) suggest differential migration in *Catharus* thrushes along the Gulf coast based on declining wing chord measurements as spring migration progressed. However, I only found a significant decline in wing chord measurement for Gray Catbird during the second year. Still, there is too much overlap to reliably sex the specimens from St. George Island based solely on wing chord and tail measurements (Pyle 1997). Technical limitations were most likely the culprit for the molecular sexing failure. A touch-down protocol during PCR DNA amplification resulted in ~80% success for Swainson's Thrush (Covino 2012), a stark difference compared to my ~10% success rate. Given my inability to reliably determine sex structure for a significant number of individuals, I could not adequately test the hypotheses of sex-effects on arrival dates, initial body condition, and refueling rate.

## 5.7 Conservation implications

Many migratory songbird species have experienced population declines linked to decreasing habitat availability during the breeding, wintering, and migratory periods. In the northern Gulf of Mexico, the availability of coastal stopover habitats is particularly important for migrant birds that have completed ~1000 km flights *en route* to the breeding grounds. Migratory songbirds arrive at stopover sites in various physiological states due to conditions experienced prior to departure or during flight. This research confirms, not only that migratory songbirds use Apalachicola Bay barrier islands during migration, but more significantly, that they may be especially important for long-distance migratory birds. Long-distance migratory songbirds represent species of international conservation concern, and these species arrived on St. George Island in extremely poor body condition. Continued loss of habitat on these barrier islands would certainly represent decreased survival at the individual level; however, the population level repercussions are unknown. These coastal habitats are an important link for these migratory species that span international boundaries; therefore, management and conservation decisions should be aimed at addressing this role in the context of a changing climate and ever-decreasing habitat at the continental-scale.

## 5.8 Conclusions

For many years there have been numerous studies of the importance of the coastal habitats of the northern Gulf of Mexico for migratory birds. I present the first

study to quantitatively determine the energetic importance of a barrier island in the northeastern Gulf coast for migratory birds during spring. My research has confirmed that barrier islands of the Apalachicola Bay are used by trans-Gulf migratory songbirds. St. George Island is energetically important to migratory birds because it serves as an important stopover site for both short-distance and long-distance migrants, and may therefore be considered of international conservation importance. Furthermore, long-distance migratory *Catharus* thrushes consistently arrive in poorer condition than the short-distance migrant Gray Catbird. However, refueling rates are consistently similar between the two groups. Age-related effects did not drive the differences observed in arrival date, body condition, and refueling rate. Refueling rate on St. George Island was significantly different between years. This finding highlights the importance of long-term studies to understand the dynamics of migration at varying spatial and temporal scales, especially in the context of climate change. St. George Island functions as an important fire escape stopover site during a vulnerable stage of the migratory life cycle, serving as a refuge from weather, and a place to rest and refuel prior to continuing migration.

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- ZyGEM. DNA Extraction Using prepGEM Blood Quick-Start Guide.

## APPENDICES

**Appendix A:** Gel electrophoresis for molecular sex determination of study species.



```
#Model for all birds in study
MODEL=lm(Metabolite~Year+Fat+Age+Genus+Ordinal_Date+Hrs_Sunrise+min.time
+Size_Corrected_BodyMass+Muscle, data=Data)
step(MODEL, direction="backward")

#Model for long-distance birds
MODEL1=lm(Metabolite~Year+Fat+Age+Ordinal_Date+Hrs_Sunrise+min.time+Size_
Corrected_BodyMass+Muscle, data=thrush)
step(MODEL1, direction="backward")

#Model for short-distance birds
MODEL2=lm(Metabolite~Year+Fat+Age+Ordinal_Date+Hrs_Sunrise+min.time+Size_
Corrected_BodyMass+Muscle, data=catbird)
step(MODEL2, direction="backward")

#Model for year 1
MODEL3=lm(Metabolite~Fat+Age+Genus+Ordinal_Date+Hrs_Sunrise+min.time+Siz
e_Corrected_BodyMass+Muscle, data=2013)
step(MODEL3, direction="backward")

#Model for year 2
MODEL4=lm(Metabolite~Fat+Age+Genus+Ordinal_Date+Hrs_Sunrise+min.time+Siz
e_Corrected_BodyMass+Muscle, data=2014)
step(MODEL4, direction="backward")

#Regression
Reg.1<-lm(Size_Corrected_BodyMass~Ordinal_Date, data=data)

#Shapiro test for normality
shapiro.test(catbird$Size_Corrected_BodyMass)

#ANOVA
anova.1<-aov(Metabolite~Year, data=catbird)

#Mann-Whitney
wilcox.test(thrush$Ordinal_Date~thrush$Year)

#Chi-square test
chisq.test(catbird13$Fat, catbird13$Age)
```

**CURRICULUM VITA**

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**OBJECTIVE**

Inform natural resources conservation, particularly of migratory species, by integrating scientific research, education and outreach, and international collaboration.

