**December 2012 Wormsloe Report**

Emily Cornelius

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**Interplay between stress, lipids, parasites and immunity in migrating songbirds**

## Introduction

Animal migrations are among the world's most fascinating phenomena, and much recent and historical work has centered on understanding the causes and mechanisms of long-distance migration. Although some animals undertake non-stop journeys, most species migrate in a series of short trips with stops in between. During a stopover, animals use resources in the local habitat for shelter and foraging. Birds, in particular, use stopover sites to replenish their lipid reserves, which is the main source of energy for their migratory flights[1]. Past work has suggested that prior to and during migratory journeys, animals might divert resources away from immunity and other bodily functions and towards fat storage and muscle to prepare for long distance movement[2]. Although much stopover research has examined habitat quality and resource use during migration, less attention has focused on energetic tradeoffs faced by individual birds in allocating resources to migratory success. The goal of my project is to examine the energetic tradeoffs that underlie migratory success, and their interactions with infection and immunity. Specifically, I will ask: (1) Do parasitized animals experience reduced lipid reserves and greater stress levels during migration? (2)Do measures of immune defense correlate negatively with lipid reserves among migratory animals? and (3) Do birds with lower fat reserves and poor body condition show greater stress levels?

## General Approach and Background

To explore the links between stress, fat, parasites, and immunity I will capture migrating songbirds in the fall of 2011 and 2012. Stress levels will be estimated by measuring corticosterone in fecal samples and from counts of specific white blood cells[3]. Using blood smears, I will quantify the presence of hematozoan blood parasites, and where possible, will confirm infection status with avian blood parasites via PCR. Then, I will score the level of subcutaneous fat on all birds, examine hematocrit scores to identify individuals with anemia, and will quantify leukocytes and lymphocytes as proxies for investment in immune defense. Finally, I will use stable isotopes ($\delta^{13}$C, $\delta^{2}$H and $\delta^{14}$N ) to estimate the natal origins of birds using feather samples that retain the isotopic signature of the habitat in which they developed, prior to migration.

**General Predictions**

Host condition, stress, infection status and immunity, can drastically change during the course of the migratory season. During migratory stopover, birds with smaller fat stores may be more likely to have higher stress levels owing to a reduced ability to find food or if they are suboptimal foragers. Additionally, birds that have a high level of blood parasites may have higher levels of stress and possibly lower fat reserves[4] due to parasite use of host resources for their own replication, their damage of host tissues, and the indirect costs of immune defenses[5]. Moreover, migrating animals might show a negative relationship between lipid reserves and measures of immune defense if resources necessary for migration are diverted away from immune function[2]. Lastly, by using stable isotopes we can determine the distance each bird traveled from its breeding grounds to examine whether this difference plays a key role in the three main research questions.

**1)** Blem, C. R. 1990. Avian energy storage. Current Ornithology 7: 59-113. **2)** Altizer, S., R. Bartel, B.A. Han. 2011. Animal migration and infectious disease risk. Science 331: 296-302. **3)** Davis, A.K., D.L. Maney, and J.C. Maerz. 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Functional Ecology (22): 760-772. **4)** Garvin, M.C, C.C. Szell, and F.R. Moore. 2006. Blood parasites of Nearctic-Neotropical Migrant Passerine Birds during Spring Trans-Fulf Migration: Impact on Host Body Condition. The Journal of Parasitology 92(5): 990-996. **5)** Owen, J.C. and F.R. Moore. 2006. Seasonal differences in immunological condition of three species of thrushes. The Condor 108(2): 389-398. **6)** Norris, D.R., P.P Marra, R. Montgomerie, T.K. Kyser, and L.M Ratcliffe. 2004. Molting latitude, and feather color in a migrating songbird. Science 306: 2249-2250.

**Goals for the spring**

Over Christmas break I plan to finalize the methods for the PCR of parasites. Again, this test will tell me if the malaria parasites are present or absent within a bird. At the end of January I plan to have all white blood cell counts completed for the Gray Catbirds. This will allow me to examine the differences in immune investment in relation to age, sex and body condition. Secondly, I will submit the Gray Catbird feathers for stable isotope analysis to a laboratory at UC Davis. This data will tell me where the Gray Catbirds spend the summer and breed. This will allow me to determine if distance traveled is a factor in migratory trade-offs. For the time being, I am focusing on completing as many analyses as possible for one species, and then once done, will move onto the next species.

At the end of February, I plan to have all laboratory components and analyses completed. Having all tests complete will allow me to determine the best way to analyze the data and begin writing my thesis. As I plan to graduate in August, my thesis will be submitted to the graduate school by the end of June.

