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SEASONAL DIFFERENCES IN IMMUNOLOGICAL CONDITION OF THREE SPECIES OF THRUSHES

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Abstract. Migratory birds are exposed to a diverse pathogen fauna by virtue of their long-distance travels. Although the immune system is an organism's primary defense against pathogenic microorganisms, few studies have investigated avian immune function during migration, much less shown seasonal differences in immune function. We describe the immunological condition of three intercontinental migratory species, Swainson's Thrush (*Catharus ustulatus*), Veery (*C. fuscescens*), and Wood Thrush (*Hylocichla mustelina*) during spring migration. In addition, we compare their immunological condition with conspecifics captured during fall migration and during the breeding season to provide a frame of reference for the spring immunological data. Measures of immunological condition presented include total leukocyte count, heterophil:lymphocyte (H:L) ratio, heterophil and lymphocyte counts, hematocrit, and IgG titers. In addition, we assessed each bird's energetic condition by measuring body mass and calculating a size-corrected condition index. Migrating birds had lower leukocyte and lymphocyte counts, lower hematocrit, reduced fat stores, and higher H:L ratios relative to nonmigrating individuals sampled during the breeding season. We also found that birds sampled during spring migration had lower lymphocyte counts and reduced fat stores, and higher heterophil counts and H:L ratios than fall migrants. Our results suggest that migrating birds are immunocompromised compared with conspecifics during the breeding season. Furthermore, compared with conspecifics sampled after a shorter overland flight, migrants sampled after crossing the Gulf of Mexico exhibited higher H:L ratios, which is indicative of heightened energetic stress.

Key words: body condition, hematocrit, immune function, immunoglobulin, migration, seasonal differences, white blood cell count.

Diferencias Temporales en la Condición Inmunológica de Tres Especies de *Turdidae*

Resumen. Las aves migratorias están expuestas a una diversidad de fauna patológica debido a sus viajes de larga distancia. Aunque el sistema inmunológico es la principal defensa contra los microorganismos patógenos, pocos estudios han investigado la función del sistema inmunológico de las aves durante la migración, y mucho menos han mostrado las diferencias estacionales en la función inmunológica. Este estudio describe la condición inmunológica de tres especies migratorias intercontinentales durante la migración de la primavera: *Catharus ustulatus, C. fuscescens* y *Hylocichla mustelina*. Además, comparamos la condición inmunológica de estas aves con la de aves de la misma especie capturadas durante la migración del otoño y durante la época de reproducción, a fin de proveer un marco de referencia para los datos inmunológicos obtenidos en la primavera. Las mediciones que se presentan sobre la condición inmunológica incluyen un conteo total de leucocitos, la proporción de heterófilos:linfocitos (H:L), conteos de heterófilos y linfocitos, hematocritos, y los niveles de IgG. Además, evaluamos la condición energética de cada ave por medio de la medición de la masa corporal y el cálculo de un índice de condición corregido de acuerdo con el tamaño. Las aves migratorias tuvieron conteos más bajos de leucocitos y linfocitos, hematocritos más bajos, reservas de grasa reducidas y proporciones más altas de H:L en relación con las aves no migratorias muestreadas durante la temporada de reproducción. También se encontró que las aves que se examinaron durante la migración de la primavera tenían un menor conteo de linfocitos, reservas de grasa reducidas y un conteo mayor tanto de heterófilos como de la proporción de H:L que las aves que migraron en el otoño. Nuestros resultados sugieren que las aves migratorias están inmunocomprometidas en comparación con aves de la misma especie examinadas durante la temporada en la que no hay migración. Por otra parte, las aves migratorias examinadas después de que cruzaran el Golfo de México mostraron proporciones de H:L más elevadas comparadas con aves de la misma especie examinadas durante la migración del otoño, lo cual indica un elevado estrés energético.