**EDUCATION**

- 2015-present PhD in Organismic and Evolutionary Biology, University of Massachusetts Amherst  
 2012-2015 Master of Science in Natural Resources, Delaware State University  
 Pending graduation: Spring 2016 GPA: 4.0  
 2001-2007 Bachelor of Science in Biology, Universidad Nacional Autónoma de Nicaragua. GPA: 3.35

**EMPLOYMENT**

- 8/2015 – present Graduate Research Assistant  
 University of Massachusetts Amherst  
 12/2014 – 08/2015 Visiting Wildlife/Forestry Biology Specialist  
 University of Illinois Urbana Champaign  
 08/2012 – 12/2014 Graduate Research Assistant  
 Delaware State University, NOAA ECSC  
 04/2012 – 08/2012 Field Technician  
 Louisiana Department of Wildlife and Fisheries  
 05/2009 – 07/2012 Intern Liaison (Seasonal)  
 Environment for the Americas  
 01/2007 – 10/2011 Assistant for the Park Flight Migratory Bird Program  
 National Park Foundation  
 05/2011 – 07/2011 Research Technician  
 University of Georgia  
 11/2008 – 09/2010 Biodiversity Project Assistant  
 USDA Forest Service  
 03/2008 – 10/2008 Central America Bird Banding Program Coordinator  
 SalvaNATURA  
 12/2002 – 03/2008 Seasonal Biologist  
 Fundacion Cocibolca  
 04/2006 – 07/2006 Field Assistant  
 North Carolina State University Wildlife Cooperative Unit  
 05/2004 – 07/2004 Park Flight Migratory Bird Program Intern  
 Cuyahoga Valley National Park

## **AWARDS**

93

- 2015-2016. Northeast Alliance for Graduate Education and the Professoriate (NEAGEP) graduate fellowship at University of Massachusetts Amherst.
- 2012-2014. National Oceanic and Atmospheric Administration (NOAA) Environmental Cooperative Science Center (ECSC) graduate fellowship at Delaware State University.
- 2010. US Forest Service, Wings Across the Americas, International Cooperation Award. "Bird Conservation Partnerships for Biodiversity and Ecosystem Sustainability in Mesoamerica."

## **OTHER TRAINING & WORKSHOPS**

- Golden-winged Warbler Wintering Ground Conservation Plan Workshop. Oct 2014. Honduras
- NOAA ECSC Ground-truthing Field Campaign. NOAA Environmental Cooperative Science Center. Brownsville, Texas (Laguna Atascosa NWR – Bahia Grande Unit) July 20-27, 2014.
- Center-wide Core Competency Course. Marine skills training course. NOAA Environmental Cooperative Science Center. August 5-9, 2013. St. Teresa, Florida.
- Workshop on Vigilance of Human Pathogens in Wild Birds, with emphasis in West Nile Virus and Avian Influenza. Center for Disease Control, USAID Nicaragua, and USDA Forest Service with Flora & Fauna International and MARENA. Chocoyero, Nicaragua. February 2007
- Golden-winged Warbler Conservation Workshop. GWWA Working Group & Wisconsin Bird Conservation Initiative. Siren, Wisconsin. USA. August 2005

## **RECENT PRESENTATIONS**

Gutierrez Ramirez, M., Kneidel, A.H., Lester, L.A., and C.M. Heckscher (2014). Using plasma metabolites in trans-Gulf migratory birds to assess refueling rates on a Florida Gulf coast barrier island. Northeast/Southeast Partners in Flight 2014 Conference. October 6-9, 2014. Virginia Beach, Virginia.

Gutierrez Ramirez, M., Kneidel, A.H., Lester, L.A., and C.M. Heckscher. (2014). An assessment of refueling performance by select spring trans-Gulf migrants on a Florida Gulf coast barrier island. 132nd American Ornithologists' Union, 84th Cooper Ornithological Society, and the Society of Canadian Ornithologists Joint Meeting. Sep 23-28, 2014. Estes Park, CO.

Gutierrez Ramirez, M., Lester, L.A., and C.M. Heckscher. (2013) Spring stopover ecology of migratory birds on a barrier island in Apalachicola, Florida. Association of 1890 Research Directors (ARD) 17th Biennial Research Symposium. April 6-10 2013. Jacksonville, Florida.