*Emily Cornelius*

# Wormsloe Research Report

December 6, 2012

**Introduction**

Animal migrations are among the world's most fascinating phenomena, and much recent and historical work has centered on understanding the causes and mechanisms of long-distance migration. Although some animals undertake non-stop journeys, most species migrate in a series of short trips with stops in between. During a stopover, animals use resources in the local habitat for shelter and foraging. Birds, in particular, use stopover sites to replenish their lipid reserves, which is the main source of energy for their migratory flights[1]. Past work has suggested that prior to and during migratory journeys, animals might divert resources away from immunity and other bodily functions and towards fat storage and muscle to prepare for long distance movement[2]. Although much stopover research has examined habitat quality and resource use during migration, less attention has focused on energetic tradeoffs faced by individual birds in allocating resources to migratory success. The goal of my project is to examine the energetic tradeoffs that underlie migratory success, and their interactions with infection and immunity. Specifically, I will ask: (1) Do parasitized animals experience reduced lipid reserves and greater stress levels during migration? (2)Do measures of immune defense correlate negatively with lipid reserves among migratory animals? and (3) Do birds with lower fat reserves and poor body condition show greater stress levels?

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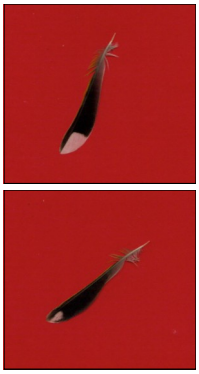
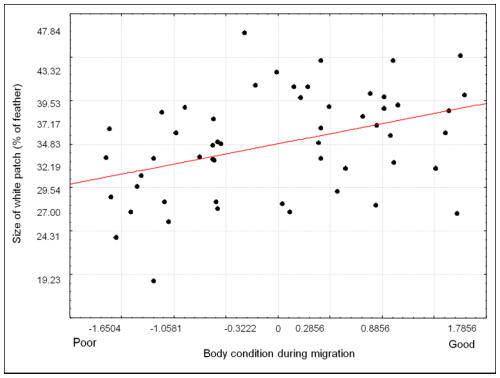
## Updates from November






Since the last meeting I have worked on examining the white patches of Western palm Warbler tail feathers. These patches are generally thought of as sexual ornamentation and are molted (grown) prior to migration. Further, body condition during migration can be used as a proxy for migratory success. Our data shows that birds with larger white patches have better body condition during migration, and thus might be the birds that are more likely to survive the journey to the wintering site.

Additionally, I have completed 10/50 white blood cell counts for the Gray Catbirds, begun DNA extraction to look for malaria parasites, and submitted all of the Common Yellowthroat feathers for stable isotope analysis.

## Goals for the spring

Over Christmas break I plan to finalize the methods for the PCR of parasites. Again, this test will tell me if the malaria parasites are present or absent within a bird. At the end of January I plan to have all white blood cell counts completed for the Gray Catbirds. This will allow me to examine the differences in immune investment in relation to age, sex and body condition. Secondly, I will submit the Gray Catbird feathers for stable isotope analysis to a laboratory at UC Davis. This data will tell me where the Gray Catbirds spend the summer and breed. This will allow me to determine if distance traveled is a factor in migratory trade-offs. For the time being, I am focusing on completing as many analyses as possible for one species, and then once done, will move onto the next species.

At the end of February, I plan to have all laboratory components and analyses completed. Having all tests complete will allow me to determine the best way to analyze the data and begin writing my thesis.

As I plan to graduate in August, my thesis will be submitted to the graduate school by the end of June.

*Emily Cornelius*

**Special points of interest:**

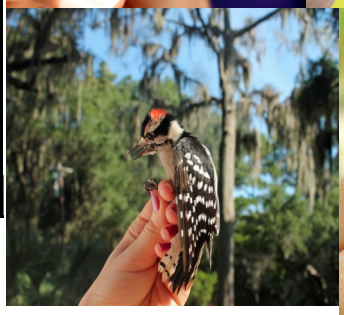
- Caught 4 new species of birds while at Wormsloe: Ruby-crowned kinglet, Black-and-white warbler, Yellow-rumped warbler and Downy woodpecker
- Caught 50-60 birds of three different species

# Wormsloe Meeting Update

November 1, 2012

## 2012 Field season

| Row Labels | Count of Species |
|---|---|
| BTBW | 2 |
| BWWA | 1 |
| CARW | 7 |
| COYE | 58 |
| DOWO | 2 |
| GCFL | 1 |
| GRCA | 46 |
| HOWA | 2 |
| NOCA | 13 |
| PABU | 25 |
| TUTI | 3 |
| VEER | 1 |
| WPWA | 77 |
| Grand Total | 238 |



I spent the last month down at Wormsloe and Jekyll Island banding station (JIBS) capturing birds for sampling. At WHS I had 8-10 mistnets running at all times of the morning hours. I was also able to conduct bird surveys in the evening to assess other potential locations or if other birds were on site. One of the last days, I also conducted a plant/habitat survey with a volunteer from JIBS.

