

How Important Are Hemoparasites to Migratory Songbirds? Evaluating Physiological Measures and Infection Status in Three Neotropical Migrants during Stopover

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Accepted 5/11/2014; Electronically Published 8/26/2014

Online enhancements: appendix tables.

ABSTRACT

Long-distance migrations are energetically expensive for many animals, including migratory songbirds. During these demanding journeys, birds likely face limitations in allocating resources to different physiological functions, including lipid reserves needed to fuel the migration and costly immune defense against pathogens. We sampled three species of long-distance migratory songbirds during their fall migration through coastal Georgia and quantified their body condition, subcutaneous fat reserves, and infection status with blood parasites (*Hemoproteus* and *Plasmodium*). We also quantified cellular immunity, on the basis of total and differential white blood cell counts, and estimated individual stress levels, using the heterophil : lymphocyte (H : L) ratio. We tested whether birds infected with blood parasites had decreased fat measures, poorer body condition, or increased stress levels (as reflected by H : L ratios). We also examined relationships between immune cell profiles and the following variables: body condition, subcutaneous fat, infection status, age, and species. Infected birds did not show greater H : L ratios, poorer body condition, or lower fat measures, but in one species infected individuals showed significantly elevated leukocyte counts. Although we found little evidence for negative relationships between immune cell counts and body condition or fat measures, as might reflect underlying trade-offs in resource allocation, our results concerning hemoparasites are consistent with past work and suggest that chronic hemoparasite infections might have minimal effects on the outcome of long-distance migratory flight.

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Introduction

Long-distance migration is strenuous and can require immense energy expenditures (Wilcove 2008). Migratory birds travel upward of 10,000 km round-trip and must acquire and store energy to fuel this flight (Blem 1990; Alerstam et al. 2003), with some birds investing up to 50% of their lean body mass in lipid reserves to prepare for migrations (Dingle 1996). These energetic demands can cause organs to atrophy, and birds may reduce investment in functions that are not necessary for flight (Piersma 1998; Møller and Szép 2011), leading some physiological systems, such as immune defense, to suffer as a result (Owen and Moore 2006). Indeed, a large body of past work showed that immune defense itself can be energetically expensive to maintain and that these defenses can trade off against other activities, such as reproduction (Demas et al. 2012). In support of the idea that migration might lower the resource pool available for immune defense, several studies showed that adult songbirds (warblers and thrushes) had lower baseline measures of several components of innate immunity (including leukocyte counts) during migration than during the breeding season (Owen and Moore 2006; Jakubas et al. 2013). In captive experiments, Owen and Moore (2008b) showed that cell-mediated immunity declined with the onset of migratory restlessness, suggesting that changes in immunity coincide with preparation for long-distance flight. Yet more work is needed to establish whether these negative relationships between migratory flight and immune defense occur across a broad range of migratory species (Buehler et al. 2010; Møller and Szép 2011).

Many animals, while migrating, harbor parasite infections (reviewed in Altizer et al. 2011) that can have negative consequences for migratory success. Parasites are known to be costly for wild animals and can lower survival, slow development, reduce reproduction, and alter behaviors important for fitness (Wobeser 2006). In terms of impacts on migration success, monarch butterflies (*Danaus plexippus*) experimentally infected with a protozoan parasite flew shorter distances and at reduced flight speeds (Bradley and Altizer 2005), and parasite prevalence decreased as wild monarchs moved southward during their fall migration (Bartel et al. 2011), consistent with the idea that infected animals migrate less well than healthy ones. Work on Bewick's swans (*Cygnus columbianus bewickii*) showed that infection by low-pathogenic avian influenza viruses delayed

the onset of migration and reduced travel distances (van Gils et al. 2007). In addition to causing direct damage to host tissues, parasites could also have indirect costs for migrating animals in the form of elevated immune defense (Hawley and Altizer 2011; Møller and Szép 2011). Thus, infectious agents could negatively affect migration success, both through direct damage and by causing animals to invest in costly defenses.

