Relationship between energetic condition and indicators of immune function in thrushes during spring migration

J.C. Owen and F.R. Moore

Abstract: Evidence suggests that the ability of an animal to maintain its immune system and (or) mount an immune response depends on its nutritional health and energetic condition. Migration is a period within an animal's annual cycle when energetic condition varies, especially after a long, nonstop flight over a large ecological barrier. Our objective was to determine if measures of immune function in migrating Wood Thrush (*Hylocichla mustelina* (J.F. Gmelin, 1789)), Swainson's Thrush (*Catharus ustulatus* (Nuttall, 1840)), Gray-cheeked Thrush (*Catharus minimus* (Lafresnaye, 1848)), and Veery (*Catharus fuscescens* (Stephens, 1817)) were related to the energetic condition of the birds at a stopover site during spring migration. We present data on total leukocyte, lymphocyte, and heterophil counts, heterophil/lymphocyte ratio, serum immunoglobulin gamma G (IgG) concentration, and immune response to phytohemagglutinin. Thrushes arriving at the stopover site in poor energetic condition had low leukocyte and lymphocyte counts. Heterophil/lymphocyte ratio, heterophil count, and IgG concentration were not related to energetic condition. Furthermore, immune response to phytohemagglutinin was positively related to change in mass and days spent in captivity, suggesting that immune function may improve during stopover. We suggest that migrating thrushes arriving at a stopover site in poor energetic condition may also be in poor immunological condition and may have increased susceptibility to disease or parasite infection.

Résumé : Des données indiquent que la capacité d'un animal à maintenir son système immunitaire et(ou) à développer une réaction immunitaire dépend de sa santé alimentaire et de sa condition énergétique. La migration représente une période dans le cycle annuel d'un animal pendant laquelle les conditions énergétiques varient, particulièrement après un long vol ininterrompu de migration à travers une importante barrière écologique. Notre objectif est de déterminer si les mesures de la fonction immunitaire chez des grives des bois (*Hylocichla mustelina* (J.F. Gmelin, 1789)), des grives à dos olive (*Catharus ustulatus* (Nuttall, 1840)), des grives à joues grises (*Catharus minimus* (Lafresnaye, 1848)) et des grives fauves (*Catharus fuscescens* (Stephens, 1817)) en migration sont reliées à la condition énergétique des oiseaux à un site d'arrêt durant leur migration printanière. Nous présentons des données sur les dénombrements totaux de leucocytes, de lymphocytes et d'hétérophiles, sur le rapport hétérophiles/lymphocytes, sur la concentration d'immunoglobuline gamma G (IgG) sérique et sur la réaction immunitaire à la phytohémagglutinine. Les grives qui arrivent au site d'arrêt en mauvaise condition énergétique ont des dénombrements bas de leucocytes et de lymphocytes. Le rapport hétérophyles/lymphocytes, le dénombrement d'hétérophiles et l'IgG ne sont pas reliés à la condition énergétique. De plus, la réaction immunitaire à la phytohémagglutinine est en corrélation positive avec le changement de masse et le nombre de jours de garde en captivité, ce qui laisse croire que la fonction immunitaire peut s'améliorer durant l'arrêt. Nous croyons que les grives en migration qui arrivent au site d'arrêt en mauvaise condition énergétique peuvent aussi être en mauvaise condition immunologique et être plus vulnérables à la maladie ou aux infections parasitaires.

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Introduction

Studies on vertebrates suggest that the ability of an animal to maintain its immune system and (or) mount an immune response depends on its nutritional health and energetic condition (Stinnett 1983; Lochmiller et al. 1993; Bachman 2003; Raberg et al. 2003). However, only one avian study has examined the relationship between direct measures of energetic condition, such as extent of fat and muscle stores, and variables of immune function (Merila and Svensson 1995). The majority of studies exploring this relationship in free-living birds have been conducted with breeding birds in which condition-dependent traits are used as a surrogate of the bird's condition (Saino et al. 1997; Gonzalez et al. 1999; Møller and Petrie 2002). For instance, birds that must maintain energetically expensive traits have reduced immunocompetence (Møller and Petrie 2002). This indirect approach to examining energetic condition may be due to the lack of variation in stored fat and muscle mass during the nonmigratory season.

