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**Seasonal Variation in Chronic Stress and Energetic Condition in Three Species of
Migratory Birds**

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July, 2015

**A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science in Environmental Science
at Rochester Institute of Technology
Rochester, NY 14623-5603**

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Abstract

Neotropical migrants face multiple challenges throughout their annual cycle. During migration, birds must avoid predation, face adverse weather, and find stopover sites with food adequate to support their needs. Upon return to the breeding grounds following migration, males must find and defend territory from other males. Females incubate eggs and both males and females may participate in feeding young leaving less time to feed themselves. After the breeding season birds undergo the energy intensive process of molt. Declines in populations of Neotropical migrants have been reported over the past several decades. In order to prevent further population declines of Neotropical migrants it is important to understand what stress and physiological changes take place between and within breeding, molt, and migration seasons in order to prioritize management of critical habitats and resources. In order to better understand these seasonal changes in stress and physiology, mass index, plasma triglyceride and uric acid concentrations were measured to determine physiological condition and the heterophil to lymphocyte ratio (H/L ratio) was determined as a measure of chronic stress in Gray Catbirds, Song Sparrows, and Yellow Warblers captured near the south shore of Lake Ontario in the spring, summer, and fall. Seasonal differences were found in plasma metabolite concentrations and H/L ratios in Gray Catbirds and Song Sparrows. These differences indicated that birds deposit more fat in spring and fall, and are more dependent on protein-rich food sources in spring and summer compared to fall. These results demonstrate that birds utilize different nutrients in different seasons and may require different habitats and food resources depending on the season. Plasma triglyceride concentrations were highest in Gray Catbirds and Song Sparrows in the fall while uric acid concentration was highest in spring and summer for all species. Gray Catbirds experienced the highest amount of chronic stress and parasite loads in the early spring when

compared with late spring, summer, and fall. Birds may be particularly vulnerable at this time of year to infection and require high quality stopover sites at which to refuel

Introduction

Neotropical migrants undergo a complex annual cycle. Neotropical migrants are birds which breed in North America before returning south to winter in the Caribbean, Mexico, Central America and South America (Hayes 1995). Many species of shorebirds, raptors, and songbirds are considered Neotropical migrants. Of the more than 700 species of birds that breed in North America, nearly half are Neotropical migrants (Cotton et al. 2009). Neotropical migrants spend the summer breeding season in North America and molt towards the end of summer before migrating back to their southern wintering grounds. The birds then return to North America in the spring to breed and the cycle repeats.

Declines in populations of Neotropical migrants have been reported over the past several decades (Rappole and McDonald 1994, Sauer and Droege 1992). Forest fragmentation of the breeding grounds and deforestation of wintering grounds have been put forth as the main causes of population decline (Robbins et al. 1989). As forests become more fragmented the proportion of edge to interior forest habitat increases, causing rates of brood parasitism and nest predation to rise, which can lead to lower rates of reproduction (Finch et al. 1991). In addition, as more birds are forced into smaller areas reproductive rates are negatively impacted. Black-throated Blue Warblers had lower fecundity in areas where the population was denser (Sillett and Holmes 2005). Migratory birds spend more time on the wintering grounds than the breeding grounds making these areas extremely important for Neotropical migrants. Habitat conversion has been occurring at greater rates on the wintering grounds than the breeding grounds removing the areas which Neotropical migrants spend the most time thereby negatively impacting survival rates (Sherry and Holmes 1996).

The migration period also represents a time of population decline for Neotropical migrants. For example, Black-throated Blue Warblers experience losses of 85% of adult birds

during migration, which is 15 times greater than mortality during stationary periods for this species (Sillett and Holmes 2002). Migratory birds can be limited by resource availability at stopover sites or extreme weather events (Newton 2006). Thus, suitable stopover sites where depleted fat reserves can be safely and rapidly replenished are vital for migrating birds (Moore et al. 1995).

Events which occur in previous seasons can also negatively impact survival in future seasons. For example, winter habitat quality affected the physical condition and spring departure date of American Redstarts and birds wintering in drier scrub habitat arrived later in the spring and were in poorer condition than birds which had wintered in better quality mangrove forest (Marra et al. 1998). Female American Redstarts which are forced by more dominant males into poorer scrub habitats had lower annual survival and raised fewer broods in the following summer as well (Marra and Holmes 2001, Norris et al. 2003). These types of studies emphasize the importance of understanding what stress and physiological changes take place between and within breeding, molt, and migration seasons in order to prioritize management of critical habitats and resources and prevent further population declines of Neotropical migrants.

The breeding season represents a large energetic cost for birds. Upon return to the breeding grounds following migration, males must find and defend territory from other males. Females incubate eggs and both males and females may participate in feeding young. Towards the end of the breeding season, feather molt begins in some species of Neotropical migrants. Molt is the periodic replacement of all or parts of the plumage (Murphy 1996). Some species may also delay molt until they have reached the wintering grounds. The process of molting is highly energy demanding and may increase energy expenditure by more than 30% (Cyr et al. 2008). Thus, fall migration will usually either follow or precede the molting period.

Migration occurs in response to seasonal changes such as day length (Ramenofsky and Wingfield 2007). Before beginning migration, birds will put on from 30-50% of their lean body mass as fat (Berthold 2001). This large amount of stored fat is required to successfully complete migration (Bairlein 2001). During migration, birds must avoid predation (Cimprich and Moore 1998), face adverse weather which limits foraging ability, cope with exposure to parasites, and find stopover sites with food adequate to support their needs while competing with other birds for limited resources (Newton 2006).

Stress and body condition play a role in a bird's ability to successfully meet these challenges and complete migration. Birds in better condition are able to maintain a more responsive immune system than those in poor condition and exhibit a greater ability to suppress stress responses (Kitayski et al. 1999). Thus, stress that affects the immune system of birds may be offset by better physiological condition. Stress is the state where stress hormones (corticosteroids) have become elevated above a defined threshold (Apanius 1998). Birds may experience both acute and chronic stress. Acute stress is caused by a sudden or short-term event such as a storm and chronic stress is due to a more predictable, long-term event such as changing energetic demands (Angelier and Chastel 2009).

There are several methods that can be used to measure stress levels in birds. Two of the more commonly used methods are to measure levels of the glucocorticoid, corticosterone, or to find the heterophil/lymphocyte ratio (H/L ratio). Heterophils and lymphocytes are types of leukocytes involved in immune response. Heterophils are the most common granulated leukocyte in the acute inflammatory response in birds (Harmon, 1998). In the lungs and air sacs, heterophils are the first line of defense against pathogens. Lymphocytes are another type of leukocyte that determines the specificity of the immune response to various pathogens.

Heterophils tend to increase in response to stress while lymphocytes decrease (Gross and Siegel 1983). H/L ratios may be ideal for studying the physiological state of wild birds for several reasons. Glucocorticoids, such as corticosterone, can increase in minutes after exposure to stress (Le Maho et al. 1992). Therefore blood must be taken shortly after capture to ensure that handling and capture stress do not affect corticosterone levels (Vleck et al. 2000). Unlike glucocorticoids, leukocyte numbers are more slow to change and can remain the same from 30 minutes to 20 hours after a stress event (Maxwell 1993). In addition, changes to the H/L ratio are less variable and multiple stress events can have an additive effect (McFarlane and Curtis 1989, McKee and Harrison 1995). Birds do not need to be bled immediately to get an accurate H/L ratio, making the H/L ratio ideal for use at a banding station (Milenkaya et al. 2013).

The H/L ratio may also be more useful than corticosterone for measuring long term stress. A study done on Adelie penguins compared the two measurements and found that H/L ratio was higher in injured penguins than in penguins with very recent injuries while corticosterone remained the same in both groups. H/L ratio also differed depending on the nesting stage. The ratio was lower in nesting birds than those that were courting or incubating. The researchers suggested that because of these changes in H/L ratio, this measurement may be a better indicator than corticosterone of stress associated with injury, reproductive cycles, or seasonal changes (Vleck et al. 2000). Corticosterone levels and the H/L ratio are also different as to what sort of stressors can cause a change. Corticosterone levels in Eurasian Kestrel nestlings changed only in response to lack of body fat and the presence of humans near the nest. The H/L ratio changed in response to more subtle stressors such as nestlings being later hatched, ectoparasite infestation, after weather events which prevented food delivery to the nest as well as lack of body fat (Muller et al. 2010).

