

Spring Stopover Ecology and Physiology of the White-Throated Sparrow
(*Zonotrichia albicollis*) in Western New York

A thesis

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Abstract

Stopover sites are an essential part of a North American migratory songbird's journey between wintering and breeding grounds, but annual variation in use and habitat conditions make it difficult to determine which sites are most critical for conservation. By learning which factors influence a bird's behavior when choosing and using a stopover site, we can target certain species or locations and more efficiently invest conservation efforts. In April-May 2013 and 2014, I studied stopover refueling rate in the White-throated Sparrow (*Zonotrichia albicollis*), a common northeastern spring migrant, at two locations near the south shore of Lake Ontario, a natural migratory barrier. To do this, I used morphological measurements and physiological techniques that measured the concentration of two important blood metabolites, triglycerides and β -hydroxybutyrate. Blood triglyceride concentration is a measure of fat deposition and feeding efficiency, and β -hydroxybutyrate concentration is associated with fat catabolism and energy loss. I found that birds captured at a location ~15 km from the shore had significantly higher blood triglyceride concentrations, as well as significantly higher body condition score, than birds captured at a stopover location within 0.5 km of the shore (1.737 mM > 1.361 mM). However, after using ANCOVA to control for the effects of body condition and time after sunrise, blood triglyceride levels did not vary significantly with location. β -hydroxybutyrate levels were not significantly higher in lakeshore-captured birds either before or after ANCOVA. Lack of statistical significance in both cases may be due to effects of small sample sizes.

My results imply that birds obtain food more efficiently at the inland location, and that birds that arrive in the area in better condition may begin their cross-lake journey directly from the inland site. Birds in poorer condition may “pile up” at the lakeshore and then must compete heavily with other migrants for available resources, slowing their fat deposition rate. These results reinforce the importance of protecting high-quality stopover habitat where birds congregate near geographic barriers, but also suggest that inland habitat patches are important stopover sites that may allow some migrants to bypass nearshore areas of intense competition.

Introduction

Background:

Avian migration is a risky and energetically costly process that has been studied extensively over the past few decades. Neotropical migrants are able to move seasonally to take advantage of plentiful resources on their spring breeding grounds while avoiding the harshest winter weather. Despite these advantages, migration may be the most dangerous part of a passerine's life and in some species can account for more than 85% of annual mortality (Sillett and Holmes 2002). During spring migration, birds weighing less than 100 g may travel distances of several thousand kilometers. It is difficult for these birds to store enough energy to complete this flight without periodic refueling, so quality stopover sites are necessary for survival. Spring migrants must quickly obtain enough food to continue migration and arrive at breeding grounds earlier than conspecifics, as earlier arrivers are at a competitive advantage (Lack 1968; Kokko 1999; Drent *et al.* 2003). For the best chance of survival, migrating birds need access to quality stopover habitat to rest and restore fat reserves, making these areas of particular importance from a conservation standpoint. Understanding how birds that are faced with limited resources use these habitats allows us to make conservation decisions that can help maintain or improve passerine populations.

While birds undergo a variety of systemic physiological and anatomical changes over their annual cycle (Farner 1986), waxing and waning fat stores are a research focus because fat stores serve as migratory birds' primary fuel source. Fats

are energy-rich molecules that can be processed efficiently in birds, shortening necessary stopover time, and are stored with little water, reducing their weight cost on flight (Weber 2009). A bird's ability to store fat before and during migration may have a huge impact on its chance of surviving and successfully breeding in a given year (Sandberg and Moore 1996a, Smith and Moore 2003).

Birds arriving at a stopover site behave differently depending on several factors, including weather conditions, distance from their destination, and body condition (Matthews and Rodewald 2010). Individuals with considerable fat stores may stay and forage only for a short period of time, continuing their journey almost immediately or in just a few days (Seewagen and Guglielmo 2010). Lean birds, however, will spend more time refueling and may stay at their initial location for several days or orient away from their expected route (Alerstam 1978, Cherry 1982, Sandberg and Moore 1996b, Seewagen and Guglielmo 2010). Birds may also delay leaving stopover sites due to inclement weather and wind direction, changing the importance of a single site from year-to-year (Åkesson and Hedenström 2000).

When encountering a barrier such as a large body of water, large concentrations of migrating birds may stop and rest, depending on fat stores, migratory inclination, and weather conditions. This concentration has been noted to be especially heavy within 3-4 km of the Lake Ontario shoreline (Agard 1994). Deutschlander and Muheim (2009) found that birds stopping over with larger fat stores tended to orient in the correct direction of migratory travel, while lean birds oriented away from a water barrier or showed no consistent orientation preference.

Birds that reorient and disperse inland may avoid competition and predation risks caused by large numbers of animals occupying a small amount of habitat (Moore *et al.* 2003). Young birds, which are less familiar with the surrounding habitat than older individuals, may be more likely to stay near the shore rather than seek better habitat farther inland (Alerstam 1978). The degree of reorientation can also be influenced by habitat quality, as large numbers of birds may not cause competitive pressure when feeding and roosting opportunities are plentiful (Deutschlander and Muheim 2009).

In any conservation effort, limited resources mean that potentially valuable or at-risk habitats must often be classified and prioritized, which is especially difficult when dealing with stopover locations. Natural weather fluctuations mean that not every stopover site will be utilized each year by the same population or species, so multiyear studies are often needed. Differences in patch size and habitat structure can also complicate prioritization, as stopover sites can range from small woodlots and city parks to large tracts of undisturbed forest. Also, the importance of these sites can vary due to surrounding landscape structure, development, and proximity to other suitable stopover habitats (Mehlman *et al.* 2005). Thus, multi-year studies using familiar, reliable species can help us encompass this variation and generate a baseline for comparison to other locations or to assess long-term changes.

As a widespread generalist species in northeastern North America, the White-throated Sparrow (*Zonotrichia albicollis*) can serve as a model for understanding the migratory physiology and ecology of other passerines and give us some idea of how

birds use potentially important locations. My study examines energetics as a record of foraging and metabolic demands in migratory White-throated Sparrows, which can help us to understand habitat use in this and other passerine species.

Study Species (White-throated Sparrow):

The White-throated Sparrow is a widespread, well-studied passerine with a typical adult length of 16-18 cm and a mass of 22-32 g, which varies with season (Falls and Kopachena 2010). Females are generally smaller than males, and some individuals can be sexed using wing chord measurement (Falls and Kopachena 2010). Adult *Z. albicollis* have a plumage pattern defined by bold striping on the head and a light gray or white bib that contrasts with darker face and chest plumage. The back is dark rufous streaked with black, and the wing feathers have buffy edges. The flanks are light brown with dark streaks, and belly is light gray.

An interesting trait of this species is the existence of a plumage dimorphism. The sexes are not dimorphic, but two alternate-plumage color phases exist: white-striped and tan-striped morphs, which refer to the distinctive median crown and eyebrow stripes. Most birds in alternate plumage can be easily separated by morph on sight, but difficult birds or those in basic plumage can be sorted by molecular means (Michopoulos *et al.* 2007). The phenotype also manifests as behavioral differences, where white-striped individuals of both sexes tend to be more aggressive and white-striped males sing more frequently (Kopachena and Falls 1993). This polymorphism is controlled by a set of alleles on chromosome 2 that were produced by a chromosome inversion (Thornycroft 1966; Tuttle *et al.* 2016). Except in

extremely rare cases, white-morph birds are heterozygous for the associated alleles, while tan-striped birds are homozygous (Tuttle *et al.* 2016). A nearly 1:1 ratio of color morphs is maintained through negative assortative mating, wherein white-striped males will almost always mate with tan-striped females, and vice-versa (Tuttle 2003). Behavioral differences may drive this mating strategy, and there is also evidence of deleterious effects of other gene combinations, such as two white-striped parents being more likely to produce “super-white”, slow-growing offspring (Tuttle *et al.* 2016). This “supergene” assembly functions almost like a second sex chromosome, and its numerous effects on physiology, survival, and behavior make it a fascinating addition to migration studies.