Migration is a period of exceptional energetic demand. Long-distance migrants negotiate large ecological barriers that may require nonstop flights sometimes exceeding 18–24 h duration. In anticipation of the energy requirements of

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migration, a bird in migratory disposition becomes hyperphagic and deposits almost half of its total body mass in fat, the primary source of energy during migration (Berthold 1975; Blem 1990). In addition, protein stores may also be used as a source of energy after lipid stores have been depleted (Biebach 1998).

Few landbird migrants carry sufficient stores to complete their migration nonstop, and must stop periodically to redeposit fat stores. Upon arrival at stopover sites, birds vary in energetic condition (Moore and Kerlinger 1987; Wang and Moore 1997); some birds have no fat stores and depreciated muscle mass, whereas others have sufficient fat and muscle stores to continue their migration. Hence, migration is the ideal time period in which to examine the relationship between indicators of immune function and energetic condition. The present study investigates immunological status of migratory birds and their health in relation to energetic condition during migration. Birds that arrive in poor energetic condition following a flight across the Gulf of Mexico have experienced heightened energetic demand relative to birds that arrive with sufficient fat stores to continue their migration, and as a consequence may suppress or alter immune function. We test our expectations with four migratory species: Wood Thrush (*Hylocichla mustelina* (J.F. Gmelin, 1789)), Veery (*Catharus fuscescens* (Lafresnaye, 1848)), Swainson's Thrush (*Catharus ustulatus* (Nuttall, 1840)), and Gray-cheeked Thrush (*Catharus minimus* (Stephens, 1817)). The life histories of these species are similar in many respects and most individuals of populations breeding in eastern North America make a trans-Gulf flight during spring passage (Rappole et al. 1979), which provides substantial constructive replication. The species also differ in some important ways, including migratory distance and breeding and wintering distributions (e.g., Wang and Moore 1997). Their mean migratory distances between wintering and breeding grounds are approximately 2200, 5200, 5500, and 6200 km, respectively, which is thought to influence their migratory strategy (see Wang and Moore 1997).

The measures of immune function used in this study include (*i*) absolute and differential leukocyte counts (*ii*) heterophil/lymphocyte (H/L) ratio, (*iii*) serum immunoglobulin gamma G (IgG) levels, and (*iv*) T-lymphocyte proliferation immune response. The first three measures listed reflect the status of the bird's immune system at the time of capture. Although indirect, they do provide information on the preparedness of the bird's immune system and have been used as a substitute for direct measures of immune function (Gustafsson et al. 1994; Boonstra et al. 2001; Bachman 2003). Phytohemagglutinin (PHA), a T-lymphocyte-dependent mitogen, stimulates the proliferation of lymphocytes and can be used to assess the immune system of the bird (Smits et al. 1999). It has both an innate and an acquired component (Martin et al. 2006). PHA induces dermal infiltration of lymphocytes and phagocytes, which cause increased swelling of the tissue at the inoculation site (Stadecker et al. 1977; Cheng and Lamont 1988). If immune function is condition-dependent in migrating birds, we expect that birds in poor condition to exhibit signs of immunosuppression. Given what we know about the above variables of immunological status, we expect birds in poor condition to have low leukocyte and lymphocyte counts and serum IgG titer and higher heterophil counts and H/L ratios. Furthermore, we expect a bird's immune response to PHA to be positively related to mass gain.