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INTRODUCTION

Migration is a period of heightened energetic demand. Long-distance, intercontinental migrants must negotiate ecological barriers, which often requires nonstop flights of 18–24 hr. In anticipation of the energy required for migration, a bird becomes hyperphagic and within a few days deposits up to 30%–50% of its lean body mass as fat (Berthold 1975). Fat is the primary source of energy for long-distance flight (Blem 1990) and is often depleted during such flights (Bairlein 1985, Moore and Kerlinger 1987). In addition to the energetic costs of flight, migrating birds face many challenges *en route* including variation in quality and availability of food (Bibby and Green 1980, Moore et al. 1995), competition for resources (Rappole and Warner 1976, Moore and Yong 1991), predation risk (Cimprich and Moore 1998), adverse weather (Gauthreaux 1971), and exposure to novel pathogens (Møller and Erritzoe 1998). A bird's ability to meet the challenges of migration is crucial to its survival and subsequent reproductive success.

The immune system is an animal's primary defense against pathogens (Zuk 1994), and is not without maintenance and activation costs (Lochmiller and Deerenberg 2000, Schmid-Hempel 2003). Some evidence indicates that immune function is suppressed when animals are engaged in energetically demanding activities (Raberg et al. 1998, Norris and Evans 2000), and immunosuppression in response to energetically demanding activities may reflect a trade-off with the immune system (Sheldon and Verhulst 1996, Lochmiller and Deerenberg 2000). Intercontinental migrants are exposed to a diverse pathogen fauna by virtue of their extensive movements (Møller and Erritzoe 1998). If immune function is suppressed in response to heightened energetic demands of migration (Raberg et al. 1998), a migrating bird may be more likely to be infected by a pathogenic microorganism.

Despite the importance of immune function to the survival and reproductive performance of migratory birds, the subject has received little attention (but see Fänge and Silverin 1985, Møller and Erritzoe 1998, Deerenberg et al. 2003, Muñoz and Fuente 2003). Much of the research on immune function in passerines has been conducted during the breeding season (Sheldon and Verhulst 1996, Norris and Evans 2000). Our objective was twofold: (1) describe the immunological and energetic condition of three species of intercontinental spring migrants sampled after flight across the Gulf of Mexico, and (2) present comparative data on immunological and energetic condition of conspecifics during the breeding season and during fall migration prior to crossing the Gulf of Mexico. Measurements of immunological and energetic condition of individual birds in the present study include: absolute and differential leukocyte counts, hematocrit, immunoglobulin gamma (IgG) levels, body mass, and a size-corrected condition index (Ellegren 1992, Yong and Moore 1994).

METHODS

Focal species were Swainson's Thrush (*Catharus ustulatus*), Veery (*C. fuscescens*), and Wood Thrush (*Hylocichla mustelina*), all of which are intercontinental landbird migrants. Birds were sampled in 1998–2001 during spring migration at a study site located near Johnson Bayou (29°45′N, 93°37′W) in southwest Louisiana on the northern coast of the Gulf of Mexico. The site is in a narrow coastal woodland (chenier) surrounded by marsh. Coastal cheniers provide a place for Nearctic–Neotropical migrants to rest and replenish fat stores following trans-Gulf flight (Moore and Kerlinger 1987). See Barrow et al. (2000) for a description of the study site.

Swainson's Thrushes and Veeries were sampled during the breeding seasons of 1999–2001 at Saint Martin's Bay, located on the northern shore of Lake Huron in the Upper Peninsula of Michigan (46°02′N, 84°35′W). Smith and Moore (2003) provide a description of the study site, mist-netting protocol, and vegetation. Swainson's and Wood Thrushes were sampled during fall migration in 1999 and 2000 at a site located in the Bon Secour National Wildlife Refuge, Fort Morgan Peninsula, coastal Alabama (30°13′N, 88°10′W). Bon Secour National Wildlife Refuge is located at the tip of an east–west peninsula bounded by Mobile Bay to the north and the Gulf of Mexico to the south. For a description of the study site, mist-netting protocol, and vegetation, see Woodrey and Moore (1997).