Although migration is physically demanding, much of what is known about competing energetic demands in the context of parasitism and immunity in birds comes from studies conducted during the breeding season. Like migration, the breeding season is a period of increased physical demand due to reproduction and parental activity. While it is unknown which season has the greater energetic demand, information can be drawn from the breeding season to develop hypotheses about the costs of energetic demands during migration. Past research on birds shows that increased work associated with breeding tends to decrease immune defense. For example, cellular immunity in barn swallows (*Hirundo rustica*) declined in response to experimentally increased brood size (Pap and Markus 2003). When brood size was experimentally increased in great tits (*Parus major*), the numbers of lymphocytes and total white blood cells decreased (Hörak 1998). Zebra finches (*Taeniopygia guttata*) with experimentally increased brood size similarly showed suppressed antibody production (Deerenberg et al. 1997), although they recovered some responsiveness when given a fortified diet. Collectively, evidence supports the idea that increased physical demand has negative physiological implications for immune functions, leading to the possibility that migratory hosts might suffer high fitness costs when long-distance flight is combined with parasite infection.

Neotropical migrant songbirds are a model system to explore potential trade-offs between infection, immunity, and energy reserves during long-distance migration. This is, in part, owing to a high level of understanding of songbird ecology and the nature of their long-distance migration. These birds typically breed in the Nearctic zone during the spring and summer, and they winter in the New World tropics. To complete this long-distance flight, most birds stop frequently along their migration paths to rest and replenish fat reserves, which are highly visible in subcutaneous deposits. In this study, we captured Neotropical migrant songbirds at a stopover site in coastal Georgia to investigate the physiological profiles (age, heterophil: lymphocyte [H:L] ratios [stress], fat measures, body condition) associated with infection by blood parasites and to test for relationships between innate immune defense (based on numbers of circulating leukocytes) and energetic reserves (fat deposits). Specifically, we predicted that (1) infection with blood parasites would be associated with decreased fat reserves, poorer body condition, and increased stress levels; (2) immune cell numbers would associate negatively with fat reserves and either positively or negatively with body condition, depending on energetic supply and demand (i.e., if demands are high, there might be limited resources to invest in both activities); and (3) immune cell numbers would be higher among infected individuals. Our broader goal was to advance scientific understanding of the

physiological processes associated with infection that are crucial to migration success.

Material and Methods

Study Location

This study was conducted at the Jekyll Island Banding Station, located in coastal Georgia (fig. 1), where mist netting and bird banding have occurred every October, coinciding with the timing of fall migration, since 1978. While a large number of songbird species are typically captured each year at this site, in this study we specifically focused on three Neotropical migrants—gray catbirds *Dumetella carolinensis*, common yellowthroats *Geothlypis trichas*, and western palm warblers *Dendroica palmarum palmarum*—because of their high abundance during the time of our sampling. Birds were sampled during a 2-wk period from October 7 to October 21, 2012. At this banding station there were 18 mist nets (Avinet, Dryden, NY) located in areas with little or no overhead tree cover. Nets were open from 20 min before sunrise until 12:00 (Eastern Daylight Time) each day and checked at least every 20 min. All captured birds were placed in individual paper or cloth bags and were carried to a central banding facility. Sampling procedures were approved by the University of Georgia (animal use protocol A2011 08-006).

Data Collection

For the purposes of this project, all birds were classified as hatch year (HY) or after hatch year (AHY), following Pyle (1997). Sex determination was possible only for common yellowthroats, because of similar plumage of the sexes for the other species (Pyle 1997). Birds were weighed to the nearest 0.01 g with a standard electronic scale, and their wing chord (wing length) was measured to the nearest millimeter with a wing ruler. The level of subcutaneous fat in the furculum of each bird was visually scored on a six-point scale (0–5), with a score of 0 denoting no fat present and 5 denoting the highest fat load (fat bulging from furculum), following Helms and Drury (1960). We collected a blood sample (no more than 50 μ L) from the brachial vein, using a 26-1/2-gauge needle and a microcapillary tube. A thin blood film was made immediately, and the remaining blood was frozen at -20°C for polymerase chain reaction (PCR) detection of hemoparasites (below).