Materials and methods

The study was conducted during April and May of 1999–2001 at a stopover site for landbird migrants in southwest Louisiana (29°45′N, 93°37′W), near Johnson Bayou, Louisiana. The field site, located on the northern coast of the Gulf of Mexico, was within a narrow coastal woodland (chenier) surrounded by marsh. Coastal cheniers provide critical spring stopover habitat for birds having just traversed the Gulf of Mexico (Rappole and Warner 1976; Moore and Kerlinger 1987; Moore et al. 1995). The four focal species are abundant at the Johnson Bayou study site (see Wang 1993; Wang and Moore 1993, 1994, 1997); Wood Thrush were not sampled in 1999.

Thrushes were captured by mist nets (12 m × 2.6 m with 30 mm mesh) that were operated from 0700 to 1600 CST every day between 28 March and 7 May of each year of the study. Only first captures of individuals were included in the analysis. Thrushes were banded with a USFWS aluminum leg band (FRM USFWS permit no. 21221). Unflattened wing chord and body mass to the nearest 0.1 g were recorded. In addition, a blood sample (100–250 μL) was collected via the brachial vein into microhematocrit capillary tubes using a 26 gauge needle. Capillary tubes were placed upright in hematoseal and stored in a cooler at approximately 10 °C for 4–9 h. Blood was then spun in a clinical centrifuge for 9 min at 14 000 rev/min (5125*g*). Plasma was drawn from the capillary tubes using a Hamilton syringe, stored in a microcentrifuge tube, and frozen at –20 °C during the field season, and then at –70 °C following the field season until assays were performed.

Assessment of energetic condition

Three interrelated measures were used to assess energetic condition: (1) subcutaneous fat deposits were estimated according to a six-point scale (Helms and Drury 1960), (2) muscle mass present on the keel was estimated using a four-point scale (Bairlein et al. 1995), and (3) size-corrected condition index was calculated (see Ellegren 1989, 1992; Wang and Moore 1997). To calculate the size-corrected condition index, individuals from 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 the specific wing-chord class. We produced a second regression model by regressing fat-free mass on corresponding wing-chord lengths. Using the equation generated by the second regression model, we calculated size-specific fat-free mass for each individual. To obtain an index of energetic condition, we subtracted fat-free mass from actual body mass.

Variables of immunological status

To identify and count leukocytes, 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 counts. Absolute counts were all white blood cells, that is, lymphocytes, monocytes, heterophils, eosinophils, and basophils, per 100 fields of view (FOV), using oil immersion. Total leukocyte count is expressed as the number of leukocytes per 10 000 red blood cells. Differential counts were conducted using the same method. H/L ratio was calculated by dividing the number of heterophils per 100 FOV by the number of lymphocytes per 100 FOV.