White-throated Sparrows are short-distance migrants, with wintering grounds across the eastern, central, and southern US and breeding grounds across boreal Canada and parts of the northeastern United States. This species travels in loose flocks, usually nocturnally, and spring migrants’ behavior is strongly influenced by weather-related factors like wind direction and barometric pressure (Falls and Kopachena 2010). Sexes and age classes migrate north at slightly different times, with males arriving at breeding areas 1-2 weeks before females (Mills 2005; Falls and Kopachena 2010), although this temporal difference has not been seen in some studies (Caldwell and Mills 2006) and may vary with population or year.

White-throated sparrows are ground-feeders and generalists that typically consume grains and arthropods when available. During migration, foraging birds remain close to cover and feed in openings in dense vegetation or along shrubby

edges and hedgerows (Falls and Kopachena 2010). A migrating flock's dominance hierarchy can influence feeding behavior, restricting low-ranking individuals to foraging areas far from cover and placing them at higher predation risk (Schneider 1984). Birds must balance food availability with exposure to predators, all while facing the selective pressure to arrive at breeding grounds quickly and in good condition.

Energetic Condition:

Energetic, or body, condition is an index of an individual's energetic state. An animal's body condition is a representation of its energy reserves, meaning that animals in better condition may have higher survival or reproductive rates. There are several different methods researchers can use to measure the body condition of birds, which include lethal and nondestructive approaches, with different levels of precision.

Fat score is a commonly used metric that estimates subcutaneous fat deposits, which are easily visible in most passerines. First developed by Helms and Drury (1960), this method is nondestructive and relatively quick, reducing handling stress and processing time for researchers. Unfortunately, the usefulness of this method may vary based on the species, the individual observer, and the number of different observers throughout a study. Krementz and Pendleton (1990) tested the reliability of visual fat scoring by comparing reported fat score with total body fat of dissected birds. They found that the precision of fat scoring varied with species, and that there was an unacceptable level of between-observer variation, but that within-observer variation was at an acceptable level. Thus in studies such as mine, in which there are

multiple observers, it is best not to rely solely on fat score for statistical analyses of body condition.

A more reliable method of estimating energetic condition is by comparing mass to body size, which can be measured in several different ways. One method of calculating an energetic condition index is ($CI = \text{mass [g]} / \text{wing chord [mm]} * 100$; Winker *et al.* 1992). This method is inappropriate when comparing multiple species, but one can obtain a reliable measure of overall body condition by comparing only conspecifics, as in my study.

Measuring Fat Deposition and Mobilization:

Traditionally, measuring rates of change of energetic condition required that individual birds be recaptured and weighed multiple times within a short period of time. This method has some problems relative to both practicality and scientific validity, including the difficulty of capturing the same bird several times, the increased stress inflicted on these birds, and the possibility that low-quality animals will be those most likely to be recaptured (Guglielmo *et al.* 2005). Other investigators have used regression analysis on birds captured over several hours at one location to determine whether birds of a certain species are gaining mass at that site (Dunn 2001; Bonter *et al.* 2007).

In contrast, measuring blood metabolites such as triglyceride and β -hydroxybutyrate provides a method of determining fat deposition and mobilization rates without recapturing individual birds (Jenni-Eiermann and Jenni 1994, Guglielmo *et al.* 2005). This decreases the effort needed, since same-day recaptures

are rare and sample sizes typically low, and also reduces the stress associated with capturing and handling birds, which may affect metabolism (Mueller and Berger 1966; Guglielmo *et al.* 2005). Measuring blood metabolites provides a snapshot of metabolic activity, allowing researchers to profile foraging effort and habitat quality (Smith and McWilliams 2010).

While birds are foraging, plasma triglyceride concentrations increase as lipids from food are absorbed into the body, processed in the liver, and deposited into adipose stores. During fasting, the body increases lipolysis of these fat stores and β -hydroxybutyrate levels increase as ketone formation increases (Guglielmo *et al.* 2005). Comparing levels of these metabolites among individuals gives researchers insight into short-term changes in body condition, both in general (Cerasale and Guglielmo 2006) and when investigating diet components (Smith *et al.* 2007). Jenni-Eiermann and Jenni (1994) field-tested this technique and found that triglyceride and β -hydroxybutyrate levels explained 61% of the variation in body-mass change in a sample of migratory Old World warblers. Since it provides a qualitative measure of energy gain and loss, this process has also been used to compare birds at different sites (Smith and McWilliams 2010, Smith *et al.* 2015) and spring and fall migrants (Smith 2013).

The main aim of my study was to determine how a stopover barrier influenced blood triglyceride levels, as a measure of refueling rate, in migrating White-throated Sparrows. Western New York provides a valuable opportunity to observe the effects of a stopover barrier, Lake Ontario, on competition and food availability in habitats

adjacent to the lakeshore. For example, if there is greater pressure on food resources near the lake, birds there would have lower refueling rates than those farther inland. I also examined differences in refueling rate between White-throated Sparrow plumage morphs, which could be caused by the behavioral traits associated with each morph. Finally, to add to our general knowledge of migration energetics and ecology, I sought to compare the blood metabolites themselves and examine their relationship to body condition.

As a relatively widespread, generalist species, the White-throated Sparrow can serve as a model organism for various other passerine birds, especially in migration studies where stopover site condition and use may vary from year to year. Research on this species can be compared to other birds and locations as our knowledge and use of blood metabolites in physiology studies increases. Though studies of free-living organisms cannot typically encompass every factor influencing their behavior and biology, focusing on a few important variables hopefully enables researchers, managers, and conservation organizations to make more informed decisions about wildlife and habitat management.

Study Sites

I conducted my research at two sites south of Lake Ontario in Monroe County, New York (Figure 1). The lakeshore site was Braddock Bay Bird Observatory (BBBO, 43°19'N, 77°43'W), an established constant-effort bird banding station located within 0.5 km of the shoreline. The habitat at BBBO was characterized by abandoned agricultural fields maintained by regular mowing and cutting, shrubs, and

forests of varied successional stages. Common plants include dogwood (*Cornus* spp.), honeysuckle (*Lonicera* spp.), viburnum (*Viburnum* spp.) and early-successional trees such as alder (*Alnus* spp.) and ash (*Fraxinus* spp.). There were some surrounding residential homes inland and along the shoreline. Netting here took place among the different habitat types, within net lanes that have been consistent since the late 1980s.

The inland site was Cobble Creek Farm (CCF, 43°13'N, 77°48'W), a private mixed-use farm in Spencerport, NY located approximately 14.5 km SSW from Braddock Bay. This location consisted mostly of active ornamental tree and crop fields, fallow fields, hedgerows, and some shrubby patches. Netting at this site took place along a network of dirt tractor lanes connecting the fields, among trees and shrubs, approximately 400 m south of New York Route 104. Surrounding vegetation included maple (*Acer* spp.), cherry (*Prunus* spp.), and coniferous trees; many of the tractor lanes were bordered by dense stands of honeysuckle and some multiflora rose (*Rosa multiflora*).