I am happy to say that I captured four new species of birds and two species in greater numbers than last year: Hooded warblers and Veerys .

The next steps are to do the white blood cell and parasite counts under the microscope and submit the feathers for stable isotope analysis. Currently I have enough birds for each fat score for the GRCAs and COYEs to examine my initial 'trade-off' hypothesis.

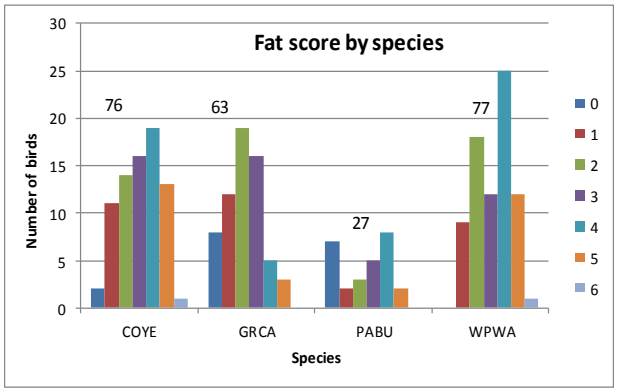
I'm hoping to have more hard results to share during the last meeting, as I am still getting things organized after the trip!

## Wormsloe Photo Book

My field tech and I are putting together a photo book of the birds that have been captured at WHS. This will be organized by resident species, partial migrants, and migratory birds. Each page will also have descriptions of the type of 'markers' that make the bird identifiable. In addition to pictures, it will have pages about the project, conservation issues, and field techniques.

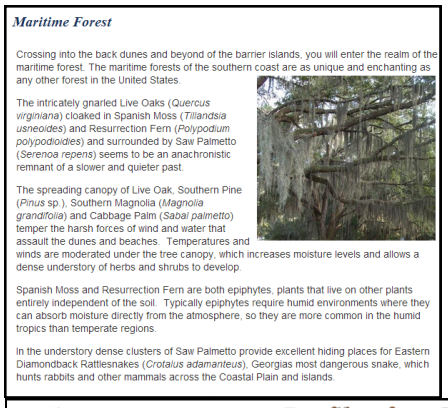


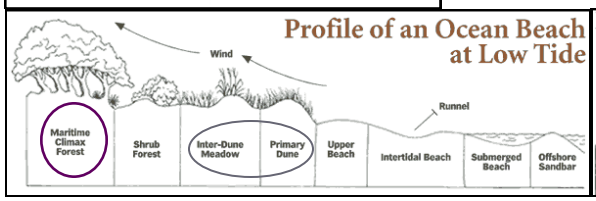
# Summary of results

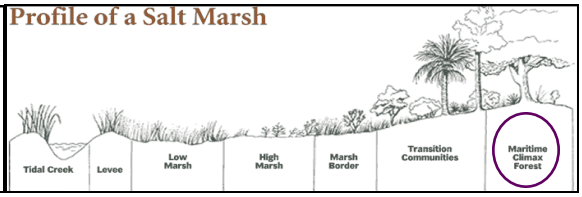



*After coming back and analyzing the data, I have about 60 Common yellowthroats, Gray catbirds and Western-palm warblers that have all of the samples that I need. Tentatively, I am going to use the tail feather samples from the yellowthroats and the catbirds for the stable isotope analysis. For the palm warblers, I am interested in examining the differences in the size of the white patches on the tail feathers to see if that correlates with body condition, parasite loads, or whether it is only age/sex related.*

*In addition to looking at bird physiology, I think it is important to look at site characterisitcs of Wormsloe versus other stopover areas, like Jekyll island. Therefore, I plan on doing a bit more research on the differences in forest types, and how that might affect bird community dynamics.*



# September update

**SUMMER 2012** **EMILY CORNELIUS**

## My two goals for this summer were to catch breeding birds and also finalize all methods for lab and field work...

I traveled down to Wormsloe three times over the course of the summer to try to catch Gray Catbirds, Common Yellowthroats and other residential birds. This first time down, in May, I caught 1 Catbird and 5 Yellowthroats, showing that these birds are also migrating back to their breeding grounds and using WHS as a stopover site.

**I also mist netted breeding birds** in Athens. Over the course of the summer I was able to capture a handful of the species I needed. I was able to catch 3 Catbirds of which I have an inital blood and fecal sample, which could potentially be used later for stress data.