We captured birds using mist nets (12 × 2.6 m with 30-mm mesh). The same mist net

protocol was used at each study site. Each bird was banded with a U.S. Fish and Wildlife Service aluminum leg band. Age, subcutaneous fat, unflattened wing chord, and weight (to the nearest 0.1 g) were recorded. Visible subcutaneous fat and muscle mass were quantified according to scales developed by Helms and Drury (1960) and Bairlein (1985), respectively. Grams of fat were estimated according to Ellegren (1989, 1992). Specifically, we used data from birds captured at the Johnson Bayou study site, 1998–2001 to estimate species- and size-specific fat-free mass. Individuals of each species were grouped according to common wing chord classes (1 mm increments). For each wing chord class, we regressed body mass on fat score. In each regression, the intercept (equivalent to fat score = 0) provided an estimate of fat-free mass for that particular wing chord length. We performed linear regressions for all wing chord lengths. In a second analysis, we regressed fat-free mass on corresponding wing chord lengths. Using the equation generated by this second regression model we estimated size-specific fat-free mass for each individual. To obtain a size-corrected condition index we subtracted fat-free mass from actual body mass. The regression model developed for each species was applied to conspecifics captured in the other seasons. Body mass, fat and muscle scores, and condition index provided information on energetic condition of the birds.

The same blood collection protocol was followed at all three sites. Immediately following capture, a blood sample (100–250 μl) was taken from the brachial vein using a 26-gauge needle. For white blood cell identification and counts, a drop of blood was placed on a glass slide and a thin smear was made using a beveled slide. Blood smears were air-dried, fixed with 100% methanol, and stained with a combination Wright-Giemsa stain. Using the blood smears, we conducted both absolute and differential leukocyte (white blood cells; WBC) counts. Absolute counts consisted of tallying all WBCs, i.e., lymphocytes, monocytes, heterophils, eosinophils, and basophils, per 100 fields of view, using oil immersion. Total WBC count is expressed as the number of WBCs per 10 000 red blood cells (RBC). Total leukocytes may provide information on the health and immunocompetence of an individual bird (Campbell and Dein 1984, Amand 1986). Changes in numbers of leukocytes, whether elevated or depressed, reflect ongoing disease processes of bacterial, parasitic, or viral origin. Reduced leukocytes may also indicate systemic stress caused by a nonetiological process such as malnutrition (Gershwin et al. 1985), strenuous exercise (Hoffman-Goetz and Pedersen 1994), or significant weight loss (Stinnett 1983).

Lymphocytes operate entirely in acquired immune responses; therefore, they provide an indirect measure of acquired immune function, i.e., cell-mediated and humoral immunity (Blount et al. 2003). Heterophil:lymphocyte (H:L) ratio was calculated by dividing the number of heterophils per 100 fields of view by the number of lymphocytes per 100 fields of view. This method differs from similar studies (Ots and Hõrak 1996, Hõrak et al. 1998, Vleck et al. 2000), in which H:L ratio was calculated by counting a total of 100 WBC under oil immersion and then taking the relative proportion of each blood cell type. WBC counts in migrating birds are extremely low (JCO, unpubl. data), therefore counting 100 WBCs is not efficient. We compared the two methods using blood smears from Veeries and Wood Thrushes and a paired *t*-test. H:L ratio did not differ between methods (Wood Thrush, $t_{79} = 0.6$, $P = 0.58$; Veery, $t_{58} = -0.4$, $P = 0.67$). During periods of stress, birds may experience heterophilia and concomitant lymphopenia, resulting in high H:L ratios (Gross and Siegel 1986, Ots and Hõrak 1996, Dabbert et al. 1997, Work et al. 1999).