Leukocyte Profiles

Dried blood smears were stained with a Giemsa-Wright (Sigma-Aldrich, St. Louis, MO) stain (Owen 2011) and examined with a compound microscope at 1,000 \times magnification. Leukocytes were identified as heterophils, lymphocytes, eosinophils, basophils, or monocytes (table A2; tables A1–A3 available online). At least 100 white blood cells (WBCs) were counted per slide. Alternatively, in cases where WBCs were infrequent, 150 microscope fields were assessed (Dunn et al. 2013; Campbell 1988). Only fields of view with even monolayers of erythrocytes



Figure 1. Map showing the location of the site where migratory birds were captured (Jekyll Island, GA, 31.0°N, -81.4°W; star) in relation to the overall distance of the migratory journey for Neotropical migrants. The dotted line represents southern limit of estimated breeding range, and the dashed lines represent the estimated wintering range of common yellowthroats, gray catbirds, and western palm warblers (Cimprich and Moore 1995; Wilson 1996; Guzy and Ritchison 1999). A color version of this figure is available online.

were included during the counting procedure. The final WBC count was then divided by the total number of fields of view, which served as an estimate of immune cell abundance (total WBCs). Full descriptions and functions of each leukocyte type are provided in Davis et al. (2008) and Clark et al. (2009). Briefly, lymphocytes are the most common WBC type in passerines (60%–73% of WBCs, depending on species) and are differentiated into B-cells and T-cells, which are morphologically indistinguishable and are involved in the adaptive immune response and immune memory. Heterophils are phagocytic cells that are the next-most-common WBC type in songbirds (7%–20% of WBCs; Davis 2009). Monocytes are generally rare, are the largest WBC type, and are also phagocytic. Eosinophils are involved in defense against multicellular parasites, and basophils are important for the inflammation response (although their precise function is unclear). Collectively, lymphocytes and heterophils make up 80% of passerine WBCs (Davis et al. 2008). In our study, 98.4% of the cells were made up of lymphocytes, heterophils, and basophils (table A2).

From the counts of heterophils and lymphocytes, we calculated H : L ratios as a hematological index of stress (Davis et al. 2008). This measure has been shown to scale positively

with plasma levels of corticosterone (Davis et al. 2008), the primary vertebrate stress hormone. Unlike plasma corticosterone, which can increase within 2 min of acute stress associated with animal capture (Romero and Romero 2002), prior research has shown that H : L ratios in songbirds increase more slowly and remain at baseline levels during routine handling for up to 1 h after capture (Davis et al. 2008).

Determining Blood Parasite Infection

Nested PCR was used to identify infection status with hemoparasites (*Hemoproteus* and *Plasmodium*). This method has been shown to be more sensitive than blood smear analysis for parasites (Waldenström et al. 2004; Bradley 2009), with the potential to detect one infected erythrocyte per 10,000 cells. While PCR is more sensitive, it detects both active and chronic infections. Here, a chronic infection would mean that the bird currently has parasitemia for avian malaria but no clinical signs of infection. Generally, birds do not show any clinical signs of avian malaria infection. Methods and conditions for determining the presence of avian malarial parasites were adapted from the protocol developed by Waldenström et al. (2004).