Infection by parasites may affect a bird's overall condition. Blood parasites appear to be fairly prevalent in both migratory (Garvin et al. 2006) and breeding birds (Deviche et al. 2001). Avian blood parasites are obtained from blood-sucking arthropods such as mosquitoes (Noble et al. 1989) and common avian blood parasites include *Haemoproteus*, *Plasmodium*, *Leucocytozoon*, and *Trypanosoma* (Scheuerlein and Ricklefs 2004). Overall prevalence of parasite infection can vary drastically among bird species (Garvin et al. 2006, Young et al. 1993). A study measuring parasite prevalence in 54 species of Neotropical migrants during spring migration found that infection rate varied from 0-78% depending on the species (Garvin et al. 2006). The overall prevalence of blood parasites in avian populations may also be influenced by reproductive effort (Norris et al. 1994, Richner et al. 1995), age and sex, year or seasonal effects (Weatherhead and Bennett 1991, 1992, Forbes et al. 1994) and geographical distributions (Merilä et al. 1995). Male Great Tits with larger clutch sizes also had a higher prevalence of parasites than those with lower reproductive effort (Norris et al. 1994). Differences in local parasite vector populations between years and geographic location affect the prevalence of parasites in local bird populations. Highly disturbed areas were less capable of supporting parasite vectors resulting in a lower infection rate in area birds (Merilä et al. 1995).

Blood parasites can negatively affect body condition of birds (Valkiunas et al. 2006) and reproductive success (Marzal et al. 2005, Tomas et al. 2007). House Martins and Blue Tits treated with antimalarials had increased clutch sizes and fledging rates over birds not treated with the drugs. Chronic infections can also decrease foraging ability (Jenkins et al. 1963) and increase susceptibility to predation (Hudson et al. 1992). Infection by *Haemoproteus* may cause a decrease in the H:L ratio due to an increase in immune system activity, which may make chronic stress levels appear to be lower (Dunn et al. 2013). However, *Haemoproteus* infection also

reduces the ability of the immune system to respond to a secondary infection potentially lowering survival rates in infected birds (Norte et al. 2009).

Body condition has an effect on a bird's health, ability to complete migration, and reproductive success. One way to assess physiological condition is to measure the levels of plasma metabolites. The amount of certain plasma metabolites that are present can be used to determine refueling rates because the level of circulating metabolites reflects the metabolic state (Williams et al. 1999, Jenni-Eiermann and Jenni 1994). Some metabolites such as triglycerides, indicate fat deposition, while others like β -hydroxybutyrate, indicate fasting and both are related to short-term mass change (Jenni and Schilch 2001, Jenni-Eiermann et al. 2002). Triglyceride is the most abundant lipid in birds and is stored mainly in the adipose tissues (Stevens 1996). Dietary protein is obtained from what the bird is eating whereas endogenous protein is synthesized within the fed bird itself. The liver is the main site of lipid biosynthesis in birds and the rate of lipid biosynthesis is regulated through nutritional and hormonal controls. Triglycerides serve as the main energy reserves in birds and they are used by many tissues. During migration in birds the lipid reserves of the adipose tissue are mobilized and transported to muscular tissue (Stevens 1996). Plasma uric acid is usually indicative of dietary protein breakdown in foraging birds, but may also be elevated in fasting birds (Smith et al. 2007a).

Plasma metabolite profiling of triglycerides, β -hydroxybutyrate, and uric acid have been used to assess fuel deposition rates and habitat quality of stopover sites for migrating passerines (Guglielmo et al. 2005, Smith and McWilliams 2010). Migrants need high quality stopover sites in order to refuel after a strenuous flight. Fuel deposition rate is an important measurement as it can determine whether a bird is adequately prepared for the next stage of migration. Fuel deposition rate can also indicate site quality because birds at a high quality site will generally

have a higher rate of fat deposition than those at a poor site (Jenni and Schilch 2001, Dunn 2000). Using plasma metabolites, fuel deposition rate can be determined in birds that have been caught only once (Guglielmo et al. 2005). Plasma metabolite concentrations can also be used to determine the quality of a habitat for breeding birds as well (Owen et al. 2005). Habitats where birds have lower plasma metabolite concentrations are likely to be areas with fewer resources available for breeding birds.

Body mass and amount of body fat are other commonly used indicators of body condition in birds. Birds have greater amounts of subcutaneous fat in the furcular region as they build up fat deposits (Blem 1980). The fat in that area is given a score, with a higher score given to birds with more fat (Helms and Drury 1960). Birds with a greater score of fat are likely to be in better condition because they have the fuel necessary to continue migration. Fat scoring generally has a high correlation with body fat even in studies involving multiple species of bird (Labocha and Hayes 2012). Body mass is an easily measured indicator of condition and is also often strongly correlated with fat mass, in some cases explaining more than 50% of the variation in fat mass (Labocha and Hayes 2012). Body mass and wing length can be used to find a scaled mass index which is a good indicator of the relative size of energy reserves and compensates for birds that are unusually small or large for their species (Peig and Green 2009).

Both chronic stress and levels of circulating plasma metabolites change among seasons throughout a bird's annual cycle. The high energy requirements of reproduction can result in less energy available for immune system function (Norris and Evans 2000). During breeding, female Pied Flycatchers had low triglyceride, and high B-hydroxybutyrate and corticosterone (an indicator of stress) when raising young (Kern et al. 2005). Highly stressful conditions during the breeding season may also have prolonged effects during future breeding seasons. Higher H/L

ratios were observed in burrowing parrots in the breeding season after a La Niña event than during the event (Plischke et al. 2009). Other breeding factors such as clutch size also cause the H/L ratio to change. Female Tree Swallows with larger clutch sizes had higher H/L ratios than those with smaller clutch sizes. Inferior body condition in female Tree Swallows was also associated with elevated H/L ratios (Ochs and Dawson 2008). Higher H/L ratios during the breeding season may also have an effect on future survival. For example, Great Tits with low H/L ratios during the previous breeding season had a higher return rate than those which had higher H/L ratios the previous year (Kilgas et al. 2006).

Stress levels also tend to be lower and immune function higher during molt compared to the breeding season (Machado-Filho et al. 2010, Pap et al. 2010) and predation may have an effect on stress levels as well. Predation risk during non-migratory feeding caused birds to reduce the amount of time spent foraging and lowered body reserves which could cause an increase in stress levels due to lack of adequate nutrition (Bednekoff and Houston 1994).

Stress was found to be higher during migration than any other time of year in three species of thrush (Owen and Moore 2006). Stress during spring migration was higher than during fall migration. This anomaly was explained by the birds' recent flight over the Gulf of Mexico and the lack of fruit resources in the spring. Fruit is abundant in the fall and provides an easy resource for birds recovering at stopover sites (Parrish 1997). Food is less available in the spring, particularly early in the season when it may be too cold for many insects. Similar to thrush, the Lesser Elaenia experienced increased stress during migration compared to the breeding season (Machado-Filho et al. 2010). Stress levels may also be related to arrival date in the spring. Gray Catbirds which arrived earlier in the spring were found to have higher H/L ratios than those that

arrived at the breeding grounds later. Earlier arrivals were in better energetic condition than later arrivals but seemed to experience more stress (Hatch et al. 2009).