Within 9 hr of blood collection, we spun capillary tubes in a clinical centrifuge for 9 min at 14 000 RPMs, to achieve maximum packing of red blood cells. Hematocrit was calculated by dividing the red blood cell volume by the total blood volume (erythrocytes + plasma; Campbell and Dein 1984). Hematocrit is frequently used to assess a bird's nutritional state (Amand 1986). Low hematocrit (anemia) is indicative of malnutrition as well as bacterial and parasite infections (Campbell and Dein 1984). High hematocrit may be caused by dehydration or elevated oxygen consumption (Carpenter 1975).

Plasma was drawn out of the capillary tube using a Hamilton syringe, stored in a microcentrifuge tube, and frozen at −70°C until analysis. We measured immunoglobulin gamma (IgG) titers in the plasma using a sandwich ELISA (enzyme linked immunosorbent assay) devel-

oped specifically for thrushes (Owen 2004). Plasma IgG levels provide a general assessment of humoral immune function (Apanius and Nisbet 2003). High concentrations of immunoglobulins are indicative of an event (e.g., parasite infection) that stimulated increased activity of the immune system (Kuby 1997). Extremely low levels of immunoglobulins may indicate reduced immune function.

STATISTICAL ANALYSIS

We determined whether parameters of immunological and energetic condition differed among seasons using a one-way analysis of variance (ANOVA). Swainson's Thrush data were available for all three seasons, but only two seasons of data were available for Veery (spring migration and breeding) and Wood Thrush (spring migration and fall migration). We followed the Swainson's Thrush ANOVA with a Tukey's HSD (honestly significant difference) test for pairwise comparisons between seasons.

The amount of blood we were able to collect varied among individuals. Therefore, not all parameters were measured for all individuals within a season and sample sizes vary as a result. Significance levels, which were set at a more conservative alpha of 0.01 because of multiple univariate comparisons, refer to two-tailed tests. White blood cell count and heterophil:lymphocyte ratio did not meet the assumptions of normality. Log-transforming WBC count did not affect analysis results; therefore, we used the untransformed data in the analysis. Transforming H:L ratio did influence results and all analyses were conducted using the transformed data (1/[H:L ratio + 1]). For clarity, we present all raw data in untransformed state. Data are presented as mean ± SE. We performed all analyses using SPSS 12.0 (SPSS 2004).

RESULTS

We collected immunological and condition data on 230 Swainson's Thrushes, 191 Veeries, and 226 Wood Thrushes during spring migration. In addition, we sampled 38 Swainson's Thrushes and 33 Veeries during the breeding season and 96 Swainson's Thrushes and 91 Wood Thrushes during fall migration. Of the 187 birds captured during fall migration, 166 (89%) were juvenile (hatch-year) birds. We found no age-related differences in any of the immune parameters (JCO, unpubl. data). At the breeding site only 3 of 78 (4%) captured birds were juveniles. In addition, while we were able to sex birds reliably during the breeding season, low statistical power prevented detection of sex-specific differences. Therefore, we combined age classes and sexes for all further analyses.

MIGRATION–BREEDING SEASON COMPARISION

We found significant differences in the immunological condition of migrating thrushes compared to thrushes sampled during the breeding season (Table 1). Migrating thrushes had lower hematocrits and leukocyte and lymphocyte counts, and higher heterophil counts (except Veery) and H:L ratios (Table 1). We did not detect a seasonal difference in plasma IgG titers (Table 1). Furthermore, birds during migration were in poorer energetic condition than conspecifics sampled during the breeding season (Table 2).