This protocol amplifies a 520-bp segment of the mitochondrial cytochrome-b gene. After DNA extraction from whole red blood cells with the Qiagen DNEasy blood and tissue kit (Qiagen, Valencia, CA), we conducted a nested PCR procedure. For the first round of PCR, primers HAEMF (5'-ATGGTGCTTTCGATATATGCATG-3') and HAEMR2 (5'-GCATTATCTGGATGTGATAATGGT-3') were used. All samples were run in 27- μ L volumes with 12.5 μ L of GoTaq Green Master Mix (M7122, Promega), 8.5 of μ L deionized water, 1 μ L of each primer, and 4 μ L of the extracted DNA (Waldenström et al. 2004 call for only 2 μ L of extracted DNA). The second round of PCR used the primers HAEMNF (5'-CATATATTAAGAGAAATATGGAG-3') and HAEMNR2 (5'-AGAGGTGTAGCATATCTATCTAC-3'). Nested reactions were run with the same total volumes, with 4 μ L of the PCR product from round 1. Each set of PCR reactions was run in duplicate, with positive and negative controls, and results were visualized by separation on a 2% agarose gel using GelRed (Biorad, Hayward, CA) under ultraviolet light. Positive controls were obtained from Bradley (2009), and deionized water was used in place of extracted DNA for the negative control. Bands in the 520-bp region were considered positive for parasites.

In addition, each blood smear was examined with light microscopy for the presence of blood parasites. This was completed by the same methods as outlined above and at 1,000 \times magnification. Each of the fields of view (the same number as for the leukocyte profiles outlined above) was examined for the presence of *Hemoproteus* and *Plasmodium*. Further, larger parasites such as trypanosomes, microfilaria, and *Leucocytozoon* were noted if present.

Statistical Analyses

Data were analyzed in STATISTICA, version 6.1 (Statsoft 2003). All leukocyte count data and H : L ratios were \log_{10} transformed to normalize the error variance. For each bird, we calculated a size-corrected body mass (hereafter referred to as "body condition") index, following Owen and Moore (2008a), by regressing weight (g) onto wing chord (mm) and extracting standardized residual values. Regressions were performed separately for each species; positive values denote birds that weigh more than expected, given their body size (indexed by wing chord), and negative values denote birds that weigh less than expected. Condition indices were normalized to account for differences in magnitude across the three species (i.e., because gray catbirds had a broader range of condition scores, we divided their scores by 3 to place them within the same range as the other two species). This type of condition estimate has been used widely in avian studies and has been examined further and validated in small mammals by Schulte-Hostedde et al. (2005). Capture/sample date was converted to Julian date and included in initial analyses; because date was not found to influence either analysis, we removed the term from the final models. To examine whether species, age, H : L ratio, body condition, or fat scores predicted hemoparasite infection status (uninfected vs. infected, based on PCR detection), we first used logistic regression, treat-

ing species identity and age as categorical predictors and H : L ratio, body condition, and fat score as continuous variables. Because of sample size limitations, interaction effects were not included in the logistic-regression model. A priori, fat and body condition were analyzed for a correlation, and none was found. Next, we conducted a multivariate ANOVA (MANOVA) to determine whether our immune measures (total leukocyte counts and individual counts of the three most common leukocyte types, excluding monocytes and eosinophils because of their low numbers) were associated with species identity, age, and infection status (as categorical variables) and with body condition and fat scores (as continuous variables). In particular, energetic trade-offs faced by animals might be evidenced by a negative association between leukocyte counts and body condition or fat scores. To account for potential differences in baseline leukocyte counts between species, we included the interaction effects between species identity and all categorical and continuous predictor variables. After testing for overall model significance, we conducted univariate ANOVAs to determine which individual WBCs (lymphocytes, heterophils, or basophils) contributed most strongly to significant effects, and we used Bonferroni correction ($\alpha/4 = 0.013$) for multiple comparisons. We tested lymphocytes, heterophils, and basophils independently, in addition to total WBCs. In all models, two-way interaction effects were included, but they were later removed if not significant. Finally, Student's *t*-tests were used to compare the difference in means between uninfected and infected birds on the species level.