Breeding and molt can also have an effect on the ability of birds to successfully complete migration. Delayed breeding can cause a subsequent delay in molt. Late molting occurs at a faster rate than molt that takes place earlier in the summer resulting in feathers which are weaker and less resistant to wear or shorter than normal, and these resulting weaker feathers can then impact flight ability (Hall and Fransson 2003, Echeverry-Galvis and Hau 2013, Dawson et al. 2000). Delayed breeding can also result in reduced lean tissue mass and lower condition in the period before migration (Mitchell et al. 2012).

Plasma metabolite concentrations also vary between the breeding season, molt and migration. Feeding passerines were found to have higher triglyceride and free fatty-acid plasma concentrations during migration than during the breeding and molting periods, and migrating birds which had fasted overnight were also found to have lower β -hydroxybutyrate and uric acid concentrations than molting or breeding birds (Jenni-Eiermann and Jenni 1996). Similar to the breeding season, perceived predation risk may cause migrating birds to reduce body mass thereby losing valuable fuel needed for migration (Fransson and Weber 1997).

In addition to changes in plasma metabolite concentrations between seasons, metabolite concentrations can change daily within a season or even diurnally due to different factors. Time of day and fasting are important factors in explaining the concentrations of some plasma metabolites. For example, plasma triglycerides increase throughout the day, particularly in the morning in migrating birds (Jenni and Jenni-Eiermann 1996). Feeding rate can also have an effect on plasma metabolites. Pied Flycatchers show a more moderate increase in triglycerides after sunrise than other birds because their foraging activity is more evenly distributed over the

day rather than concentrated in the morning (Jenni and Jenni-Eiermann 1996). In addition, plasma concentrations of triglycerides are higher and β -hydroxybutrate is lower in birds feeding at a high rate than those feeding at a low rate (Zajac et al. 2006).

Other factors, including body mass, local weather conditions, and predation may also have an effect on plasma metabolite concentrations. Heavy birds have been found to have higher fattening rates than lighter birds which may lead to higher triglyceride concentrations (Schaub and Jenni 2001). Temperature may also affect circulating metabolite concentrations, though results of studies that have investigated the relationship between temperature variables and plasma metabolites have been mixed. For example, migrating passerines that are feeding on insects attain higher fattening rates at higher temperatures when insects are more active (Schaub and Jenni 2001). However, Garden Warblers, which feed mostly on fruit in autumn, do not show the same change in fattening rates due to temperature because fruit availability does not change with temperature. Predation may also play a part in the daily variation in metabolite levels. Blackcaps that detected an imminent risk of predation increased their food intake and fuel deposition rate during the first period of stopover, whereas those that did not fear predation did not show such a pattern (Fransson and Weber 1997). It follows that increased food intake would cause triglyceride concentrations to potentially be higher in birds exposed to higher predation levels compared to birds exposed to lower predation levels with lower food intake.

Stressful events such as breeding and migration can have repercussions that can continue even after the event has ended. For example, higher reproductive effort has been found to reduce immune system response in Pied Flycatchers (Ilmonen et al. 2003). Migration may cause lowered immune capacity as well if the bird is experiencing chronic stress (Owen and Moore 2006). However, few studies have tracked how stress levels change throughout the year in

Neotropical migrants and how seasonal changes in stress may be linked with physiological condition.

Purpose

Studies of stress and plasma metabolite concentrations in migratory birds over the migration and breeding seasons have rarely been performed (Owen and Moore 2006). This study differs from previous studies of this nature by using the same study location for all three seasons of the study and examining how parasite infection may affect chronic stress and body condition throughout the year. Examining how stress levels vary throughout the year can help in understanding how birds respond to major life events (migration, breeding). This study will investigate how stress in migratory passerines changes over the course of the spring, summer, and fall on the south shore of Lake Ontario and how this seasonal variation in stress may be linked with physiological condition.

Hypotheses and Predictions

Based upon the findings of Owen and Moore (2006), birds will likely exhibit higher stress levels during migration than during the breeding season. Higher stress levels could potentially be observed in spring due to lower food availability compared to fall (Owen and Moore 2006). Stress could also be higher in spring because birds may migrate earlier and faster in order to claim an optimal territory for breeding before others arrive. High levels of stress experienced during the spring could also leave birds open to infection by parasites, leading to higher prevalence of parasite infection during that season. As birds recover from the rigors of migration, stress levels are likely to drop in the summer. Triglyceride concentrations are likely to be high during both migration seasons due to the active fattening of birds to maintain large fat reserves that are not present during breeding, but uric acid concentrations may be higher in

spring compared to fall as migratory birds will be more reliant upon insects, a protein source, than fruit which is available in the fall. Finally, the concentrations of uric acid will likely be the highest in summer when birds rely upon insects as a food source for breeding requirements and to feed offspring, and thus have high protein requirements.

Methods

Study Site

This study was conducted at Braddock Bay Bird Observatory (hereafter BBBO, -: 43° 19'N, 77° 43'W) during the spring, summer, and fall of 2014. BBBO has been in operation since 1985 as a long-term passerine migration banding station and participates in the monitoring avian production and survival program (MAPS) during the summer. The observatory is situated on the south shore of Lake Ontario to the northwest of Rochester and is used by thousands of birds each season as a stopover site (Bonter et al. 2007). Lake shore habitats such as Braddock Bay were identified as being more heavily used by migratory birds than more inland habitats by the Lake Ontario Migratory Bird Stopover Project conducted by The Nature Conservancy. The vegetation is a mix of taller ash trees (*Fraxinus spp.*) and alder (*Alnus spp.*) with an understory of dogwood (*Cornus spp.*) and viburnum (*Viburnum spp.*). Certain dogwoods and viburnums present at the site supply high energy fruit for migrating birds in the fall (Smith et al. 2013). Midges were found to be plentiful at other lake shore habitats making it likely that they will be available to spring migrants at Braddock Bay as well (Smith et al. 2007b). A second study site was also used during the summer season. High Acres Nature Area (hereafter HANA, 43° 5'24.77"N, 77°22'39.48"W) is owned by the High Acres Landfill and Recycling Center in Perinton, NY and has been developed for wildlife habitat. The site includes several small ponds and both emergent and forested wetland areas. The overstory is composed mainly of American basswood (*Tilia americana*) and ash trees (*Fraxinus spp.*) with an understory of honeysuckle

(*Lonicera spp.*) and autumn olive (*Eleagnus umbellata*). Samples were taken at the MAPS station that has been run there since June 2011.

Gray Catbirds (*Dumetella carolinensis*), Song Sparrows (*Melospiza melodia*), and Yellow Warblers (*Dendroica petechial*) are present at both study sites in all three seasons that were focused on in this study and were selected for this reason. The three focal species in the study also represent different migration distances, breeding strategies, and diet preferences. Song Sparrows winter in the southern United States while Gray Catbirds winter farther south in Florida and Central America. Some Song Sparrows may remain in New York year-round. Yellow Warblers travel the farthest south wintering in Central America and into the northern part of South America. Yellow Warblers migrate south in late July-early August while the other two study species leave later in the fall (Poole 2005). Yellow Warblers raise 1-2 clutches in a summer while Gray Catbirds raise 2-3 and Song Sparrows may have up to 7 broods in a summer (Poole 2005). Gray Catbirds and Song Sparrows will eat both insects and fruits while Yellow Warblers are primarily insectivorous.

Capture and Timing

Birds were caught in mist nets following standard operating procedure at BBBO (<http://braddockbaybirdobservatory.files.wordpress.com/2012/03/protocol.pdf>). Birds were captured during spring (late April – early June), summer (mid-June – early August) and fall (late August – late October). In the spring and fall banding takes place daily. In the summer, MAPS stations are run once every ten days at each site. On each banding day, birds are captured beginning at sunrise and ending after six hours. Immediately following capture birds were bled by brachial vein puncture and 100 μ L of blood was taken for analysis. Blood samples were taken starting two hours after sunrise to ensure that birds had been foraging. In addition to collecting

blood samples, age and sex was determined when possible according to Pyle (1997), the amount of fat was rated on a six (Helms and Drury 1960) or seven point scale (MAPS manual 2014), wing length and body mass were recorded for each bird. Birds were aged by looking for molt limits in the feathers. Molt limits indicated a younger bird (either hatch year or second year) while lack of molt limits usually indicated an older bird (after second year). The bird was typically aged after hatch year if no specific age could be determined. The capture time was recorded as the hours past sunrise of capture of the bird and bleed time was recorded as the number of minutes between capture and collection of the blood sample. During the summer the molt score, cloacal protuberance, and brood patch extent were recorded following MAPS protocol.