SPRING–FALL MIGRATION COMPARISON

We detected significant seasonal differences in the immunological condition of Swainson's and Wood Thrushes during spring migration compared with fall migration. Spring migrants had more heterophils and higher H:L ratios than fall migrants (Table 1). In addition, Wood Thrushes had significantly lower lymphocyte counts in the spring compared with conspecifics in the fall (Table 1). We also found significant differences in energetic condition between the two migratory seasons (Table 2). Wood Thrushes captured in spring were in poorer energetic condition than those captured in fall (Table 2). A Mann-Whitney U-test (one-tailed) indicated spring migrants had lower fat (1.92 ± 0.05, $n = 225$) and muscle (1.85 ± 0.03, $n = 226$) scores than individuals captured in fall (fat: 2.53 ± 0.11, $n = 90$; $U = -5.3$, $P < 0.001$; muscle: 2.05 ± 0.05, $n = 90$; $U = -4.5$, $P < 0.001$). In contrast, the fat (spring: 2.05 ± 0.05, $n = 226$; fall: 2.36 ± 0.15, $n = 96$) and muscle (spring: 1.98 ± 0.03, $n = 222$; fall: 1.90 ± 0.06, $n = 97$) scores of Swainson's Thrushes did not differ significantly between fall and spring (fat: $U = -1.5$, $P = 0.06$; muscle: $U = -0.7$, $P = 0.23$).

DISCUSSION

Thrushes captured during migration had reduced immunocompetence compared to non-

TABLE 1. Average (mean ± SE) seasonal differences in hematological and immunological variables for three species of thrushes during three different periods in the annual cycle: spring migration (Johnson Bayou, Louisiana, 1998–2001), breeding season (St. Martin's Bay, Michigan, 1999–2001), and fall migration (Fort Morgan Peninsula, Alabama, 1999–2000). Statistical significance is based on a one-way ANOVA (two-tailed). Superscript letters refer to significant differences ($P < 0.05$) according to a Tukey's HSD *post-hoc* procedure. Means with the same superscript letters do not differ significantly according to Tukey's HSD *post-hoc* procedure. Tukey's HSD tests were only performed for Swainson's Thrushes. Abbreviations: WBC = white blood cells; H:L = heterophil:lymphocyte; IgG = immunoglobulin gamma. Units of measure: WBC count = WBC count per 10 000 red blood cells (RBC); Lymphocytes = lymphocyte count per 10 000 RBC; heterophils = heterophil count per 10 000 RBC; IgG titer = μg mL^{-1}.

Species Immune parameters	Spring migration (*n*)	Breeding (*n*)	Fall migration (*n*)	df	*F*	*P*
Swainson's Thrush						
Hematocrit	0.48 ± 0.01 (207)[a]	0.54 ± 0.01 (26)[b]	0.48 ± 0.01 (82)[a]	2, 316	12.3	< 0.001
WBC count	21.70 ± 1.07 (201)[a]	50.20 ± 9.05 (18)[b]	20.20 ± 1.65 (90)[a]	2, 306	23.7	< 0.001
Lymphocytes	11.02 ± 0.72 (203)[a]	40.42 ± 8.07 (19)[b]	10.79 ± 1.02 (90)[a]	2, 309	45.9	< 0.001
Heterophils	6.32 ± 0.43 (203)[a]	2.43 ± 0.41 (19)[b,c]	3.30 ± 0.35 (89)[c]	2, 308	12.5	< 0.001
H:L ratio	1.04 ± 0.12 (200)[a]	0.08 ± 0.01 (18)[b]	0.71 ± 0.14 (89)[c]	2, 304	6.4	0.002
IgG titer	4.10 ± 0.13 (49)	3.64 ± 0.19 (16)	4.31 ± 0.14 (40)	2, 102	3.3	0.04
Veery						
Hematocrit	0.48 ± 0.01 (171)	0.53 ± 0.01 (18)	N/A	1, 197	10.3	0.002
WBC count	16.30 ± 1.09 (145)	26.80 ± 5.66 (18)	N/A	1, 160	10.0	0.002
Lymphocytes	7.41 ± 0.70 (154)	20.71 ± 5.52 (17)	N/A	1, 169	23.1	< 0.001
Heterophils	5.52 ± 0.47 (154)	2.47 ± 0.55 (16)	N/A	1, 168	4.3	0.04
H:L ratio	1.37 ± 0.13 (145)	0.26 ± 0.07 (16)	N/A	1, 154	7.6	0.007
IgG titer	4.10 ± 0.15 (47)	3.88 ± 0.29 (16)	N/A	1, 61	0.5	0.46
Wood Thrush						
Hematocrit	0.47 ± 0.01 (221)	N/A	0.48 ± 0.01 (88)	1, 310	2.7	0.10
WBC count	26.30 ± 1.45 (199)	N/A	29.00 ± 2.13 (81)	1, 280	0.8	0.36
Lymphocytes	10.60 ± 0.52 (203)	N/A	15.10 ± 1.34 (81)	1, 283	5.7	0.02
Heterophils	9.57 ± 0.65 (203)	N/A	5.80 ± 0.61 (89)	1, 282	13.3	< 0.001
H:L ratio	1.19 ± 0.09 (199)	N/A	0.68 ± 0.12 (79)	1, 280	16.3	< 0.001
IgG titer	6.05 ± 0.30 (70)	N/A	5.62 ± 0.37 (38)	1, 106	0.8	0.37