Results

A total of 86 birds across the three focal species were sampled (28 common yellowthroats, 38 gray catbirds, and 20 western palm warblers). Of the 86 birds, 73 were classified as HY (20 common yellowthroats, 34 gray catbirds, and 19 western palm warblers), and 13 were AHY (8 common yellowthroats, 4 gray catbirds, and 1 western palm warbler). Among all birds captured, a total of 14% ($n = 12$) were infected with either *Hemoproteus* or *Plasmodium*, based on PCR detection, and more positive samples were from HY birds ($n = 8$) than from AHY birds ($n = 4$). Because no birds that tested positive for parasites via PCR detection showed signs of parasitemia on blood smears, we were unable to assign levels of parasite intensity. Of the birds that tested positive for blood parasites, seven were common yellowthroats, three were gray catbirds, and two were western palm warblers (25%, 7.9%, and 10%, respectively, of the sampled birds from those species). The prevalence of hemoparasite infection measured here was generally lower than values reported for passerine birds (including the three focal species) sampled during the breeding season (table A1). Further, leukocyte profiles (including H : L ratios) of birds sampled here during the fall migration were within the ranges reported from other published studies of passerine birds sampled during the breeding season (table A2).

In our logistic-regression analysis, none of the categorical or continuous predictor variables measured, including species,

Table 1: Summary of recent studies focused on blood parasite infection and avian migration

Species common name	Migration	Parasites examined	Effect of parasite(s)	Detection method	Source
Magnolia warbler	Spring	<i>Plasmodium, Hemoproteus, Leucocytozoon, Trypanosoma</i>	No effect on energetic condition or migration timing	Microscopy	Boone et al. 2010
Magnolia warbler	Spring	<i>Plasmodium, Hemoproteus, Leucocytozoon, Trypanosoma</i>	No effect on refueling rate, energetic condition, or migration timing	Microscopy	DeGroot and Rodewald 2010
Yellow-rumped warbler	Spring	<i>Plasmodium, Hemoproteus, Leucocytozoon, Trypanosoma</i>	No effect on refueling rate; negative effect on energetic condition in infected second-year birds and a later capture date in all infected birds	Microscopy	DeGroot and Rodewald 2010
Yellow warbler	Spring	<i>Plasmodium, Hemoproteus, Leucocytozoon, Trypanosoma</i>	No effect on refueling rate, energetic condition, or migration timing	Microscopy	DeGroot and Rodewald 2010
Blackcaps	Spring	<i>Plasmodium, Hemoproteus, Leucocytozoon</i>	No effect on fat or refueling rate	PCR	Arizaga et al. 2009
Blackcaps	Spring, fall	<i>Plasmodium, Hemoproteus, Leucocytozoon</i>	No effect on fuel load	Microscopy	Arizaga et al. 2010
Blackcaps	Spring	<i>Plasmodium, Hemoproteus, Leucocytozoon</i>	No effect on body condition	PCR, microscopy	Santiago-Alarcón et al. 2013
Yellowhammer	Nonbreeding	<i>Plasmodium, Hemoproteus</i>	Reduced H : L ratio in infected birds and elevated WBC counts	PCR	Dunn et al. 2013
Gray catbird	Fall	<i>Plasmodium, Hemoproteus</i>	No effect on fat, body condition, or H : L ratio; elevated WBC count in infected birds	PCR	This study
Common yellowthroat	Fall	<i>Plasmodium, Hemoproteus</i>	No effect on fat, body condition, H : L ratio, or WBC count	PCR	This study
Western palm warbler	Fall	<i>Plasmodium, Hemoproteus</i>	No effect on fat, body condition, H : L ratio, or WBC count	PCR	This study

Note. Details on particular methods and descriptions of variables are provided within each publication. Search criteria focused on papers published since 2009 that quantified the effect of blood parasites on avian physiology (fat, body condition, energetic condition, refueling rate, migration timing, H : L ratio, or WBC counts). H : L ratio = heterophil : lymphocyte ratio; PCR = polymerase chain reaction; WBC = white blood cell.