Heterophil/Lymphocyte Ratio

The H/L ratio was used as a measure of chronic stress in this study. In order to find the H/L ratio, a drop of blood from each sample collected from captured birds as described above was placed on a slide and a smear was made, and then stained with Wright-Geisma solution (Hema-3 manual staining system, Fisher Scientific; Owen 2011). The H/L ratio was determined by counting heterophils and lymphocytes using a compound microscope at 1000x power until the combined total leukocytes equal 100 (Vleck et al. 2000). Blood smears were also examined for the presence of parasites and the types of parasites on each slide were noted. Slides were counted twice to ensure that the ratio obtained from the initial count was accurate. Counts were accepted as accurate if the coefficient of variation between the two counts was less than ten and then an average of the two counts was used in the final H/L ratio.

Plasma Metabolites

Plasma metabolite concentrations of triglycerides and uric acid were used as a measure of energetic and nutritional condition in this study. Plasma collected from individuals of the focal species as described above was analyzed for triglyceride concentration using modified colorimetric and kinetic microplate assays following Guglielmo et al. (2005) and Smith and McWilliams (2010) for uric acid concentrations. Plasma was diluted 1:3 with 0.9% saline and run in duplicate wells for each assay. Measurements were repeated until the coefficient of variation was $\leq 10\%$ for replicate wells. Three triglyceride measurements could not be brought within 10% variation even after the samples had been run four times. These three wells were under 15% coefficient of variation and were retained in the study.

Statistical/Data Analyses

Chronic stress levels and plasma metabolite concentrations in all three species were compared across seasons. H/L ratio did not meet assumptions of normality in this study and were logit transformed ($\ln(\text{H/L ratio}/(1-\text{H/L ratio}))$). Triglyceride concentrations were also not normal and were log transformed ($\text{Log}(\text{triglyceride}) + 1$). These transformations normalized both variables. Body mass was scaled for wing length in each target species to compensate for birds which were above or below average size for the species using a scaled-mass model to obtain mass index (Peig and Green 2009). General linear models with a backward selection were used to determine which confounding variables may cause variation in metabolite concentrations (uric acid and triglyceride) and H/L ratios. Variables with p-values < 0.1 were retained in the models and were considered significant covariates. Models were generated separately by species for each analysis. Variables used for Gray Catbirds included capture day of year, season (spring, summer, and fall), site (BBBO or HANA), age (Second Year or After Second Year), mass index, bleed time and capture time. Only season was retained by any of the models for Gray Catbirds,

thus comparisons were made between seasons using ANOVA tests followed by a Tukey's HSD test.

Variables included for plasma metabolites and the H/L ratio model for Song Sparrows were slightly different with age being excluded from the model (most were aged After Hatch Year), and only summer and fall seasons being used due to lack of samples in the spring. No variables were retained in the backward selection procedure and so ANOVA tests were then used to compare uric acid concentrations and the H/L ratio between the two seasons. Variables retained in the model for triglyceride were capture time, bleed time, and season; however, there was heterogeneity of regression relationships for bleed time. Therefore, we corrected for bleed time by using residuals of linear regression of bleed time and transformed triglyceride values. These corrected triglyceride values were then used in an ANCOVA with capture time as a covariate to compare triglyceride concentrations between seasons.

The model for Yellow Warblers focused upon within season differences for summer in metabolite concentrations due to a lack of plasma samples in spring and fall. Variables in this model included age (Second Year and After Second Year), sex, site (BBBO and HANA), capture day of year, bleed time, and capture time. Season was included in the model for H/L ratio as an adequate number of blood smears were made in both spring and summer. ANCOVA was then used with the retained variables, (capture day of year, age, bleed time, and capture time), entered as covariates.

Comparisons were also made in plasma metabolite concentrations and H/L ratios within seasons using ANOVA tests for all species. Migration seasons were divided into an "early" and "late" period by choosing points where there was an increase in the frequency of capture rate of a species for Gray Catbirds and Song Sparrows. Capture rates tended to be lower early in the

season than later. Summer was similarly split into an early and late period for Yellow Warblers only because this was the only species that began molting on the breeding grounds, allowing the effect of molt on chronic stress and body condition to be examined.

Seasonal variation in the prevalence of parasites for each target species was investigated using ANOVA to compare the number of individual birds with parasites present in blood smears between the seasons. Spring, summer, and fall were compared for Gray Catbirds while summer and fall were compared for Song Sparrows. ANOVA was also used to test whether the presence of parasites was related to H/L ratio or plasma triglyceride concentration. For ANOVA analysis birds were grouped into either having parasites or not having parasites.

Results

Blood samples were collected from 57 Gray Catbirds and 11 Yellow Warblers in the spring, 32 Gray Catbirds, 29 Song Sparrows, and 11 Yellow Warblers in summer, and 31 Gray Catbirds and 13 Song Sparrows during the fall. Four Song Sparrows and four Gray Catbirds were sampled on more than one occasion during the summer. The data from each capture was included in the analysis because it represented different points in the season and birds that were bled more than once were bled at least ten days apart. If the birds had parasites, the bird was only included once in the overall number of birds having parasites. Sample sizes within a species for plasma metabolites vary depending on the amount of plasma available for tests (Tables 1, 2, 3).

Backward selection retained only season for triglycerides, uric acid and H/L ratio in Gray Catbirds. Seasonal differences were found to be significant for triglycerides ($F_{2,89} = 12.148$, $P = <0.0001$), uric acid, and H/L ratio. Follow-up Tukey tests revealed that fall migrating Gray Catbirds had higher plasma triglyceride concentrations than those capture during the breeding season or spring migration (Figure 1A). Uric acid concentration was significantly different across seasons ($F_{2,89} = 42.4$, $P = <0.0001$) and was found by follow-up Tukey tests to be

significantly lower during fall migration as compared to spring or summer but similar during spring migration and the breeding season (Figure 1B). Stress levels also differed by season ($F_{2,112} = 6.59$, $P = 0.002$) and were highest during spring migration and lowest during fall migration as indicated by follow-up Tukey tests (Figure 1C). Fall and spring migration were also divided into an “early” and “late” period and metabolites and H/L ratio was compared within the seasons (Table 4). The only variable with any significant difference between early and late periods for Gray Catbirds were spring H/L ratios ($F_{1,51} = 6.8327$, $P = 0.0118$), birds had higher stress levels early in the spring than later in the season.

Bleed time, capture time, and season were retained in the backward selection models for triglycerides for Song Sparrows. Season was the only variable retained for uric acid and no variables were retained for H/L ratio. Plasma triglyceride concentration was not significantly different among seasons after correcting for the covariates ($F_{1,38} = 1.01$, $P = 0.32$; Figure 2A). Bleed time ($F_{1,38}=4.906$, $P=0.033$) and capture time ($F_{1,38} = 3.98$, $P = 0.054$) were positively related to triglyceride concentration. Uric acid concentration was significantly higher during the breeding season than fall migration ($F_{1,40} = 8.06$, $P = 0.007$; Figure 2B). The H/L ratio did not differ significantly between the summer and fall seasons ($F_{2,32} = 1.335$, $P = 0.256$; Figure 2C) . When the fall migration season was split into early and late season there were no significant differences between early and late fall season for H/L ratio ($F_{2,8} = 0.258$, $P = 0.625$; Table 4) , plasma triglyceride ($F_{2,11} = 0.014$, $P = 0.91$; Table 4) or uric acid concentration ($F_{2,11} = 0.234$, $P = 0.64$; Table 4).