migratory conspecifics, as indicated by lower hematocrit, lower leukocyte and lymphocyte counts, and higher heterophil counts and H:L ratios. Furthermore, the thrushes in this study had lower leukocyte counts and higher H:L ratios than those reported by similar studies of nonmigrating passerines (Hõrak et al. 1998, 1999, Ilmonen et al. 2003). The hematological

TABLE 2. Average (mean ± SE) seasonal differences in energetic condition variables for three species of thrushes during three different periods in the annual cycle: spring migration (Johnson Bayou, Louisiana, 1998–2001), breeding season (St. Martin's Bay, Michigan, 1999–2001), and fall migration (Fort Morgan Peninsula, Alabama, 1999–2000). Statistical significance is based on a one-way ANOVA (two-tailed). Superscript letters refer to significant differences ($P < 0.05$) according to a Tukey's HSD *post-hoc* procedure. Means with the same superscript letters do not differ significantly according to Tukey's HSD *post-hoc* procedure. Tukey's HSD tests were only performed for Swainson's Thrushes.

Species Condition parameters	Spring migration (*n*)	Breeding (*n*)	Fall migration (*n*)	df	*F*	*P*
Swainson's Thrush						
Mass (g)	28.4 ± 0.2 (230)[a]	30.4 ± 0.4 (38)[b]	31.9 ± 0.6 (96)[b]	2, 361	29.7	< 0.001
Condition index (g)	3.0 ± 0.2 (230)[a]	5.5 ± 0.5 (38)[b]	6.6 ± 0.6 (96)[b]	2, 364	37.7	< 0.001
Veery						
Mass (g)	26.4 ± 0.2 (191)	30.5 ± 0.3 (33)	N/A	1, 222	73.6	< 0.001
Condition index (g)	3.4 ± 0.2 (191)	7.8 ± 0.3 (33)	N/A	1, 222	88.4	< 0.001
Wood Thrush						
Mass (g)	42.0 ± 0.3 (226)	N/A	49.8 ± 0.6 (91)	1, 315	169.2	< 0.001
Condition index (g)	3.8 ± 0.3 (226)	N/A	11.6 ± 0.6 (91)	1, 315	179.0	< 0.001

values we observed in migrant thrushes were most similar to those of energetically challenged individuals, such as birds feeding nestlings or caring for an experimentally enlarged clutch (Hõrak et al. 1998, 1999, Illmonen et al. 2003).