**My other goal for the summer was to** finalize all laboratory methods. I wanted to do this so that I could quickly process all the samples I collect from the upcoming fall field season without any hiccups. The main task was to work out all the kinks in the nested-PCR methods. This PCR will determine if any birds caught have malarial parasites. This analysis gives presence, and the blood smears will give intensity. In other words, when samples are determined to have malaria parasites present, I can then go back and look through the blood smears to determine intensity. I am doing this for ALL samples, not just the migrating birds. I finally have the methods worked out for this, and will have some results to present at the next meeting.

**I also determined that for the next field** season I will try to obtain a few other samples that could give us clearer results. First, I plan to measure hematocrit, which will tell us whether or not the bird is anemic. This is another measure of condition. Secondly, I will use a field centrifuge to spin down the blood and separate red blood cells from blood plasma. The red blood cells will be used for PCR and the plasma will be used for a

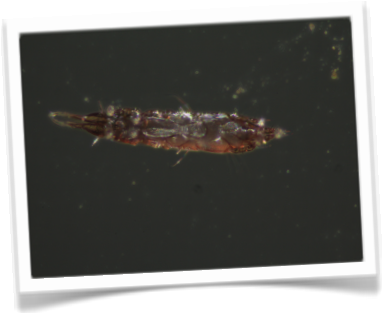
bacterial killing assay. This assay measures the blood's ability to produce an immune response to bacteria. It will tell us the differences between the immune systems of the various birds sampled. Thirdly, I also plan to conduct

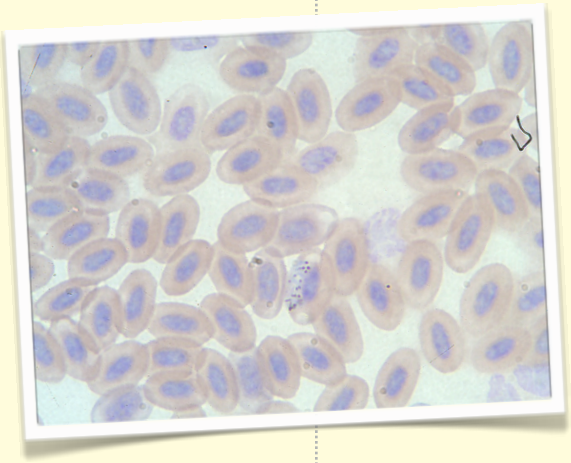
the fecal corticosterone validation during the fall season. In order to do this I will capture a bird, take an initial fecal sample and then hold the bird for 5 hours, continuously collecting fecal samples. This will be the pilot study of fecal corticosterone. This has never been done in a songbird before. I predict that this will be most feasible in Gray Catbirds because they are very robust birds.

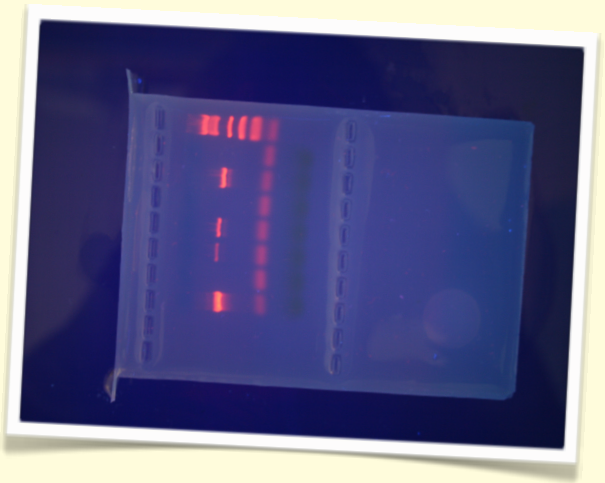
Lastly, I will be taking some feather samples in order to do stable isotope analysis. This analysis could potentially show us where the migrating songbirds are breeding during the summer. Locations have distinct nutrient profiles, specifically levels of Hydrogen, Carbon and Nitrogen. The feathers can show this nutrient profile by measuring for the combination of H, C and N and matching it to a database for the United States. This could potentially show us that birds within the same species are traveling a variety of distances to reach stopover sites. This might explain the differences in body condition, parasite load, etc.

## Plans for the fall field season (coming up!):

I will be down at Wormsloe for the end of September and most of October. While there, I will be conducting the white blood cell counts while I'm down there to maximize time. I hope to capture at least 50 birds from 2-3 species of migrants, including Gray catbirds and Common yellowthroats. I plan to have lots of lab work to do when I come back, and plenty of results to share!







*Blood smear from a Gray catbird on the left. One cell is infected with plasmodium (malaria parasite). PCR gel on the right. Amplified bands are positive samples.*