Table 2: Statistical relationship between leukocyte counts and predictor variables

Predictor variables	Wilks's λ	F	Effect df	Error df	P
Species	.396	11.041	8	150	.000
Age	.903	2.014	4	75	.101
Infection status	.733	6.801	4	75	.000
Fat	.959	.804	4	75	.526
Body condition	.943	1.125	4	75	.351
Species \times infection status	.622	5.016	8	150	.000

Note. MANOVA results testing whether species identity, age, infection status, fat score, or body condition index was associated with leukocyte counts (total white blood cell count and counts of lymphocytes, heterophils, and basophils). Interaction effects were included in the initial model but were removed if they were not found to be significant.

age, body condition, fat score, and H:L ratio, significantly predicted hemoparasite infection status (P values for all predictor variables were >0.1). Thus, in the absence of measurable parasitemia, hemoparasites appeared to have little effect on the birds sampled here. To put this result in the context of other studies, we reviewed the recent ornithological literature, which is presented (along with our results) in table 1. Collectively, most recent studies also demonstrated that blood parasites seem to elicit minimal effects on physiological variables during migration (table 1).

Our MANOVA analysis showed evidence for significant differences in leukocyte profiles in relation to infection status, species, and the species \times infection status interaction (table 2). Univariate ANOVAs using individual WBC measures as dependent variables showed that species identity, species \times infection, and infection status were all significant predictors of total WBC count, lymphocyte count, and heterophil count (table 3). These three measures were all higher in infected than in uninfected gray catbirds, but no associations were observed for common yellowthroats or western palm warblers (table 3; fig. 2). This result was comparable to that of the Student t -tests, which showed that differences in WBC counts (total WBCs, lymphocytes, heterophils, and basophils) between uninfected and infected birds were significant only for gray catbirds (table A3). No other predictors tested (i.e., body condition, fat scores) were significant predictors of WBC counts (table 3).

Discussion

Results of this study provided little evidence that hemoparasites negatively affect migratory songbirds, consistent with findings from other recent published work (e.g., Arizaga 2009, 2010; DeGroot and Rodewald 2010; Dunn et al. 2013; Santiago-Alarcón et al. 2013). None of the physiological variables we measured predicted infection status in the birds examined here, suggesting that subcutaneous fat, body condition, and hematological stress do not differ between hemoparasite-infected and uninfected birds. Moreover, we found no evidence to support a negative association between leukocyte measures and body condition or energy reserves, as might be predicted by resource-based energetic trade-offs during periods of active migration. We also found no evidence for a positive association between immune measures (total WBC count) and body condition, as might be expected if birds in better condition overall are able to maintain higher levels of defense. The exception to these negative results was that for one species (gray catbirds), blood parasite-infected birds had elevated total WBC counts and higher counts of lymphocytes and heterophils when compared to uninfected birds of the same species.

White blood cells are an important component of the innate immune system, serving to phagocytize extracellular parasites and destroy infected host cells, and certain leukocytes (lymphocytes) play a role in adaptive immunity (Figueroa et al.

Table 3: Univariate results of the statistical relationship between leukocyte counts and predictor variables

Predictor variables	df	Total WBCs		Lymphocytes		Heterophils		Basophils	
		F	P	F	P	F	P	F	P
Species	2	49.84	.000	49.68	.000	24.93	.000	2.634	.078
Age	1	.963	.330	.134	.716	3.893	.052	3.818	.054
Infection status	1	12.028	.001	11.33	.001	12.56	.001	2.055	.156
Fat	1	.037	.849	.0124	.912	.514	.476	.723	.398
Body condition	1	.863	.356	.620	.433	.002	.967	.536	.466
Species \times infection status	2	7.677	.001	7.224	.001	4.900	.010	5.636	.005

Note. Univariate ANOVA results examining associations between white blood cell (WBC) counts and all predictor variables (performed after the MANOVA results in table 1). Bonferroni correction was applied ($\alpha/4$), with significance at the $P < 0.013$ level. Interaction effects were included in the initial model but were removed if they were not found to be significant.