We measured IgG titers using a sandwich ELISA (enzyme-linked immunosorbent assay) developed specifically for thrushes. Initially, IgY was isolated from *Catharus* thrush eggs (Pierce Eggcellent Chicken IgY Purification Kit). Antibodies were then developed by immunizing a rabbit with the purified *Catharus* thrush IgY. First, block titration ELISA assays were set up to determine the working concentration or dilution for each component of the ELISA. In the first step of the sandwich ELISA, a 96-well microtiter plate (Nunc, flat-bottomed, low evaporation) was coated with 50 μL of 30 μg/mL rabbit anti-*Catharus* thrush IgG (RaTH) antibodies in coating buffer, pH 9.6 (15 mmol/L sodium carbonate, 35 mmol/L sodium bicarbonate, and 3 mmol/L sodium azide). The plate was covered and incubated overnight at room temperature. The wells of the plate were then washed with wash buffer, pH 7.4 (8 mmol/L dibasic sodium phosphate, 1.1. mmol/L monobasic potassium phosphate, 137 mmol/L sodium chloride, 2.7 mmol/L potassium chloride, 3.0 mmol/L sodium azide, and 0.05% Tween 20), using an automated 96-well plate washer. In the second step, 5-fold serial dilutions (1:50, 1:250, and 1:1250) were made of individual plasma samples from captured birds in incubation buffer, pH 7.0 (10 mmol/L dibasic sodium phosphate, 7.8 mmol/L potassium phosphate, 137 mmol/L sodium chloride, 0.05% Tween 20). Wells on each plate were filled in triplicate with 50 μL of the diluted samples. Each plate was incubated for 30 min at 37 °C before washing the wells 5 more times. In the third step, 50 μL of biotin-labelled RaTH (biotin with an amino-linker added to purified RaTH; constructed in the laboratory) diluted 1:1000 in incubation buffer was added to each well. Plates were incubated for 30 min at 37 °C, then wells were washed again 5 times with wash buffer. In the fourth step, 50 μL of eAAP (ExtrAvidin-labelled alkaline phosphatase; Sigma, St. Louis, Missouri) diluted 1:9000 in incubation buffer was added to each well. The plate was incubated again for 30 min and the wash step repeated. In the fifth step, 50 μL of *p*-nitrophenyl phosphate (2 mg/mL; Sigma 104® phosphate substrate tablets in substrate buffer, pH 9.8 (9.7% diethanolamine and 0.5 mmol/L $MgCl_2 \cdot 6\ H_2O$)) was added to each well. Plates were covered and incubated at room temperature for 30 min. The color development was measured at a wavelength of 405 nm with a microtiter plate spectrophotometer (BIO-TEK Instruments ELx808 Microplate Reader).

A negative control was done for each sample by coating wells, in triplicate, with a coating buffer (alone) instead of RaTH. Nonspecific binding may occur in some samples depending on the concentration of other proteins in the serum. A standard curve was run in triplicate on two, separate 96-well plates as follows: IgY (300 μg/mL) purified from *Catharus* thrush eggs was assayed, at concentrations ranging from 22 to 2000 ng/mL, in triplicate with one negative control where only incubation buffer was added to the well. The interassay coefficient of variation was 14.0%. The absorbance of the unknown samples minus the absorbance for the negative control was plotted on semi-log standard curve. The concentrations of the unknown samples were determined by using the equation from the standard curve ($y = m \cdot \log x + b$) and the dilution factors of the concentrations.

We measured PHA-induced immune response in Swainson's Thrush and Wood Thrush. Immediately following capture, thrushes were brought into captivity and held in individual cages (30 cm × 38 cm × 38 cm) in an enclosed aviary located near the study site. All birds were fed ad libitum a mixed diet of yellow mealworms (*Tenebrio molitor* L., 1758), sand blackberry (*Rubus cuneifolius* Pursh), and moistened monkey biscuits (ZuPreem). Within 1–3 days following capture, the right and left wing webs (patagium) of each individual were measured three times to the nearest 0.01 mm using a pressure sensitive dial thickness gage. Phosphate-buffered saline (PBS; 0.05 mL) was injected into the right wing web as a control, while PHA (0.25 mg/0.05 mL PBS) was injected into the left wing web. Twenty-four hours following the injection, each wing web was measured again three times to the same level of precision. All injections and handling of the birds occurred 2–3 h after sunrise and this was kept constant throughout the study. The coefficient of variation within each of the three measurements was low (range 0.1%–3.0%); therefore, we took the mean of the three wing-web measurements to calculate PHA response. Birds were immediately released following the postinjection measurement. The PHA response was determined by subtracting the change in thickness for the right wing web (pre- to post-treatment thickness) from the change in thickness in left wing web (Lochmiller et al. 1993). Mass and fat score were recorded at the same time each day and corresponded with the timing of the injections and measurements. The methods described here were approved by the University of Southern Mississippi's Institutional Animal Care and Use Committee (protocol no. 06011201).