For Yellow Warblers, capture day of year and bleed time were retained in the backward selection models for both plasma metabolites for the summer season analyses, and age was also retained for uric acid. Capture day of year, season, and capture time were retained for H/L ratio.

Triglyceride concentration did not differ significantly by capture day of year when bleed time was accounted for using ANCOVA ($F_{5,4} = 2.89$, $P = 0.206$)(Figure 3A). Uric acid also did not differ significantly by either capture day of year ($F_{5,4} = 1.64$, $P = 0.42$) or age ($F_{5,4} = 3.48$, $P = 0.135$)(Figure 3B,C) when bleed time was corrected for. Triglyceride concentration was negatively related to bleed time ($F_{5,4} = 9.0325$, $P = 0.057$) and uric acid was positively related to bleed time ($F_{5,4} = 3.47$, $P = 0.204$). Stress levels also did not have a significant seasonal difference when capture time was controlled for ($F_{13,7} = 1.55$, $P = 0.31$); Figure 3D). The H/L ratio was positively related to capture time ($F_{13,7} = 2.07$, $P = 0.167$) There were also no differences in H/L ratios when early and late summer periods were compared ($F_{2,7} = 0$, $P = 0.99$; Table 4).

Parasites were detected in 39 Gray Catbirds and 6 Song Sparrows, but there were not parasites detected in Yellow Warblers (Table 5). The parasites found on the blood smears were either *Haemoproteus* spp. or microfilaria (Figure 5). The most common infection was by *Haemoproteus* with microfilaria being present mainly in the summer (Table 5).

Spring had a significantly higher parasite infection rate than either summer or fall in Gray Catbirds ($F = 10.25$, $P = <0.0001$; Figure 4). Song Sparrows had a higher prevalence of parasites during the summer than the fall ($F = 4.005$, $P = 0.053$; Figure 4). H/L ratio was not significantly different in Gray Catbirds with or without parasites ($F = 0.549$, $P = 0.46$) or Song Sparrows ($F = 1.55$, $P = 0.221$).

Discussion

Overview

Few studies have looked at seasonal changes in stress and plasma metabolite concentrations in migratory birds. Each season presents distinct challenges making differences in physiological condition and chronic stress between seasons likely. Plasma metabolite concentrations reflect changes in diet and the metabolic state of birds (Guglielmo et al. 2002), both of which can affect chronic stress. In this study of passerines near the south shore of Lake Ontario, seasonal differences were found in plasma metabolite concentrations indicative of energy metabolism and H/L ratio, an indicator of chronic stress, in some of the study species. In general, triglyceride concentration was highest in fall, uric acid concentration was highest in summer, and chronic stress was highest in the spring. Birds were least stressed during fall migration which coincided with high plasma triglyceride concentrations. Parasite infection was greatest in spring migration for Gray Catbirds and summer for Song Sparrows.

Between Season Variation

Plasma metabolite concentrations were found to have significant differences between seasons in some of the study species. Plasma triglyceride concentrations were found to be highest in the fall and lowest in the summer for both Song Sparrows and Gray Catbirds. These results are consistent with expected patterns of fattening and feeding in terms of the annual cycle. Migrating birds need to build and retain fat reserves in order to fuel long flights and can do this by increasing triglyceride concentrations (Berthold 2001). During the breeding season the extra weight of retained fat can instead become a liability, leaving birds more open to predation

(Kullberg et al, 1996). Breeding birds consume a lower fat diet in the summer when retained fat is no longer necessary, which then lowers triglyceride concentrations (Bairlein 2001).

Spring triglyceride concentrations were slightly lower than those in fall for Gray Catbirds. The type of food available during the different migration seasons may help explain this pattern. Many types of high fat content fruit are available for birds to feed upon during fall migration at the study site (Smith, et al. 2013). The high fat content of the fruit could cause migratory birds to have higher triglyceride concentrations in the fall than in the spring when no fruit is available resulting in higher fattening rates in the fall (Bairlein 2001).

Uric acid concentration showed a significant seasonal difference in both Song Sparrows and Gray Catbirds. Uric acid concentrations were higher in the summer than during the fall in both species, suggesting that birds were consuming more protein in the summer. Spring uric acid concentrations were similar to those of summer for Gray Catbirds. Food availability may explain this seasonal pattern as well because birds switch to a higher protein insect diet in the spring and summer (Bairlein 2002). The protein content of insects is typically higher than that of fruits which causes an increase in plasma uric acid concentrations in birds feeding on insects (Izhaki and Safriel 1989). Breeding females may also need more protein in the summer to meet the requirements of egg production (Meijer and Drent 1999).

Chronic stress levels of birds displayed seasonal variation as indicated by H/L ratios. H/L ratios were significantly higher in the spring compared to summer or fall in Gray Catbirds. There was a trend toward higher H/L ratios in the spring versus summer in Yellow Warblers, though not significantly so. The low chronic stress levels during fall migration in both Gray Catbirds and Song Sparrows is in contrast to a study of thrushes where H/L ratios were found to be higher

during both migration seasons compared to breeding season (Owen and Moore 2006). Breeding Gray Catbirds and Song Sparrows may experience greater chronic stress than Wood Thrush, Veery, or Swainson's Thrush due to differences in life history. Gray Catbirds raise 2-3 broods and Song Sparrows can raise up to 7 each season, whereas the previously listed Thrush species may raise only one brood a summer (Poole, 2005) and this added breeding effort may have contributed to the higher stress levels in the former species.

Chronic stress may have been elevated in the spring compared to the fall for multiple reasons. The breeding range of Gray Catbirds and Song Sparrows extends only a little farther north than the north shore of Lake Ontario creating a relatively short flight to the study site. Thus, the birds in this study may not have been very far into their migration route when they reached the study site. Birds were predicted to have slightly elevated H/L ratios in the fall due to the flight across Lake Ontario, but this may not have been a large obstacle for the birds compared to the longer flight over the Gulf of Mexico (Diehl et al. 2003).

Changes in diet lipid concentration, food availability, and body condition can cause an increase in chronic stress response. For example, food-restricted Red-Legged Kittiwake chicks had chronically elevated baseline stress and the effects were exacerbated in those chicks which were also on a low lipid diet (Kitaysky et al. 2001). The lower quantity of food available in the spring may have played a part in elevated H/L ratios in the spring. Fruit is plentiful at BBBO in the fall, while few insects may be present in the cool climate of early spring at inland sites putting greater stress on early arrivals by restricting food resources (Smith et al. 2007b).

The number of birds infected with parasites varied significantly across seasons. More than half of the Gray Catbirds sampled in the spring were found to have parasites while less than

a quarter of those sampled in the summer were infected. The percentage of Gray Catbirds infected with parasites was high compared to another study which sampled many species of Neotropical migrants for parasites (Garvin et al. 2006). Samples were taken from birds in that study on the northern coast of the Gulf of Mexico. Birds arriving on their breeding grounds have been reported to have a high prevalence of blood parasites potentially due to the stress of migration (Deviche et al. 2001). Birds sampled in this study were likely reaching the end of their migration route and would therefore have a higher prevalence of parasites due to parasite exposure during their journey north as compared to birds sampled early in migration that had less exposure to parasites. Birds were also more stressed in the spring which may be an indicator of immunosuppression leaving birds more open to parasite infection (Owen and Moore 2006).

Parasite infection was low in Gray Catbirds (Garvin et al. 2003) and Song Sparrows compared to some studies and high compared to others which focused on different species (Deviche et al, 2001, Kirkpatrick et al, 1988). In these studies, the percentage of birds infected by parasites on the breeding grounds varied greatly across species and locations involved in the studies. In this study, the low level of infection in the fall for Song Sparrows and Gray Catbirds agrees with parasite levels found in Reed and Sedge Warblers sampled during fall migration (Wojczulanis-Jakubas et al. 2012). Parasite infections are likely to become latent or cleared away by the time birds initiate fall migration causing fewer birds to be infected (Garvin et al. 2006).