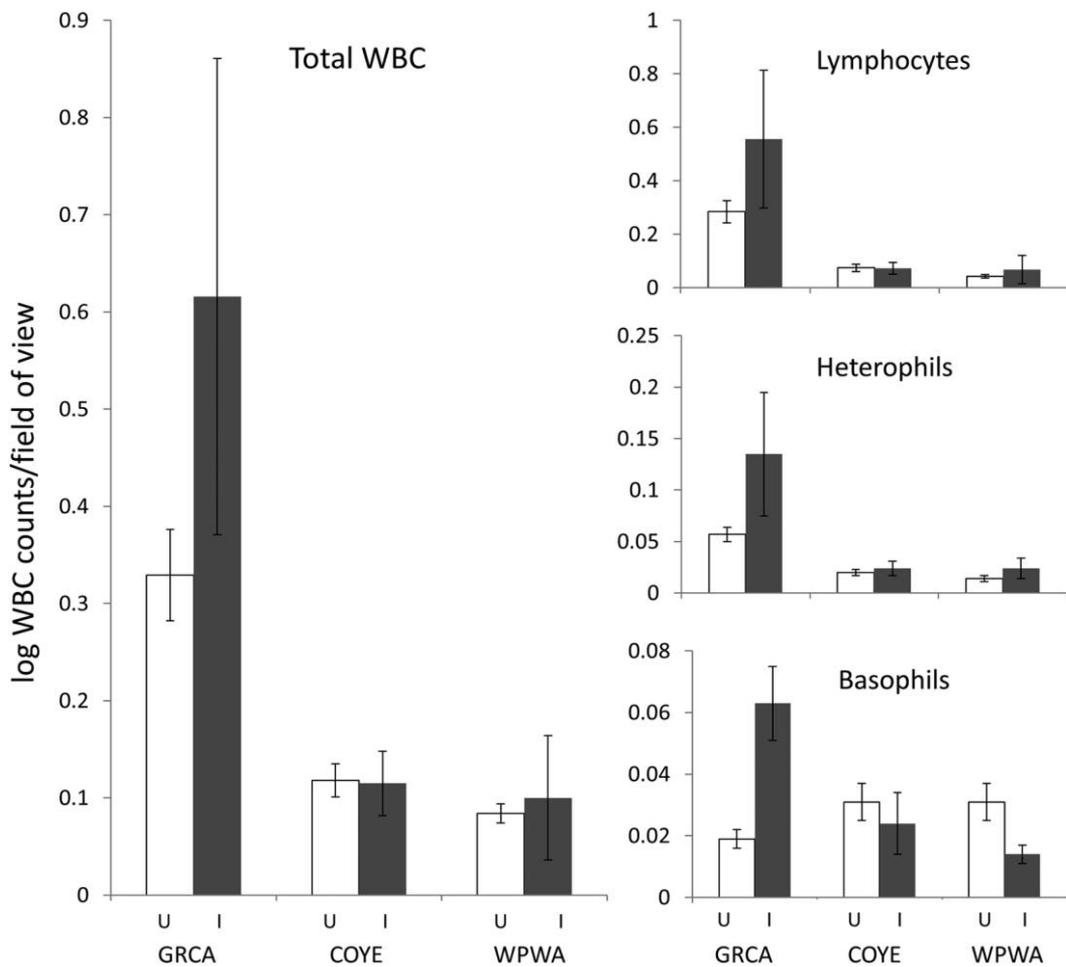


Figure 2. Leukocyte abundance relative to hemoparasite infection status for the three focal bird species sampled here. WBC = white blood cells; GRCA = gray catbird; COYE = common yellowthroat; and WPWA = western palm warbler. White bars represent uninfected birds (U), and dark gray bars represent infected birds (I).