Data analysis

The three measures of energetic condition (fat score, muscle score, and condition index) were entered into a principal component analysis (Tabachnick and Fidell 1996). A separate analysis was conducted for each species. In each case, the first principal component (PC1) explained 60%–72% of the variance (see Table 1). The remaining two components only accounted for an additional 12%–24% of the variance. Loadings of the variables and percentage of variance for PC1 are shown in Table 1. The PC1 score (hereafter called condition score) was saved and used as a global measure of energetic condition for all subsequent analyses.

The majority of the birds passing through the study site leave on the same evening the day they arrive (J.C. Owen, unpublished data) and resume migration in a northward direction (Moore et al. 1990; Moore and Kerlinger 1992). There is very little east–west movement of migrants that stopover in these coastal woodlands. For example, the mean

Table 1. Factor loadings and percentage of variance for principal factors extraction on body condition variables for Swainson's Thrush (*Catharus ustulatus*), Wood Thrush (*Hylocichla mustelina*), Veery (*Catharus fuscescens*), and Gray-cheeked Thrush (*Catharus minimus*) captured at Johnson Bayou, Louisiana, 1998–2001.

Variable	Swainson's Thrush (factor loadings)	Wood Thrush (factor loadings)	Veery (factor loadings)	Gray-cheeked Thrush (factor loadings)
Fat	0.83	0.79	0.87	0.85
Muscle	0.79	0.82	0.85	0.87
Fatgrams	0.72	0.79	0.83	0.83
Percentage of variance	60.82	64.29	72.40	72.05

east–west movement of 24 radio-tracked Hooded Warblers (*Wilsonia citrina* (Boddaert, 1783)) during stopover was 150 m and never more than 500 m (Z. Nemeth, personal communication). During our study, 15%–20% of the birds captured stopover for more than 1 day, and among those individuals stopover length ranges from 2 to 9 days (J.C. Owen, unpublished data). The first capture of an individual is assumed to be on the day that they arrived, unless the capture time is prior to 1000 h in which case they likely arrived the previous day (Moore and Kerlinger 1987). We only included initial captures after 1000 h in the analyses to ensure that the birds we were capturing had just arrived.

Partial correlation was used to examine the relationship between the condition score and the different variables of immunological status while controlling for Julian date. Multivariate analyses were not performed owing to variation in sample sizes for some of the immunological variables. The amount of blood collected varied between individuals preventing us from conducting all the assays. Basophils represented less than 0.05% of the total leukocyte count for all four species and were not included in the analyses.

The relationship between energetic condition and PHA response was measured using a standard multiple regression analysis with PHA response as the criterion and days in captivity and change in mass as predictor variables. Time in captivity was included as a predictor variable because birds were held in captivity for a varied length of time and rest may also positively influence immune response (J.C. Owen, unpublished data). We used change in mass as a surrogate for energetic condition in this analysis because the measurement of PHA took place over a 24 h period at which time a bird's mass can change (J.C. Owen, unpublished data), which may influence their ability to mount an immune response. Change in mass was calculated by subtracting mass at time of second measurement from mass at capture.

Leukocyte data did not meet the assumptions of normality and were transformed: white blood cell count was log-transformed and all other variables were natural-log-transformed. The transformed variables met assumptions of normality and (or) passed visual inspection of normality. Because of the multiple univariate comparisons, we used a Bonferroni-corrected α value of 0.007 for all variables except PHA response. We used different individuals for the PHA injection, as PHA may alter an individual's leukocyte count and profile (Hõrak et al. 2000). The α value for PHA-induced immune response was set at 0.05. We performed all analyses with SPSS version 12.0 (SPSS Inc., Chicago, Illinois).