Methods

Data collection took place during the same time period at the two study sites. I collected data at BBBO from 20 April – 14 May 2013 and 17 April – 10 May 2014, using the BBBO migration monitoring protocol (Braddock Bay Bird Observatory 1999), as part of regular mist netting and banding activities associated with the spring songbird migration. I collected data at CCF from 18 April – 2 May 2013 and 25 April – 5 May 2014. To allow birds time to forage in the morning, I only collected

blood from birds netted 1-6 h after sunrise. I checked nets approximately every 20-30 min, or more frequently in cooler weather, and did not band on especially cold or windy days.

To account for environmental and procedural variables, I recorded year, Julian date, capture time after sunrise (HAS), and time between capture and blood draw for each bird. Each bird was fitted with a metal United States Fish and Wildlife Service numbered band following physical measurements. I collected blood by piercing the brachial vein with a 27-gauge needle and collecting blood in heparinized capillary tubes. Total blood collected varied by bird, but no more than 2 full tubes (2 x 70 μ L) were taken from any individual (Fair *et al.* 2010). Tubes were sealed and stored in a cooler until the end of the banding day and were then centrifuged in the laboratory for 10 min at 10,000 rpm to separate blood cells from plasma. Cells and plasma samples were stored in a -80°C freezer until later analysis.

Bird banding and blood collection were performed under U.S. Geological Survey subpermits 20539-Q (BBBO) and 10539-E (CCF).

Morphological Measurements:

After blood draws, birds were aged and sexed when possible, measured, and banded with a US Geological Survey numbered aluminum band according to Pyle (1997), and White-throated Sparrow color morph was recorded. I measured mass (\pm 0.1 g), tibiotarsus length (\pm 0.1 mm), and wing chord (\pm 0.5 mm). Fat score was recorded on a 0-5 scale following Helms and Drury (1960): 0 = no visible fat, 1 = trace fat visible in furcular region, 2 = furcular region filling but concave, 3 = furcular

region filled with fat, 4 = furcular region bulging and fat visible on abdomen, 5 = furcular region bulging and abdomen mounded. I calculated condition index (CI), an estimate of fat storage adjusted for overall body size, for each bird as $(CI = \text{mass (g)} / \text{wing chord (mm)} * 100$, Winker *et al.* 1992). Birds were immediately released following measurement, banding, and blood draw.

Blood Metabolite Analysis:

I analyzed blood metabolites at the Rochester Institute of Technology, Rochester, NY. Before analysis, most plasma samples were diluted with 0.9% saline solution, typically at a 1:2 ratio or a 1:3 ratio for smaller samples.

Blood triglyceride analysis followed the sequential endpoint assay protocol described in Guglielmo *et al.* (2002). In this protocol, blood triglyceride concentration is measured by colorimetric assay before and after addition of lipase that converts the triglyceride into fatty acids and glycerol molecules. For analysis, I produced a standard curve (0.085 – 2.816 mM) using known concentrations of glycerol standard solution (Sigma-Aldrich, St. Louis, MO). I pipetted samples and standards onto a 96-well microplate. Glycerol reagent (Sigma-Aldrich, St. Louis, MO) was added to determine free glycerol concentration (Glycerol), and the plate allowed to incubate at 37° C for 10 min, after which absorbance was read at 540 nm in a microplate spectrophotometer (BioTek EON, Gen5 2.01 software). This was followed by addition of a triglyceride reagent (Sigma-Aldrich, St. Louis, MO), which catalyzes the catabolism of triglyceride and releases glycerol. After incubation at 37° C for 10 min to complete the reaction, the absorbance was recorded again to determine total

glycerol concentration after the reaction. Actual blood triglyceride concentration was calculated by subtracting free glycerol from total glycerol following the lipase reaction.

β -hydroxybutyrate analysis followed the kinetic assay protocol, also described in Guglielmo *et al.* (2002). In this protocol, β -hydroxybutyrate concentrations are determined by kinetic assay that measures the oxidation of the ketone in the presence of the D-3-hydroxybutyrate dehydrogenase enzyme. I used a D-3-hydroxybutyrate assay kit (R-Biopharm, Darmstadt, Germany) for analysis and used Stanbio TDM linearity standards (Stanbio, Boerne, TX) to produce standard curves for each assay (0.25 – 2.00 mM). I pipetted standards and samples onto a 96-well microplate and then added the working reagent (buffers and dye) to each sample and measured absorbance at 492 nm for 2 min in a microplate spectrophotometer (BioTek EON, Gen 5 2.01 software) to check background curves. After quickly adding 3-hydroxybutyrate dehydrogenase suspension, the samples were returned to the plate reader and read at 1-min intervals for about 30 min, or until slopes leveled off, indicating completion of the reaction. Due to unanticipated differences between kits, it was difficult to produce reliable and matching curves for each plate, reducing our usable sample size. Sample readings for some plates with failed standard curves were compared to successful curves to produce β -hydroxybutyrate concentrations for samples.

To ensure accuracy, all samples were tested in pairs and were retested if blood metabolite concentration coefficient of variation (CV%) was above a 10% threshold

for triglyceride (Smith and McWilliams 2010). I used a 15% threshold for β -hydroxybutyrate to improve sample size as this metabolite was more variable in the assays. I also included two wells of a chicken plasma pool on every microplate assay as an inter-assay control.

Statistical Analysis:

Data for wing chord, mass, fat class, body condition, and blood metabolites were distributed normally per Ryan-Joiner test results ($P > 0.05$) after removal of outliers that I could attribute to procedural errors such as mis-recorded measurements or pipetting mistakes. Tibiotarsus length was not normally distributed, and was \log_{10} -transformed when included in analyses.

I used preliminary two-sample t-tests to check for between-year differences among basic morphological measurements in birds and rule out any pseudoreplication (Hurlbert 1984) that would be caused by combining years. Basic morphological differences between sites and morphs, including wing chord, tibiotarsus length, fat score, and mass, were compared using two-sample T-tests. Analysis of blood metabolite data first included a backwards stepwise selection to identify significant environmental or other methodological variables that should be included in analysis of covariance (ANCOVA) models to compare metabolite concentrations between sites and color morphs (Smith and McWilliams 2010). These variables included time of capture after sunrise, time between capture and blood draw, year, and Julian date, which must be accounted for before isolating and drawing conclusions about feeding rate (Guglielmo *et al.* 2005; Smith and McWilliams 2010). Other, physical variables

tested included body mass and body condition. Variables were retained in the models if $P < 0.10$ (Guglielmo *et al.* 2005).

I then tested for relationships between blood metabolites and body condition, location, and color morph using ANCOVA. Finally, the relationships between the blood metabolites, and the metabolites and body condition, were tested using regression analysis. My main aim was to determine the variables with the greatest influence on blood triglyceride and β -hydroxybutyrate levels and detect significant relationships between these two metabolites. For all t-tests and ANCOVA, significance level was set at $P < 0.05$. Statistical tests were performed using Minitab 17 and IBM SPSS Statistics 24 software.

Results

A total of 96 birds were banded and processed during my study; 22 at CCF and 74 at BBBO. Color morph data were recorded for 85 birds.

Blood metabolites were not significantly different between years for both triglyceride ($t = 0.46$, $df = 59$, $P = 0.650$) and β -hydroxybutyrate ($t = 1.85$, $df = 23$, $P = 0.078$) when birds from both locations were combined. Within BBBO-captured birds only, year also did not have a significant effect on triglyceride ($t = 0.82$, $df = 42$, $P = 0.418$) or β -hydroxybutyrate ($t = 1.89$, $df = 17$, $P = 0.076$). Because relatively few birds were captured at CCF (2013 $n = 13$; 2014 $n = 9$), I did not analyze between-year differences in blood metabolites at this location.