Within Season Variation

No significant variation was found in plasma metabolite concentrations between early and late migration in Gray Catbirds or Song Sparrows. However, H/L ratio showed within-season variation in Gray Catbirds in the spring and birds arriving early in the spring had

significantly higher H/L ratios than those which arrived later in the season. This agrees with findings in another study of spring migrating Gray Catbirds where H/L ratios were also higher in birds which arrived earlier in the season (Hatch et al. 2009). Birds which arrive early in the spring tend to be those which are in better condition however, the poor environmental conditions and low food availability in early spring may place more stress on the birds (Kokko 1999, Smith and Moore 2005).

Parasite infection within the spring and summer did not show any effect on H/L ratio or plasma triglyceride concentrations. Other studies have found that the presence of parasites caused the H/L ratio to rise in Reed and Sedge Warblers or fall in Yellowhammers (Wojczulanis-Jakubas et al. 2012, Dunn et al. 2013). If the presence of parasites increased the H/L ratio in infected birds during spring migration, the difference may not have been detectable if the birds already had elevated H/L ratios due to the energetic pressures of migration. The sample size of birds infected with parasites in the summer was low, which may also have limited the statistical tests and contributed to the insignificant results. Parasite infection did not seem to be related to the fat loads of birds during spring migration as both infected and non-infected birds in spring had very low fat scores (close to a fat score of one on the six point scale).

Plasma metabolite concentrations within the breeding season may be affected by age of the bird, nesting, incubation, and molt. Studies done on captive Red-legged Partridges indicate that age can have an effect on concentrations of plasma metabolites, and plasma uric acid concentrations were higher in older Red-legged partridges than younger ones (Dobado-Berrios et al. 1998). Another study conducted on six species of Neotropical landbird migrants found that hatch year Swainson's Thrushes, White-eyed Vireos, and American Redstarts carried less fat than adult birds during fall migration (Woodrey and Moore 1997). Plasma metabolite

concentrations can also vary across different time periods during the breeding season. Nesting birds may experience different stressors than those defending territories or incubating eggs and plasma triglyceride concentrations of Pied Flycatchers have been shown to steadily decrease as the breeding cycle progresses whereas uric acid concentrations were higher earlier in the summer (Kern et al. 2005). Molt at the end of summer or during fall migration could also result in within-season differences in plasma metabolite concentrations (Machado-Filho et al. 2010). Though the effects of age and date on plasma metabolite concentrations in breeding Yellow Warblers were not significant (likely due to small sample sizes of birds sampled), plasma uric acid concentration reached a peak at the beginning of July and became lower through the rest of the summer and plasma triglyceride concentrations became higher (Figure 3). Yellow Warblers begin migration in late July- early August and triglyceride concentrations should rise as birds put on fat for migration.

Conclusions

This study provides additional support for a seasonal shift in chronic stress and energy metabolism in some Neotropical migrants. Plasma triglyceride and uric acid concentrations can vary based on migration status and the type of food available during migration, and spring migrants appear to experience higher levels of chronic stress than fall migrants or breeding birds near the south shore of Lake Ontario. At this location, fall migration could be less stressful due to the quantity of food available or the slower pace at which it occurs. In addition, the prevalence of parasites was high in the spring which could be a reflection of the high stress levels experienced by birds at that time.

Examining changes in chronic stress and energy metabolism across seasons can help increase our understanding of how birds cope with the challenges of migration and the breeding season. Recent population declines in Neotropical migrants makes an understanding of how chronic stress and energy metabolism vary throughout the year vital in determining what elements are important for both migrating and breeding birds and when birds may be most vulnerable (Moore et al. 1995). Comparing seasonal changes in energy metabolism and chronic stress could also help pinpoint potentially negative carry-over effects of migration into the breeding season and vice-versa (Machado-Filho et al. 2010) and help to further identify times and locations where birds may be particularly at risk. In addition, examining blood metabolite concentrations in different seasons can show how birds utilize nutrients in different seasons and therefore may inform managers about particular habitat types that may be most useful at different times of year (Kern et al. 2005). Investigating seasonal changes in the physiology of migratory birds is also important for identifying specific times of the year when birds may be in poorer body condition and have higher chronic stress, and will therefore have a greater need for high-quality habitats at that provide adequate food resources. The results of this study indicate that the early spring is a time of high parasite prevalence and greater chronic stress for migratory birds. Lake shore sites provide midges, an important food source for migratory birds in early spring, making these sites vital for chronically stressed migrants. Further studies are needed to determine if early spring is a time of high chronic stress for other migratory bird species at other locations.

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Tables

Table 1. Age ratios (SY-second year, ASY-after second year), mass index, bleed time, plasma metabolite concentrations, and H/L ratio of Gray Catbirds captured in each season. Values for all except age ratios are mean \pm standard error, - and sample sizes are in parentheses.

Parameter	Spring	Summer	Fall
Age Ratio (sy/asy)	39/10	23/3	
Mass Index	35.43 \pm 0.327 (56)	36.15 \pm 0.915 (31)	38.72 \pm 0.992 (29)
Bleed Time (minutes)	17.5	17	15.2
Plasma Triglyceride (mM)	0.802 \pm 0.061 (38)	0.527 \pm 0.085 (23)	1.25 \pm 0.142 (31)
Plasma Uric Acid (mM)	0.865 \pm 0.034 (38)	0.884 \pm 0.071 (23)	0.381 \pm 0.032 (31)
H/L Ratio	0.359 \pm 0.037 (53)	0.239 \pm 0.036 (32)	0.189 \pm 0.023 (30)

Table 2. Mass index, bleed time, plasma metabolite concentrations, and H/L ratio of Song Sparrows captured in each season. Values for all except age ratios are mean \pm standard error, - and sample sizes are in parentheses.

Parameter	Summer	Fall
Mass Index	21.12 \pm 0.249 (28)	21.33 \pm 0.397 (12)
Bleed Time (minutes)	12.9	15.4
Plasma Triglyceride (mM)	0.805 \pm 0.105 (27)	1.103 \pm 0.115 (13)
Plasma Uric Acid (mM)	1.15 \pm 0.091 (29)	0.725 \pm 0.095 (13)
H/L Ratio	0.33 \pm 0.053 (27)	0.196 \pm 0.036 (10)

Table 3. Age ratios (SY-second year, ASY-after second year), mass index, bleed time, plasma metabolite concentrations, and H/L ratio of Yellow Warblers captured in each season. Values for all except age ratios are mean \pm standard error, - and sample sizes are in parentheses.

Parameter	Spring	Summer
Age Ratio (SY/ASY)	6/4	4/5
Mass Index	9.85 \pm 0.247 (11)	10.09 \pm 0.313 (10)
Bleed Time (minutes)	16.5	18.8
Plasma Triglyceride (mM)		0.845 \pm 0.115 (10)
Plasma Uric Acid (mM)		1.65 \pm 0.208 (9)
H/L Ratio	0.283 \pm 0.066 (11)	0.145 \pm 0.052 (10)

Table 4. Average values (+SE) of plasma metabolite concentrations and H/L ratio in the early and late parts of spring, fall, and summer. Sample size is in parentheses.