Results

A total of 646 birds of the four species were sampled during spring 1999–2001: Swainson's Thrush (n = 131), Wood Thrush (n = 273), Veery (n = 121), and Gray-cheeked Thrush (n = 102). Variability characterized the arrival condition of the four species. Over a third (38%) of the individuals captured at the study site were considered lean (fat score ≤ 1), whereas 62% of the birds (fat score ≥ 2) had sufficient fat stores to continue migration (cf. Moore and Kerlinger 1987; Wang and Moore 1997). The proportion of lean birds differed among species (Veery, 0.47; Gray-cheeked Thrush, 0.35; Wood Thrush, 0.35; Swainson's Thrush, 0.22; $\chi^2_{[3]} = 28.30$, $p < 0.0001$; Table 2). Veerys had significantly smaller fat stores compared with the other three species (Table 2). Furthermore, we found significant between-year differences in condition of Swainson's Thrush ($F_{[76,2]} = 3.49$, $p = 0.04$). Individual Swainson's Thrush were in significantly better condition in 2000 and 2001 than in 1999, so we controlled for year in species in the other analyses. There is a reasonable expectation that energetic condition will be better later in the season when conditions are more favorable for trans-Gulf flights (i.e., less inclement weather and more days with southerly winds; Buskirk 1980). Julian date was significantly related to condition score for two of the four species with all years combined (Table 3) and for three of the species in year 2000 alone (Veery, Swainson's Thrush, and Gray-cheeked Thrush). All the analyses control for Julian date using partial correlation.

We found interspecies variation in the relationship between variables of immune status and condition score. Leukocyte count was significantly higher in birds in better condition for three of the four thrush species (Table 3). Likewise, lymphocyte count was also positively correlated to the condition score in the same three species. Heterophil count was not significantly correlated to the condition score in any of the species (Table 3); neither was the H/L ratio, although it approached significance in the Gray-cheeked Thrush. Eosinophils were significantly, positively related to the condition score in Wood Thrush and approached significance in Veery and Gray-cheeked Thrush (Table 3). Monocyte number was only related to the condition score in Swainson's Thrush. Serum IgG level were not correlated to the condition score in any of the thrushes (Table 3); however, these results need to be considered with caution given the small sample size.

We assessed the relationship between total leukocyte count and condition score while controlling individually for the different blood cell types (Table 4). The relationship

Table 2. Mean (±SD) fat and muscle scores for four species of Neotropical landbird migrants (Swainson's Thrush (*Catharus ustulatus*), Wood Thrush (*Hylocichla mustelina*), Veery (*Catharus fuscescens*), and Gray-cheeked Thrush (*Catharus minimus*)) arriving at a stopover site on Gulf of Mexico coast of Louisiana, spring 1999–2001.

Species (*n*)	Mean (±SD) fat score	Mean (±SD) muscle score
Swainson's Thrush (131)	2.05±0.74a	2.02±0.39a
Wood Thrush (273)	1.92±0.73a	1.87±0.44b
Veery (121)	1.67±0.80b	1.48±0.60c
Gray-cheeked Thrush (102)	1.88±0.85a	1.78±0.57b

Note: Values with different letters are significantly different from one another based on a Dunnett's T3 post hoc procedure.

between total leukocyte count and condition score depended on lymphocyte count, whereas variation in other blood cell types, with the exception of monocytes in Swainson's Thrush and eosinophils in the Wood Thrush, did not alter the relationship.

We assessed the relationship between energetic condition and immune response to PHA in a separate sample of Wood Thrush and Swainson's Thrush and found that length in captivity and change in mass were not correlated: Swainson's Thrush, $r = -0.031$, $p = 0.410$; Wood Thrush, $r = -0.072$, $p = 0.287$. Both time in captivity and change in mass were significant predictors of wing-web index in both species (Swainson's Thrush — $F_{[2,54]} = 12.24$, $R^2 = 0.554$, adj. $R^2 = 0.307$, $p < 0.001$, Fig. 1; Wood Thrush — $F_{[2,63]} = 3.86$, $R^2 = 0.335$, adj. $R^2 = 0.112$, $p = 0.026$, Fig. 2). Change in mass ($t = 3.94$, $p < 0.001$) and days in captivity ($t = 2.86$, $p = 0.006$) significantly contributed to predicting PHA response in Swainson's Thrush (Table 5), whereas only time in captivity ($t = 2.67$, $p = 0.010$) significantly explained variation in PHA response in the Wood Thrush (Table 5). Change in mass accounted for 21% (partial r) and time in captivity accounted for 14% (partial r) of the observed variability in PHA response in the Swainson's Thrush model, while time in captivity was responsible for 10% (partial r) of the variability in PHA response in the Wood Thrush model (Table 6).