Year did not have a significant effect on wing chord ($t = 1.50$, $df = 88$, $P = 0.138$) and mass ($t = 1.64$, $df = 75$, $P = 0.104$) at BBBO, although birds captured in

2014 did have a slightly higher body condition score (2013 \bar{x} = 36.78, 2014 \bar{x} = 38.15; $t = 2.40$, $df = 74$, $P = 0.019$). Since there were very few between-year differences in morphological or physiological variables, data for birds from both years were combined for most other statistical analyses.

Physical Characteristics:

A summary of physical measurements and blood triglyceride concentrations, separated by banding location, is presented in Table 1. Wing chord did not differ significantly between locations ($t = 0.25$, $df = 33$, $P = 0.805$), but birds at the inland site had significantly greater tibiotarsus length ($t = 4.58$, $df = 32$, $P < 0.001$) and mass ($T = 2.41$, $df = 29$, $P < 0.001$) than those at the lakeshore.

Birds banded at the inland site tended to be larger and heavier, and body condition was significantly higher in inland birds (Figure 2; $t = 2.41$, $df = 27$, $P = 0.02$). Average fat score was slightly higher in birds at CCF (1.81) than at BBBO (1.50) (Figure 3), but the difference was not statistically significant ($t = 0.94$, $df = 28$, $P = 0.354$).

Stopover Location and Blood Metabolites:

Following backwards stepwise selection in preparation for ANCOVA, triglyceride levels were not significantly affected by year, mass, or time between capture and blood collection. Location was retained in the triglyceride model, along with body condition and hours after sunrise. After controlling for body condition and hours after sunrise, inland birds had a higher mean blood triglyceride concentration than lakeshore birds (location: $F_{1,69} = 3.22$, $P = 0.077$; hours after sunrise: $F_{1,69} =$

4.04, $P = 0.048$; body condition: $F_{1,69} = 8.94$, $P = 0.004$; Figure 4). Location, year, mass, time between capture and blood collection, and hours after sunrise were not retained in the β -hydroxybutyrate model; only body condition was retained in this model. After controlling for body condition, β -hydroxybutyrate did not vary significantly with location (location: $F_{1,32} = 1.33$, $P = 0.257$; body condition: $F_{1,32} = 5.41$, $P = 0.026$; Figure 5).

Color Morph:

I used capture date to compare average estimated stopover arrival date of each color morph; only BBBO data were used due to the larger sample size. White-striped birds were initially captured an average of three days earlier than tan-striped birds over both years at BBBO (Figure 6; $t = 2.07$, $df = 67$, $P = 0.042$).

I tested for the potential influence of color morph on blood metabolite concentration using ANCOVA. Since a slightly smaller subset of birds had color morph recorded, backwards selection was repeated. After testing for the influence of year, hours after sunrise, bleed time, and body condition, the triglyceride model retained hours after sunrise and body condition. After controlling for these factors, blood triglyceride was not significantly affected by color morph (color morph: $F_{1,62} = 0.16$, $P = 0.693$; hours after sunrise: $F_{1,62} = 3.16$, $P = 0.080$; body condition: $F_{1,62} = 9.83$, $P = 0.003$). I tested the same covariates against β -hydroxybutyrate and found that only Julian date was a significant covariate. After controlling for Julian date, color morph did not have a significant effect on β -hydroxybutyrate (color morph: $F_{1,25} = 0.72$, $P = 0.403$; Julian date $F_{1,25} = 2.94$, $P = 0.099$).

Body Condition:

Blood triglyceride concentration was positively and significantly associated with body condition (Figure 7; $\text{TRIG} = -1.844 + 0.08831 * [\text{body condition}]$; $S = 0.494$, $P < 0.001$, $r^2 = 21.2\%$). β -hydroxybutyrate was negatively and significantly associated with body condition (Figure 8; $\text{BUTY} = 0.1819 - 0.003233 * [\text{body condition}]$; $S = 0.023$, $P < 0.001$, $r^2 = 16.0\%$).

Blood Metabolite Relationship:

Despite the relationship between each blood metabolite and body condition, I did not find a significant relationship between the metabolites themselves. A linear regression analysis produced the equation ($\text{BUTY} = 0.04866 + 0.004628 * \text{TRIG}$; $S = 0.2516$, $P = 0.575$, $r^2 = 1.1\%$).

Discussion

Stopover Location and Blood Metabolites:

Average blood triglyceride concentrations, a measure of refueling rate and fat deposition, as well as overall body condition, were higher, but not statistically significantly so, in birds netted at the inland location than in those captured close to the lakeshore. My results approached significance ($P = 0.077$), but a larger sample size may be needed to clarify the relationship between stopover location and blood triglycerides. This trend is consistent with earlier data recorded for spring migrant White-throated Sparrow observations at BBBO and farther inland in Brockport, New York. An analysis of observations made between 1994 and 1999 revealed that birds banded in Brockport had an average body condition score of 41.8, while birds banded

at BBBO had an average score of 36.3 (C. J. Norment, unpublished data). These results imply that birds stopping over closer to the lake were not obtaining as much food as those farther away, which may be a result of physical ability, behavior, competition, habitat quality, or a combination of these factors.

A fall migration study, also based around BBBO and a comparison location, the Rochester Institute of Technology campus approximately 25 km south, found the opposite of my study: higher triglyceride concentrations and lower β -hydroxybutyrate concentrations in White-throated Sparrows at the lakeshore site than at the inland site (Smith *et al.* 2015). Since White-throated Sparrows are generalists, the difference between that study and the current one may be related to seasonal differences in available food types (fruits, grains, and arthropods) as well as total food availability in the fall. For songbirds in eastern and midwestern North America, food tends to be less abundant and overall conditions less predictable for spring migrants than fall migrants (Ewert and Hamas 1996).

In early spring, migrating birds may gather in dense groups near water to find emerging aquatic arthropods, but can spread out more in autumn when fruit is available (Ewert and Hamas 1996). Smith *et al.* (2007) found that chironomids (midges) and spiders were important foods for spring migrant warblers stopping over on the north shore of Lake Huron, and were more abundant along the lake than in inland habitats. Another spring study along the south shore of Lake Erie found that chironomid abundance was only weakly associated with refueling rate in White-throated Sparrows (MacDade *et al.* 2011), suggesting that other food sources are

probably important for this generalist species. Spring arthropod availability at Braddock Bay varies from year-to-year, with chironomid and culicid (mosquito) species most abundant in late May and early June (Jones 1997), and during this time flying insects are more abundant at the lakeshore than at locations 3-4 km directly inland (Jones 1997). White-throated Sparrows, however, migrate through in late April and early May, so they may be influenced by the distribution of other arthropod food types, or other food types in general.

Fruit availability is probably less important in the spring, since only scarce leftovers from the previous year will be present, but fall-based studies have shown that Braddock Bay has a greater diversity of fruiting shrubs, and larger overall fruit crop, than a banding site ~24 km inland (Smith *et al.* 2015). The White-throated Sparrow's generalist diet likely buffers it against shortages in individual food types, and yearly variation may influence distribution and stopover site use in a given season.