Within Season	Parameter	Gray Catbird		Song Sparrow	Yellow Warbler
		Spring	Fall	Fall	Summer
Early	Triglyceride (mM)	0.976 ± 0.163 (11)	1.107 ± 0.204 (13)	1.117 ± 0.249 (5)	0.795 ± 0.079 (4)
Late		0.731 ± 0.051 (27)	1.346 ± 0.197 (18)	1.095 ± 0.119 (8)	0.879 ± 0.192 (6)
Early	Uric Acid (mM)	0.835 ± 0.059 (12)	0.407 ± 0.046 (13)	0.785 ± 0.131 (5)	1.583 ± 0.414 (4)
Late		0.879 ± 0.042 (26)	0.362 ± 0.043 (18)	0.688 ± 0.136 (8)	1.713 ± 0.227 (5)
Early	H/L ratio	0.493 ± 0.072 (16)	0.233 ± 0.042 (12)	0.217 ± 0.054 (5)	0.166 ± 0.097 (5)
Late		0.302 ± 0.038 (40)	0.16 ± 0.025 (18)	0.175 ± 0.041 (5)	0.133 ± 0.051 (5)

Table 5. Parasite prevalence in blood smears of captured birds by species and season

Species	<i>Haemoproteus</i>	<i>Microfilaria</i>	Both
Gray Catbird			
Spring	28	0	2
Summer	6	1	0
Fall	2	0	0
Song Sparrow			
Spring			
Summer	2	2	1
Fall	1		

Figures

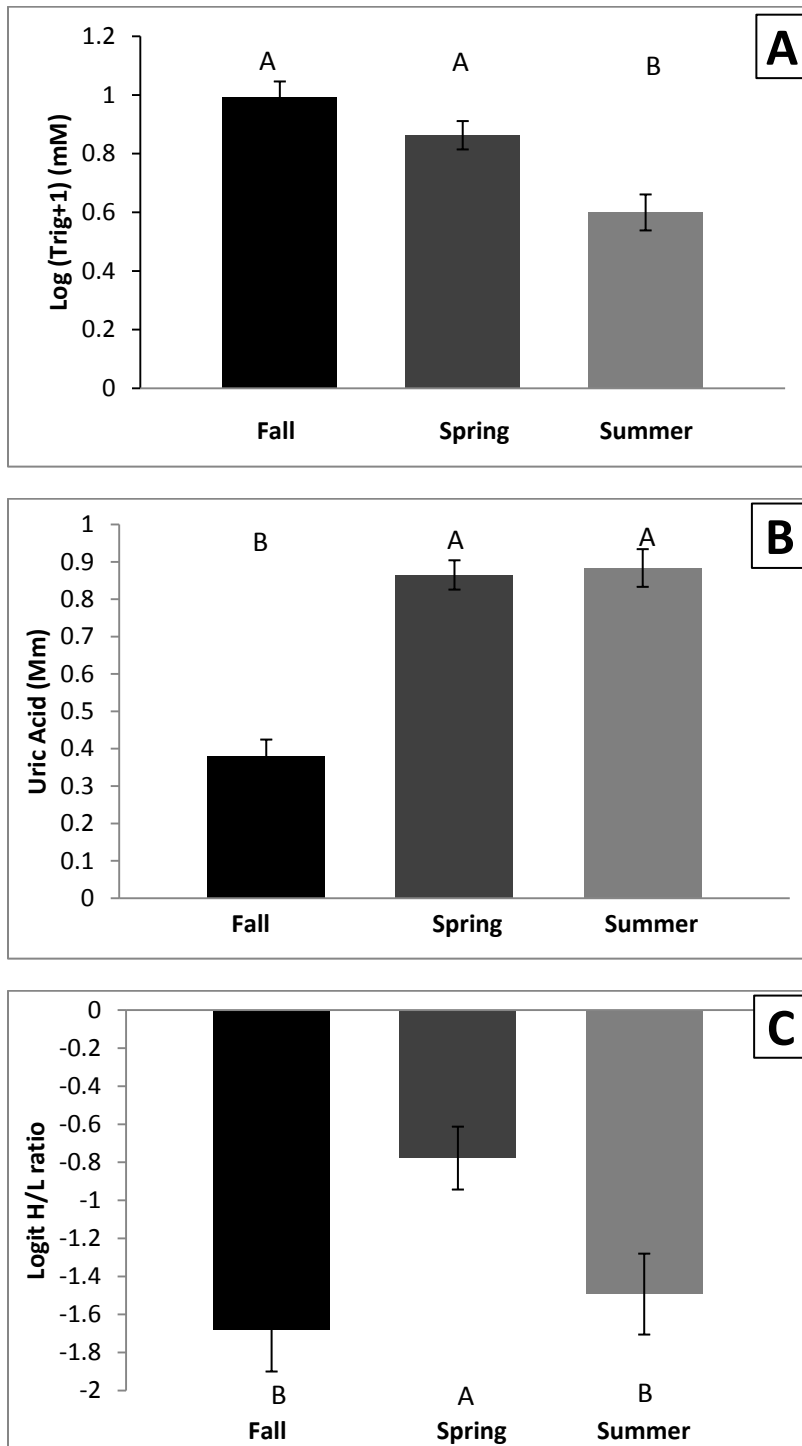


Figure 1. Mean log-transformed triglyceride concentrations (A), uric acid concentrations (B), and Logit-transformed H/L ratios (C) for Gray Catbirds in spring, summer, and fall (+SE). Bars that do not share a common letter are significantly different.

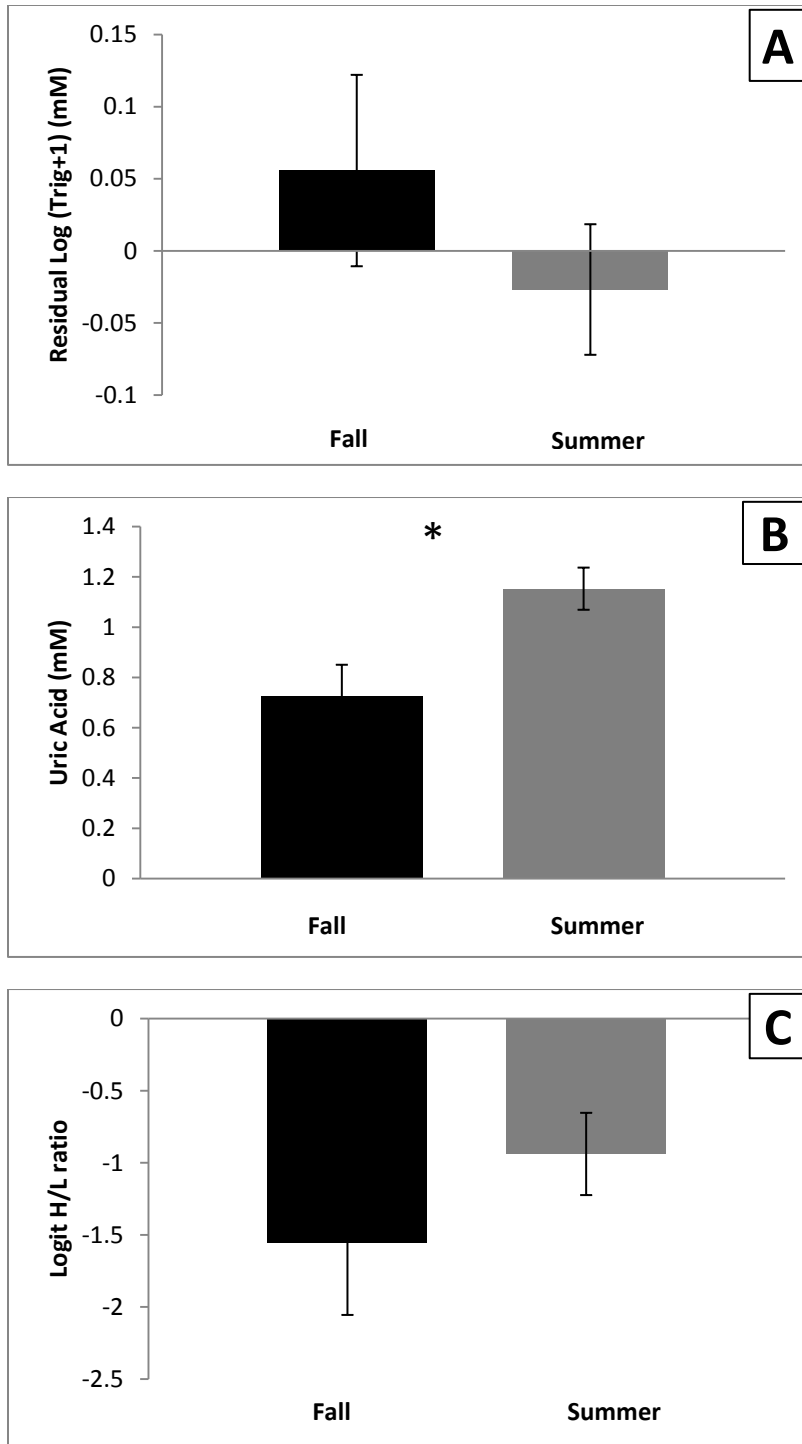


Figure 2. (A) Least mean square of residual log-transformed triglyceride concentration (+SE) (B) mean uric acid concentration, and (C) logit-transformed H/L ratio (+SE) for Song Sparrows. Bars marked with an asterisk (*) are significantly different.