The low leukocyte counts we observed in migrating thrushes could indicate stress-induced immunosuppression (Hõrak et al. 1998, Boonstra et al. 2001, Bachman 2003, Blount et al. 2003) or an ongoing disease process of viral origin (Campbell and Dein 1984, Amand 1986). However, the other parameters of health and immune function did not indicate a disease process. Low leukocyte counts in migrating birds probably reflect a reduced number of lymphocytes, since heterophil numbers differed little among seasons. Lymphocytes are responsible for acquired immunity, whereas other leukocytes, particularly heterophils, are involved in the innate immune response. The acquired immune system is more costly to maintain and deploy than innate immunity (Apanius 1998). Therefore, an animal may not suppress the entire immune system during stressful episodes of intense physical activity, but rather may suppress one component while enhancing another component (Apanius 1998). This adjustment is most often reflected in lower lymphocyte numbers, higher heterophil counts, and a high H:L ratio (Maxwell 1993).

Migrating thrushes had high H:L ratios, which also may be indicative of a disease process (Campbell and Dein 1984) or chronic stress (Maxwell 1993). Studies show that the ratio of heterophils to lymphocytes increases in response to food deprivation and malnutrition (Gross and Siegel 1986), prolonged exercise (Nieman 2000), thermal stress (Dabbert et al. 1997), injury (Vleck et al. 2000), brood enlargement (Ots and Hõrak 1996, Hõrak et al. 1998, Ilmonen et al. 2003), and translocation (Work et al. 1999). Our study is the only one of which we are aware that reports H:L ratios for migrating birds, which is surprising given that several of the factors leading to elevated H:L ratios apply to migrating birds (i.e., food deprivation, strenuous exercise, and thermal stress). In addition, H:L ratios and heterophil counts of thrushes differed between the two migration seasons. This difference may be explained by where the birds were sampled in relation to flight across an ecological barrier. In spring we sampled birds following a long-distance flight across the Gulf of Mexico, which may take a landbird migrant 18–24 hours to complete (Gauthreaux 1971, Buskirk 1980). In the fall, we sampled birds prior to flight across the Gulf of Mexico and after an overland flight of probably shorter duration (Cochran and Kjos 1985).

Neuroendocrine-immune interactions may be responsible for the differences in H:L ratios and heterophil counts between fall and spring migrants. Baseline levels of corticosterone, the primary glucocorticoid in birds (Harvey et al. 1984), are elevated during migration, and may mediate food acquisition and fat deposition (Astheimer et al. 1992). While elevated corticosterone may enhance short-term survival (Harvey et al. 1984), chronically elevated levels of corticosterone may lead to immunosuppression and stress-related disease (Vleck 2001). Holberton et al. (1999) found spring migrants had lower baseline corticosterone levels than birds sampled during autumn migration. Conversely, plasma levels of corticosterone in Semipalmated Sandpipers (*Calidris pusilla*) did not differ between birds captured during fall and spring migration (Tsipoura et al. 1999). Increased production of corticosterone leads to a reduction in numbers and activity of lymphocytes, hence H:L ratio increases (Vleck 2001). We did not measure plasma corticosterone levels in this study, and our results depict a somewhat inconsistent relationship between corticosterone levels and measures of immunological condition. On one hand, H:L ratios were higher among thrushes sampled during spring migration compared with fall migrants, which is consistent with elevated corticosterone levels. However, elevated corticosterone is sometimes linked to inhibited erythrocyte production, lymphopenia, and leukopenia (Boonstra et al. 2001, Vleck 2001), none of which were observed in spring migrants to a greater extent than fall migrants.

Immunoglobulin gamma (IgG) titers did not differ among seasons. Boyum et al. (1996) found that human military subjects engaging in strenuous activity and deprived of food exhibited declines in several classes of immunoglobulins. Likewise, House Sparrows (*Passer domesticus*) fed a protein-restricted diet exhibited reduced humoral immune function as measured by immunoglobulin concentration (Gonzalez et al. 1999). Conversely, the humoral

immune function of Northern Bobwhite (*Colinus virginianus*) chicks fed diets with varying amounts of protein did not differ (Lochmiller et al. 1993), and humoral immune function was not associated with energetic condition in Blue Peafowl (*Pavo cristatus*, Møller and Petrie 2002). Likewise, we did not find any seasonal differences in humoral immune status.