Between-site differences in refueling rate also may be a result of proximity to a migration barrier, where large concentrations of birds stopping over can increase competition for safe foraging habitat and food itself (Moore and Yong 1991). One would expect to see this effect around Lake Ontario, but the defined width of "lakeshore" habitat varies among studies (France *et al.* 2012). These concentrations seem to be especially noticeable within 3-4 km of the Lake Ontario shoreline (Agard 1994); Strobl (2010) found bird concentrations within 2 km of the shore, as well as areas 32-75 km inland, but also found high levels of bird abundance in wooded

habitat patches 2-32 km inland. Bird distribution in this mid-range area may be patchy and highly site-specific, and additional resource characterization would be needed to know exactly where Cobble Creek Farm fits into this range.

It is possible that the Cobble Creek Farm banding site and surrounding landscape are far enough inland, with resources spread widely enough, to reduce competition and allow birds that find these pockets of habitat to forage more efficiently. Birds in good condition may even begin their cross-lake flight from what is considered an “inland” site, skipping the lakeshore area entirely or stopping there only briefly. I suspect that this is the case during spring stopovers on the south shore of Lake Ontario. White-throated sparrows in good condition, with adequate fat stores, may not be staying to refuel at the lakeshore long enough to be trapped and banded. Those that need to rebuild fat stores may congregate around the barrier habitat, and even abundant food resources there may be subject to intense exploitation. Birds stopping at the lakeshore may build up just enough energy to make it over the lake, rather than accumulating the increased energy reserves that I and others have found in inland sparrows.

Competition may be the main driver of differences in refueling rate, but especially plentiful food sources may mask underlying differences linked to age, sex, or other internal variables. A quantitative study of food (fruit, grain, and arthropod) availability and other habitat characteristics at these stopover sites, such as in MacDade *et al.* (2011), and foraging behavior studies (as in Smith *et al.* 2007) would give us a better idea of what other factors are driving these differences. Blood

triglyceride concentration has proven to be a valuable indicator of food availability in these studies, and adding other blood metabolites might provide a clearer picture of an individual's activity while at a stopover site. Glycerol concentration, for instance, is another measure of fat deposition and metabolism (Guglielmo *et al.* 2005), and phospholipids can be compared to triglycerides as a measure of food quality (Guglielmo *et al.* 2005).

After analyzing triglycerides, I predicted that β -hydroxybutyrate concentrations also would differ between locations since the two typically have negative correlations (Guglielmo *et al.* 2005). I expected lakeshore birds to have higher β -hydroxybutyrate levels than inland birds, but did not find a significant or near-significant relationship between β -hydroxybutyrate and location. This may have been due to a small sample size for successful β -hydroxybutyrate determinations, because it was very difficult to produce a reliable standard curve due to differences among our β -hydroxybutyrate assay conditions. For future studies, it would be pertinent to run kinetic assays for much longer than 30 or 40 min, ensuring that the curve really does plateau its maximum level. Despite this, the metabolites did respond as expected to body condition.

In my study, birds with higher body condition scores tended to have higher blood triglyceride concentrations and lower β -hydroxybutyrate concentrations. Some individuals may have arrived at the sites in relatively good condition, and these birds may have been able to forage efficiently and continue on their way in a relatively short time. On the other hand, those in poor condition may have spent more time

resting, slowly foraging, or staying under deeper cover. Body condition during migration may be influenced not just by local stopover site quality, but also by wintering habitat (Bearhop *et al.* 2004), further complicating these relationships. The ability to follow individual birds to obtain longer-term trends of body condition would let us tease out more causality.

Color Morph:

The observation that color morph did not significantly influence triglyceride or β -hydroxybutyrate levels is interesting in comparison to other studies of White-throated Sparrow stopover ecology. Brown *et al.* (2014) found that in fall migrant White-throated Sparrows north of Lake Ontario, white-striped individuals refueled faster than tan-striped birds. A possible explanation for the difference between our study results is that the color morphs do not segregate temporally during the fall migration south (Brown *et al.* 2014), but are separated when coming north in the spring (Caldwell and Mills 2006). This segregation might mitigate competition for resources between color morphs, reducing differences in the refueling rate of spring migrants. I did find a statistically significant, three-day separation in average capture date of the morphs at BBBO, suggesting that this may be the case. Other studies have found differences in behavior and migration timing among conspecifics between spring and fall migrations (Tøttrup *et al.* 2011), reflecting the different environmental and evolutionary pressures facing these birds.

Additional Variables:

My results could be used as a starting point for landscape-level migration and stopover ecology research, on topics such as how frequently birds captured at one location travel between regional stopover patches or how they navigate there. BBBO has not recaptured birds banded at CCF during the same spring season, so birds from CCF likely stop over at a different lakeshore location, or may even begin their cross-lake flight directly from the farm. Refueling rate and body condition may also change with stopover duration, but I recaptured very few birds on multiple days at either location; intense banding effort or telemetry studies may be the only ways to analyze these factors and stopover duration with any confidence.

A few additional, observable variables that I was not able to measure may influence body condition, refueling rate, and behavioral processes. White-throated Sparrows are difficult to age accurately during spring migration, even by seasoned banders (Pyle 1997). Most of the birds banded in both locations were labeled “after hatch-year”, with only a few individuals (all at BBBO) confidently called the more specific “second year” or “after second year”. Although it is widely believed that older, more experienced birds are better able to find, or remember, quality stopover sites and migration routes, age was not a significant factor in blood metabolite levels in one study of several migrating warbler species (Seewagen *et al.* 2013).

Sex may be another influential variable due to its effects on behavior and physiology. Some White-throated Sparrows can be reliably sexed by wing chord measurement, with males at the extreme high end (>72 mm) and females at the

extreme lower end (<69 mm) (Pyle 1997), but my study included a large number of birds that fit in the middle, “unknown” area. Of the 96 birds captured, only 9 (9%) would be labeled female by this method, while 53 (55%) would be considered male, and 34 (35%) considered unknown. Males are generally the more aggressive sex; therefore they may be able to forage more efficiently, and are under greater pressure to continue migrating and arrive at nesting grounds earlier than conspecifics (Drent *et al.* 2003). Seewagen *et al.* (2013) found that in spring migrant warblers, males tended to have higher blood triglyceride and lower β -hydroxybutyrate concentrations than females. Upon arrival at the breeding grounds, females with larger fat stores may experience a reproductive advantage through increased egg mass and egg number (Smith and Moore 2003). For the White-throated Sparrow, analyzing interactions between sex and color morph, and the associated behavioral trends, would be especially interesting.

With multiple factors influencing avian behavior and physiology, it can be challenging to draw definitive conclusions about migratory bird habits and conservation needs. The White-throated Sparrow is both a reliable and fascinating subject for stopover ecology study, and has helped to shed some light on variables important to it and other species that require more protection. We can use interesting observations, such as finding noticeably heftier birds away from the lakeshore, and take advantage of ever-improving technology and techniques, to fill in more of the complicated picture of migratory ecology. Though there remains much to learn about individual birds and their movements near barriers during migration, this study adds

to a body of knowledge that can be used to compare sites, species, and seasons and hopes to provide an imperiled group of species with the best chance possible for survival.

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Tables and Figures

Table 1. White-throated Sparrow body measurements and blood metabolite concentrations taken from birds at Braddock Bay Bird Observatory (BBBO) and Cobble Creek Farm (CCF) during the 2013-2014 spring migration study periods. Blood triglyceride (TRIG) and β -hydroxybutyrate (BUTY) concentrations are measured in millimolarity (mM; mmol/L). Values are mean \pm SE, with *n* in parentheses. Values marked with * are significantly different between sites.