1999; Ricklefs and Sheldon 2007; Clark et al. 2009). Infection by a range of pathogens can cause increases in circulating leukocytes, as demonstrated by prior ecological studies (Davis 2004; Ricklefs and Sheldon 2007; Dunn et al. 2013) and clinical veterinary research (Latimer et al. 1988; Clark et al. 2009). In this study, gray catbirds with chronic blood parasite infections had higher leukocyte profiles than did uninfected birds. One interpretation of this finding is that chronic or latent infections might elicit long-term activation of innate immune defense (see also Arizaga et al. 2009, 2010; Wojczulanis-Jakubas et al. 2012). While only a handful of infected birds from this study experienced elevated WBC counts, a recent analysis by Dunn et al. (2013), using a larger sample size of actively infected birds, showed similar responses (elevated WBC numbers in migratory birds infected with blood parasites). Therefore, and as suggested by Dunn et al. (2013), more work is needed on blood parasite infection outside of the breeding season, as these infections might play important roles during times of year when mortality is high, such as during migration.

Associations between immune measures, infection, and phys-

iological condition will likely depend on the biological traits of host and parasite species and also on the dynamics of infections. For example, certain host species might be more tolerant of chronic infections than others, because of differences in life history, foraging, or behavioral ecology, which could affect the signal of elevated immune defense following initial infection. Moreover, whether infections are acute, defined as recent infections with high parasitemia, or chronic, defined as recovered infections with low parasitemia, will likely play a role in the association with physiological conditions. Finally, because bird species differ in their distances traveled, it is possible that we might have caught some birds at the start of their migration and others farther along in their travels, thus adding variation to the degree to which individuals are energetically limited. Importantly, the high differences among species in total WBC counts observed here suggest that future work should examine the baseline differences in species' leukocyte profiles and ask what, if any, biological and behavioral traits explain these differences.

We predicted that migrants infected with blood parasites would show a decreased ability to procure or store energy re-

serves, in the form of subcutaneous fat (Wikelski et al. 2003). However, we found no evidence that hemoparasite-infected birds had lower fat scores, lower body condition indices, or higher stress levels. Our literature review further supported this finding of minimal effects of parasites on physiological parameters (table 1). Collectively, these results demonstrate that migrating with a blood parasite might not impede a bird's ability to acquire the lipid reserves necessary for long-distance flight.

We also expected to find evidence of physiological trade-offs between immune status and energy reserves, consistent with the idea that when resources are limited and in the presence of energetically demanding flight, immune defense might be reduced (reviewed by Norris and Evans 2000). However, our analysis showed no relationships between leukocyte abundance and fat levels or body condition in any species examined here. This finding diverges somewhat from those of previous work, where positive associations between leukocyte counts and fat score (Hatch et al. 2010) or between leukocyte counts and energetic condition (Owen and Moore 2008a) were found in wild songbirds. In other work, Hasselquist et al. (2007) found that experimental long-distance flight in red knots (*Calidris canutus*) did not cause changes in cell-mediated immunity (when compared to control birds), which further suggests that migratory flight might not lower resources available for immune defense.

In sum, results from this study contribute to scientific understanding of the interactions between parasitism, avian physiology, and migration. We found partial support for effects of hemoparasite infection on immune cell counts, although this pattern varied with species. Future work across a broader range of bird species could determine the generality of this pattern and whether the location and time when the infection is acquired are likely to play a role in the impact of that infection on physiological state. In addition, future studies could employ multiple measures of stress (especially corticosterone) rather than relying solely on H:L ratios. We also found that chronic blood parasite infections did not influence fat reserves or body condition during migratory flight and that fat reserves were not associated with immune cell counts. Importantly, our findings do not imply that parasite infection carries no cost, and this cost might have been reflected in variables not measured here, such as duration of stopover. Nevertheless, the absence of negative relationships between immune defense and fat measures and between infection status and physiological condition suggests that migrating birds can maintain multiple physiological processes while undertaking these long-distance journeys (Jakubas et al. 2011, 2013).

Acknowledgments

This study was supported by the Wormsloe Institute for Environmental History, the Odum School of Ecology, the Georgia Ornithological Society, the Explorer's Club, Sigma Xi, and a National Geographic Young Explorers grant. We thank the Jekyll Island Banding Station for help in data collection and members of the Altizer lab for comments on earlier drafts of

this article. We also thank Diane Borden for field and laboratory support and Sarah Ross and Craig and Diana Barrow for their financial and logistical support.

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