The low lymphocyte counts found in this study are consistent with the smaller spleens found in migrating vs. nonmigrating Pied Flycatchers (*Ficedula hypoleuca*, Fänge and Silverin 1985). The spleen is a secondary organ of the avian immune system and its size is assumed to positively reflect an individual's ability to mount an immune response (Møller and Erritzoe 1998). Several studies have investigated variation in spleen size and activity throughout the annual cycle (Fänge and Silverin 1985, Deerenberg et al. 2003, Muñoz and Fuente 2003). Fänge and Silverin (1985) sampled spleen size of Pied Flycatchers captured during spring migration and throughout the breeding season. Spleen size was smallest during spring migration and slowly increased until it peaked during the egg-laying phase and nestling period for males and females, respectively. Likewise, Deerenberg et al. (2003) found spleen size to be reduced in Garden Warblers (*Sylvia borin*) after flight across an ecological barrier, compared with conspecifics sampled in winter. Muñoz and Fuente (2003) investigated lymphoproliferative response at different stages in the annual cycle of Black-headed Gulls (*Larus ridibundus*) and found that immune response was suppressed in individuals during the postmigratory period (fall) compared with birds captured during the nonmigratory (winter) and premigratory (spring) periods. Suppression of spleen function and low spleen mass during migration may explain the low lymphocyte count we observed in migrating thrushes.

Contrary to our expectations, hematocrit values of migrating thrushes were no higher than those of nonmigrating individuals. High hematocrit is more likely to be observed in migrating birds because of either the hypoxia and increased oxygen demand tied to the aerobic requirements of migration (Carpenter 1975), the possible dehydration experienced during migration (Biebach 1990, Carmi et al. 1992, but see Child 1969), or both. Lower than expected hematocrit values among spring migrants may be the result of short-term food deprivation and malnutrition (Campbell and Dein 1984, Amand 1986). Hematocrit in Bar-tailed Godwits (*Limosa lapponica taymyrensis*) at a stopover site in the Wadden Sea was low on arrival, but red blood cell numbers slowly increased during the refueling period and peaked immediately prior to departure (Landys-Ciannelli et al. 2002). An increase in red blood cells likely accommodates the increased oxygen demands of migratory flight, and may safeguard against possible depletion of red blood cells due to exercise-related stress. Therefore, we are surprised that fall birds did not display higher hematocrit values.

Energetic condition may account for some of the seasonal differences in immunological condition. Thrushes captured in spring had lower body mass and condition index values than either fall migrants or thrushes sampled during the nonmigratory season. On average, landbird migrants captured following a trans-Gulf flight had depleted fat stores (Moore and Kerlinger 1987), and at a stopover site similar to that in this study, approximately 65% of birds captured did not have sufficient fat stores to continue migration (Moore and Kerlinger 1987). Therefore, birds that have just traversed the Gulf of Mexico are likely more energetically stressed than birds sampled prior to trans-Gulf migration, which would account for the heterophilia, elevated H:L ratios, and poor energetic condition observed in spring migrants.

In conclusion, thrushes experience reduced immune capacity during migration compared with conspecifics during the breeding season. Furthermore, migratory birds are more energetically stressed and immunocompromised after crossing a large ecological barrier. If immune function is compromised during migration, birds may be more susceptible to pathogen or parasite infection during stopover. In addition to reducing the likelihood of surviving migration, a pathogen or parasite infection may delay migration because birds in poor health forage less efficiently and must direct resources normally allocated to meeting energetic requirements toward maintaining the immune system (Lochmiller and Deerenberg 2000). If a migrant stays longer than usual at a stopover site, resource levels at the next stopover site may be depressed by earlier migrants. If a migrant does not make up lost

time, arrival on the wintering or breeding grounds is necessarily delayed. Late arrival on the breeding grounds may lead to fewer opportunities to secure a territory or mate or lower reproductive success (Aebischer et al. 1996, Smith and Moore 2003).

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