	BBBO	CCF	Total Average
Wing Chord (mm)	72.5 \pm 2.7 (74)	72.3 \pm 2.7 (22)	72.4 \pm 0.3 (96)
Tarsus Length (mm)	23.7 \pm 0.1 (75)*	24.6 \pm 0.2 (21)*	23.9 \pm 0.1 (96)
Mass (g)	26.8 \pm 0.2 (73)*	28.1 \pm 0.5 (21)*	27.0 \pm 0.2 (94)
Fat Score (0-5)	1.5 \pm 0.1 (75)	1.8 \pm 0.3 (21)	1.6 \pm 0.1 (96)
Body Condition (mass/wing x 100)	37.0 \pm 0.3 (72)*	38.8 \pm 0.7 (21)*	37.4 \pm 0.3 (93)
TRIG (mM)	1.361 \pm 0.063 (59)*	1.737 \pm 0.154 (18)*	1.449 \pm 0.063 (77)
BUTY (mM)	0.063 \pm 0.004 (23)	0.042 \pm 0.004 (11)	0.056 \pm 0.004 (34)

Table 2. White-throated Sparrow body measurements and blood triglyceride concentrations taken from birds during the 2013-2014 spring migration study periods. Groups are divided by plumage morph for comparison. Blood triglyceride (TRIG) and β -hydroxybutyrate (BUTY) concentrations are measured in millimolarity (mM; mmol/L). Values are mean \pm SE, with *n* in parentheses. There were no significant differences between the groups.

	White Stripe (WS)	Tan Stripe (TS)
Wing Chord (mm)	72.8 \pm 0.4 (49)	71.7 \pm 0.4 (36)
Tarsus Length (mm)	24.0 \pm 0.1 (48)	23.7 \pm 0.2 (37)
Mass (g)	27.0 \pm 0.3 (48)	27.2 \pm 0.4 (36)
Fat Score (0-5)	1.7 \pm 0.2 (48)	1.4 \pm 0.2 (37)
Body Condition (mass/wing x 100)	37.1 \pm 0.4 (48)	38.0 \pm 0.5 (35)
TRIG (mM)	1.417 \pm 0.092 (36)	1.555 \pm 0.091 (32)
BUTY (mM)	0.057 \pm 0.005 (17)	0.055 \pm 0.006 (11)

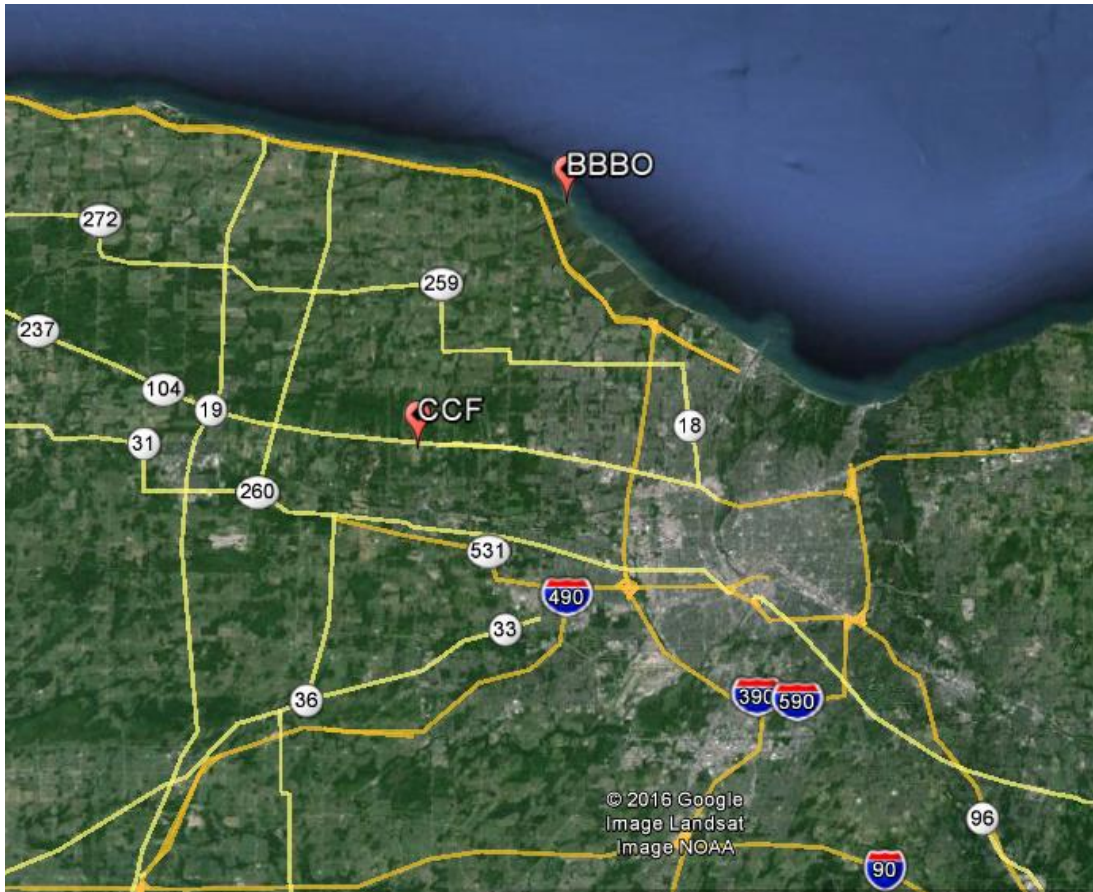


Figure 1. Relative locations of shoreline banding site (Braddock Bay Bird Observatory, BBBO) and inland site (Cobble Creek Farm, CCF) in Monroe County, New York.

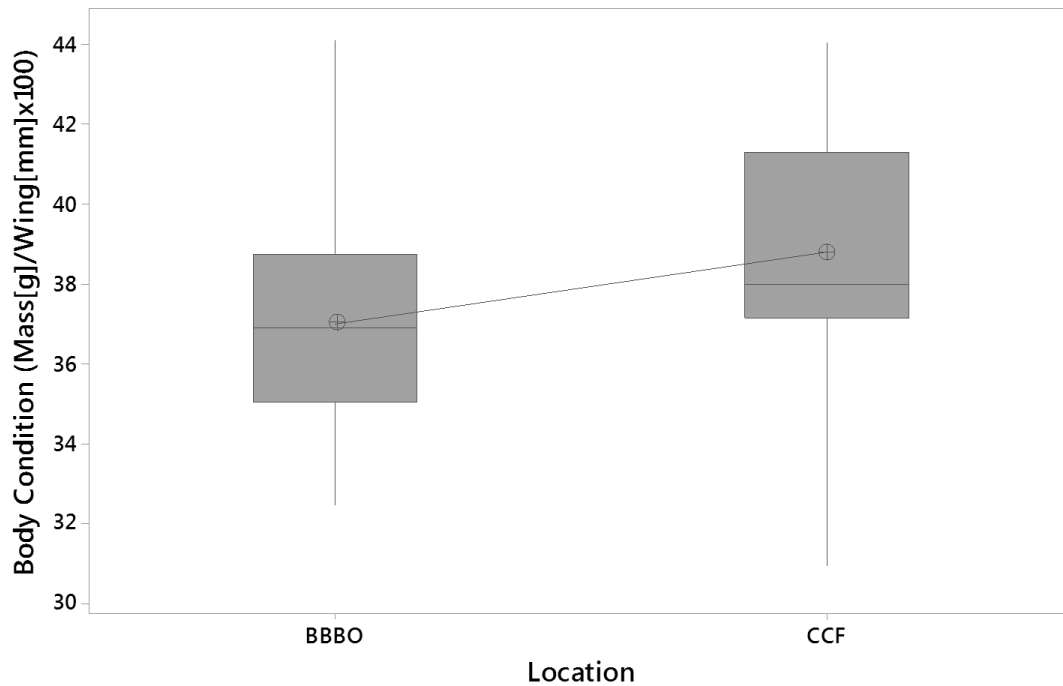


Figure 2. Boxplot comparing mean body condition (mass [g]/wing chord [mm] x100) between White-throated Sparrows caught at Braddock Bay Bird Observatory (BBBO) and Cobble Creek Farm (CCF) in the combined 2013 and 2014 spring migration seasons. Body condition was significantly higher in birds captured at CCF than at BBBO. The boxes contain the middle 50% of values, the horizontal lines represent the median value, and the lines extending from the top and bottom represent the highest and lowest 25% of values, respectively. The circles and connecting line represent and compare the mean values at each site.