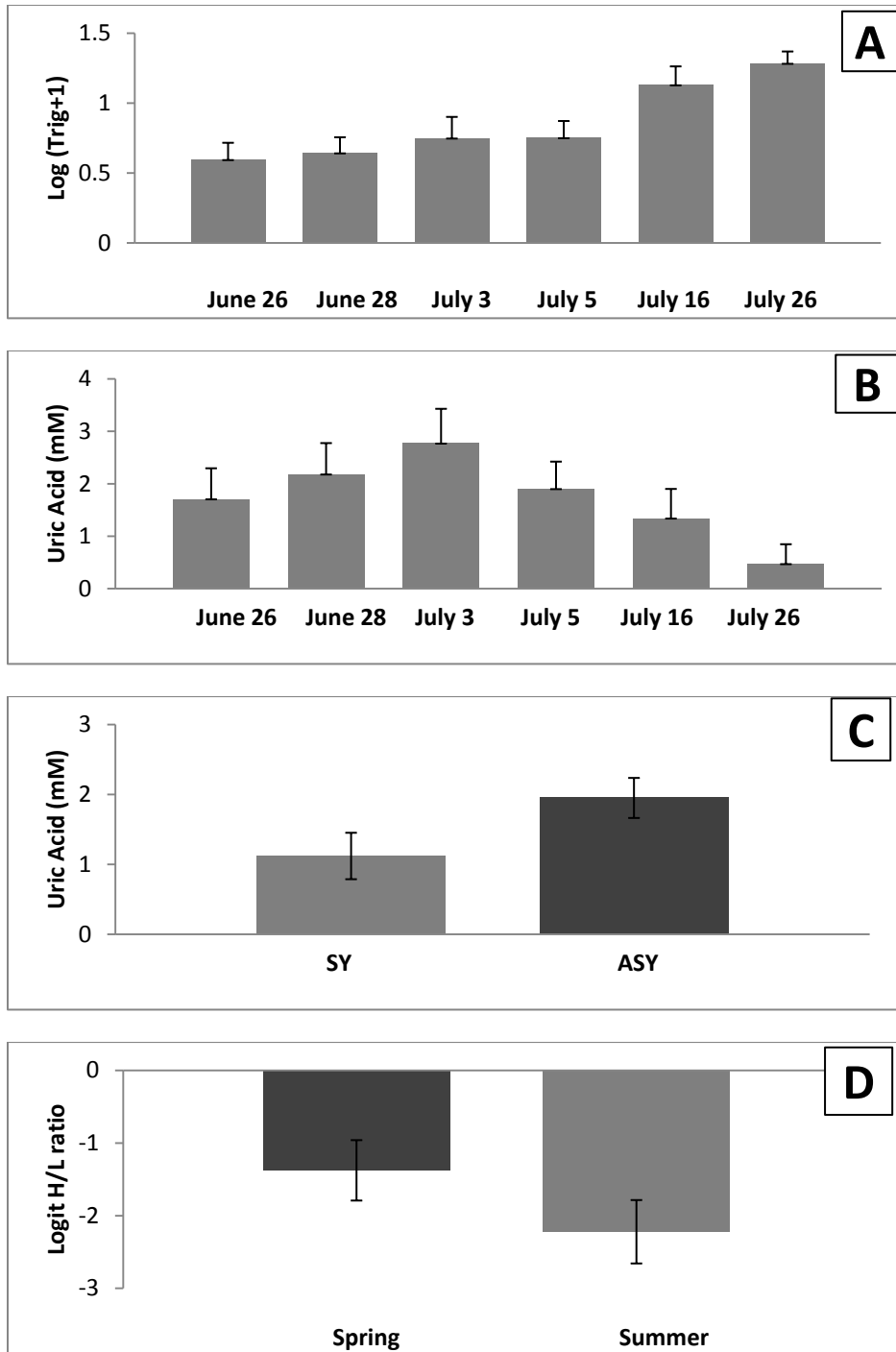


Figure 3. Within season analysis of plasma metabolites for Yellow Warblers (A) Least squares mean (+SE) of log triglyceride and (B) uric acid concentration by capture date, (C) least squares mean of uric acid concentration by age (+SE), and (D) least squares mean of logit H/L ratio by season (+SE)

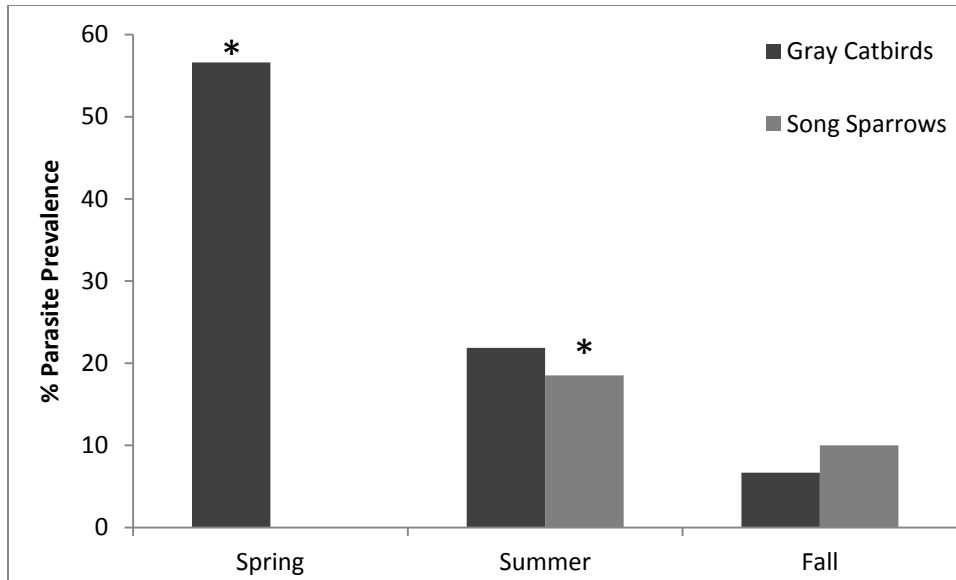


Figure 4. Percentage of Gray Catbirds and Song Sparrows with parasites by species and season, bars marked (*) are significantly different for that species

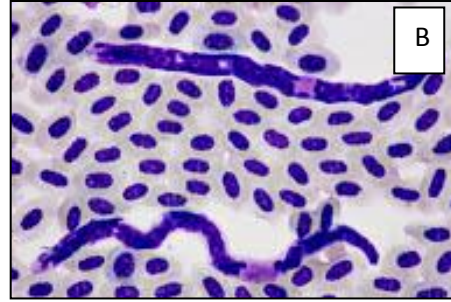
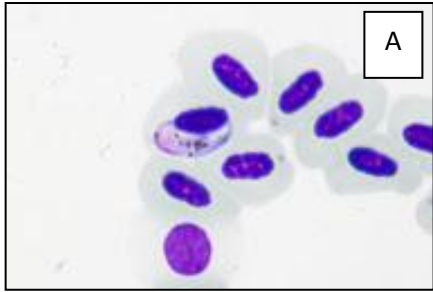


Figure 5. Parasite genera present in Song Sparrows and Gray Catbirds: (A) *Haemoproteus*, (B) *Microfilaria*