Discussion

Several indicators of immune function were related to energetic condition in thrushes captured during spring migration. Absolute leukocyte count was positively related to our condition index in all four species. Although elevated leukocyte count may be indicative of an infection (Gustafsson et al. 1994; Merila and Svensson 1995; Saino and Møller 1996; Saino et al. 1997; Soler et al. 1999), as a sufficient number of circulating leukocytes is essential for an organism to produce an immune response against a bacterial or parasite infection, it is more likely that a high number of leukocytes among birds in better energetic condition reflects an immunocompetent individual (Ots et al. 1998; Boonstra et al. 2001; Dubiec and Cichoñ 2001; Bachman 2003). Whereas leukocyte count is not a direct reflection of immune function, it does provide information on the preparedness of the immune system at a given point in time and may predict an animal's ability to mount an immune response, which is why leukocyte count has been used as a surrogate for assessing immune function (Boonstra et al. 2001; Bachman 2003).

Moreover, the relationship between leukocyte count and energetic condition is a consequence of the increase in lymphocytes, rather than the other blood cell types. The number of lymphocytes provides an indirect measure of acquired immune function (Blount et al. 2003). Three of the four species showed a significant, positive relationship between lymphocyte count and condition, suggesting that birds in poor energetic condition have reduced acquired immune function. Again, a high lymphocyte count may signal activation of the immune system in response to an infection (Campbell and Dein 1984; Moreno et al. 1998; Ots and Hõrak 1998), rather than an indication of immunocompetence. However, it is likely that the number of "unhealthy" individuals sampled was probably quite low given a clinically "sick" bird is less likely to endure the long, nonstop flight across the Gulf of Mexico. Furthermore, we argue that birds responding to an infection with an increase in populations of the various white blood cells will not be in better condition than uninfected individuals. The lymphocyte counts of the birds captured in good condition closely resemble the values observed in nonmigratory conspecifics (Owen and Moore 2006).

Low lymphocyte counts may reflect a redistribution of leukocytes. For example, Dhabhar and McEwen (1996) suggest that endocrine changes associated with acute stress may cause lymphocytes to leave circulation and sequester into peripheral organs and tissues, subsequently leading to enhanced immune function. Without knowing the internal state of the spleen and other immune organs, we cannot specifically address this issue. However, studies show that the spleen, a major site for lymphocyte storage and differentiation, is reduced during or immediately following the migratory period (Fänge and Silverin 1985; Deerenberg et al. 2003), particularly after crossing an ecological barrier (Deerenberg et al. 2003; J.C. Owen, unpublished data). Furthermore, lymphoid activity within the spleen is reduced (Fänge and Silverin 1985; Muñoz and De la Fuente 2003), which may suggest a less active or compromised immune system.

Reduction in circulating lymphocytes is frequently accompanied by an increase in heterophils (Gross and Siegel 1983; Maxwell 1993; Bachman 2003), reflecting a redistribution of leukocytes to other branches of the immune system (i.e., innate immune system) rather than overall immunosuppression. This redistribution enhances the innate immune system while the acquired system is being downregulated. Despite the decrease in lymphocyte number with poor body condition, we did not observe higher heterophil counts or H/L ratios in birds in poor condition. Thrushes captured during spring migration have higher H/L ratios compared with nonmigrating conspecifics (Owen and Moore 2006), which suggests that birds are generally more stressed during the migratory period than other phases of the annual cycle. Furthermore, we would argue that variation in H/L ratio is responding to something other than energetic condition because we did not find a relationship between heterophil count and H/L ratio and energetic condition. In short, a reduced leukocyte count probably does not reflect a stress re-