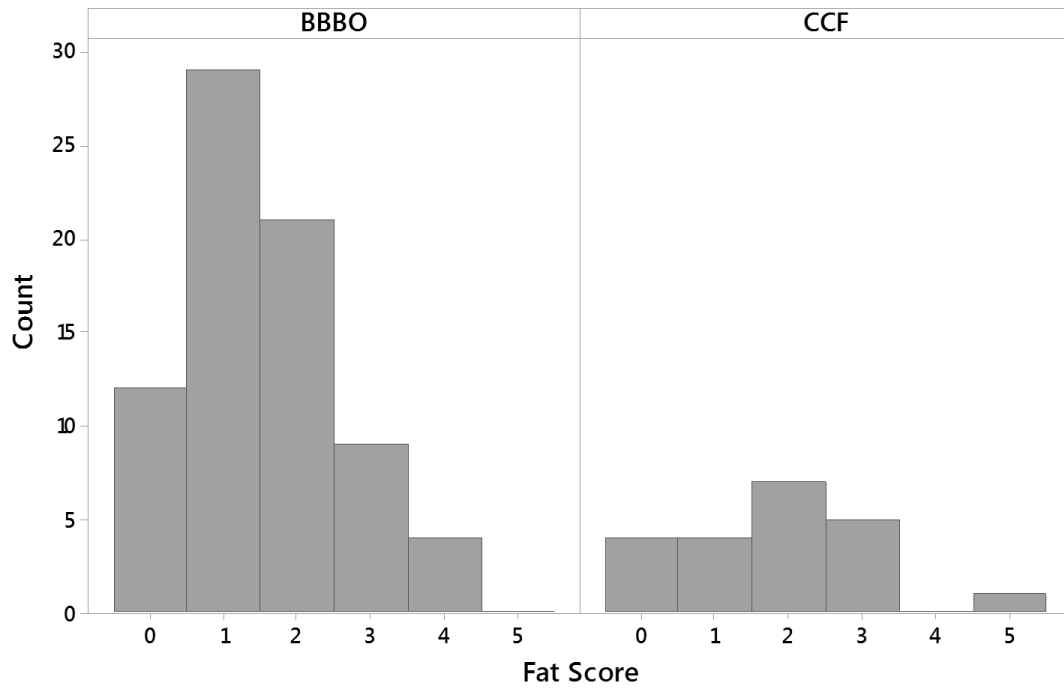


Figure 3. Fat score distribution of White-Throated Sparrows caught at Braddock Bay Bird Observatory (BBBO) and Cobble Creek Farm (CCF) in 2013 and 2014 spring migration seasons.

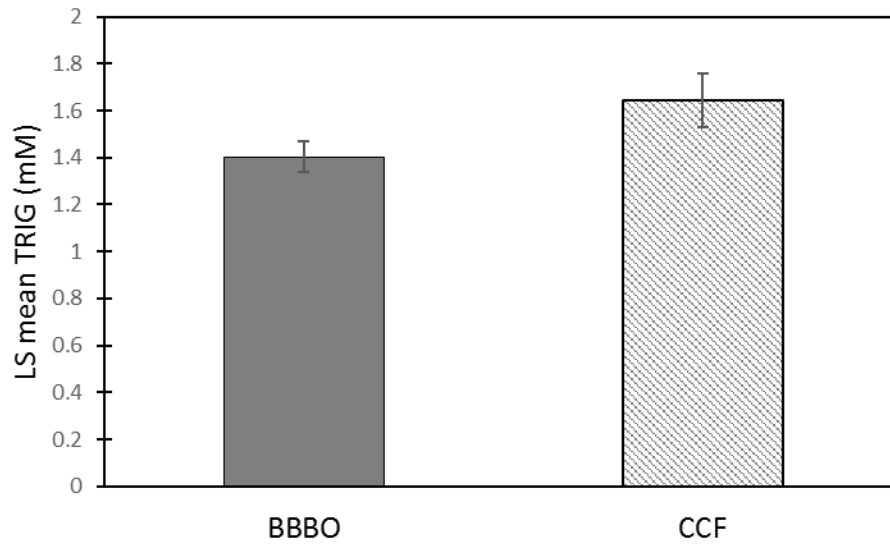


Figure 4. Bar graph comparing least squares mean (\pm SE) blood triglyceride concentration (TRIG, mM) between White-throated Sparrows caught at Braddock Bay Bird Observatory (BBBO) and Cobble Creek Farm (CCF) in 2013 and 2014 spring migration seasons, following ANCOVA (see Results). Values were not significantly different (see Results).

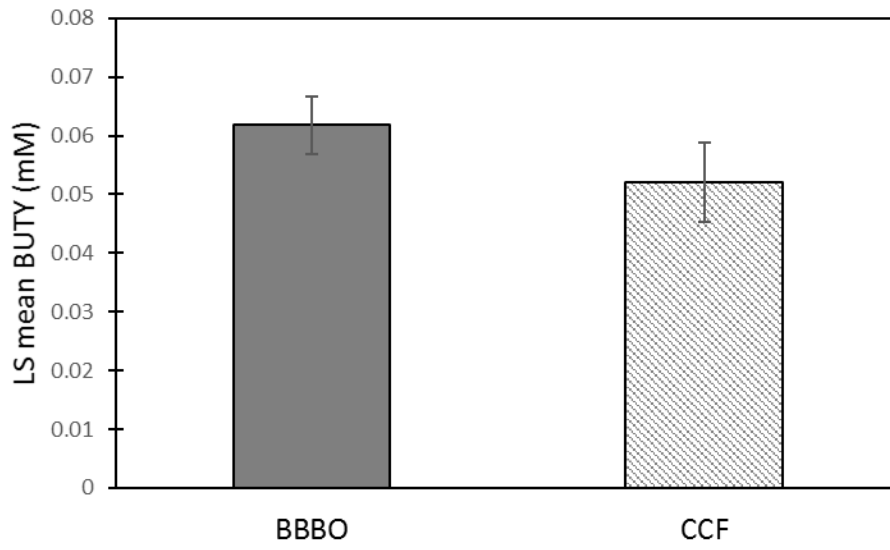


Figure 5. Bar graph comparing least squares mean (\pm SE) blood β -hydroxybutyrate concentration (BUTY, mM) between White-throated Sparrows caught at Braddock Bay Bird Observatory (BBBO) and Cobble Creek Farm (CCF) in 2013 and 2014 spring migration seasons, following ANCOVA. Values were not significantly different (see Results).

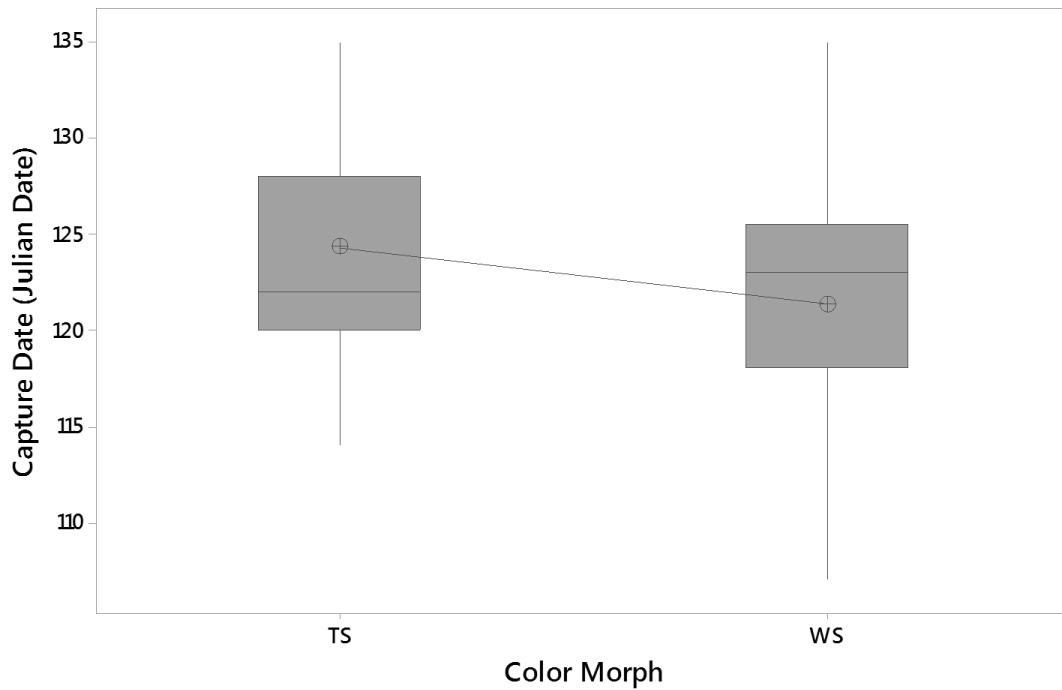


Figure 6. Boxplot comparing average capture date of White-throated Sparrow morphs (tan-striped, TS; white-striped, WS) at Braddock Bay Bird Observatory (BBBO) during the 2013 and 2014 spring migration seasons. Capture date was significantly earlier in WS birds than TS birds. The boxes contain the middle 50% of values, the center lines represent the median value, and the lines extending from the top and bottom represent the highest and lowest 25% of values, respectively. The circles and connecting line represent and compare the mean values at each site.

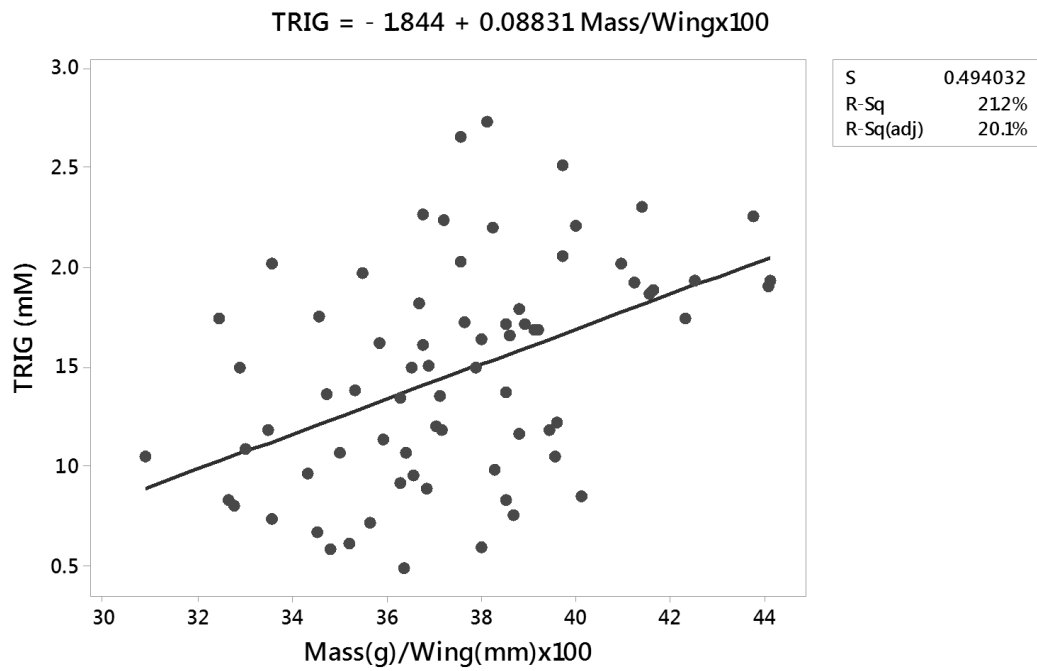


Figure 7. Regression line of blood triglyceride concentration (TRIG, mM) against body condition (mass [g]/wing[mm]x100; $P < 0.001$).

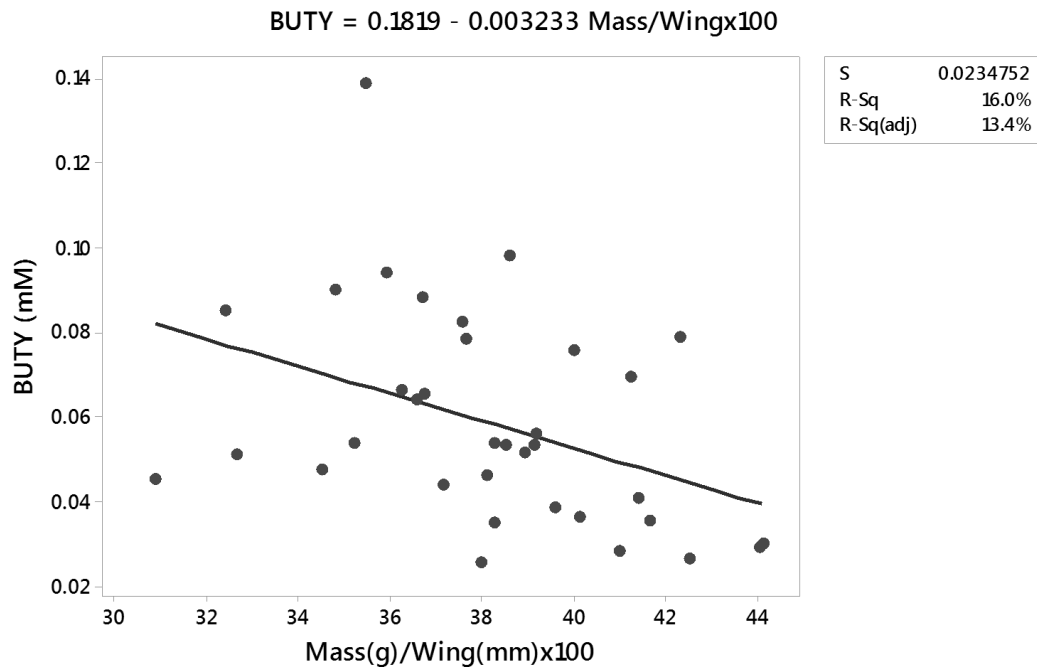


Figure 8. Regression line of blood β -hydroxybutyrate concentration (BUTY, mM) against body condition (mass [g]/wing[mm] $\times 100$; $P < 0.001$).