Table 3. Simple zero-order correlations and first-order correlations between condition score and variables of immune status while controlling for Julian date in Swainson's Thrush (*Catharus ustulatus*), Wood Thrush (*Hylocichla mustelina*), Veery (*Catharus fuscescens*), and Gray-cheeked Thrush (*Catharus minimus*) captured during spring migration in Johnson Bayou, Louisiana, 1999–2001.

		Condition score		Julian date		Condition × Julian date	
Variable species	*n*	*r*	*p*	*r*	*p*	*r*	*p*
Condition score							
Swainson's Thrush	65	1.00	—	0.19	0.13	—	—
Wood Thrush	106	1.00	—	–0.05	0.60	—	—
Veery†	91	1.00	—	0.26	0.01	—	—
Gray-cheeked Thrush†	64	1.00	—	0.37	0.002	—	—
Total leukocyte count							
Swainson's Thrush	65	0.31	0.01	0.06	0.64	0.28*	0.02
Wood Thrush†	106	0.34	<0.001	0.14	0.14	0.35	<0.001
Veery†	84	0.45	<0.001	0.35	0.001	0.40	<0.001
Gray-cheeked Thrush†	62	0.44	<0.001	0.13	0.32	0.43	<0.001
Heterophil/lymphocyte ratio							
Swainson's Thrush	63	–0.14	0.26	0.11	0.38	–0.20*	0.12
Wood Thrush	105	–0.12	0.23	0.06	0.54	–0.12	0.24
Veery	78	–0.17	0.14	–0.09	0.44	–0.15	0.19
Gray-cheeked Thrush	61	–0.34	0.007	–0.11	0.39	–0.32	0.01
Lymphocytes							
Swainson's Thrush	65	0.34	0.005	0.07	0.59	0.31*	0.01
Wood Thrush†	106	0.44	<0.001	0.12	0.21	0.45	<0.001
Veery†	88	0.51	<0.001	0.26	0.01	0.47	<0.001
Gray-cheeked Thrush†	64	0.46	<0.001	0.19	0.13	0.43	<0.001
Heterophils							
Swainson's Thrush	63	0.13	0.31	0.20	0.12	0.04*	0.74
Wood Thrush	105	0.24	0.01	0.16	0.10	0.25	0.009
Veery	87	0.20	0.06	0.15	0.16	0.17	0.11
Gray-cheeked Thrush	63	0.13	0.29	0.12	0.33	0.09	0.47
Monocytes							
Swainson's Thrush†	40	0.36	0.003	–0.02	0.85	0.35*	0.004
Wood Thrush	106	–0.08	0.40	0.08	0.39	–0.08	0.43
Veery	91	0.11	0.28	0.19	0.06	0.07	0.53
Gray-cheeked Thrush	43	–0.004	0.91	–0.25	0.04	0.10	0.44
Eosinophils							
Swainson's Thrush	65	0.18	0.16	0.02	0.90	0.13*	0.30
Wood Thrush†	106	0.31	0.001	0.01	0.90	0.32	0.001
Veery	91	0.26	0.01	0.22	0.04	0.22	0.04
Gray-cheeked Thrush	64	0.31	0.01	–0.06	0.66	0.31	0.01
Serum IgG							
Swainson's Thrush	15	–0.34	0.18	0.19	0.48	–0.38	0.14
Wood Thrush	35	0.12	0.48	0.01	0.96	0.12	0.47
Veery	38	0.03	0.84	0.41	0.008	–0.18	0.26
Gray-cheeked Thrush	27	0.13	0.50	0.18	0.35	0.05	0.78

Note: A Bonferroni-corrected α level was set at 0.007 for two-tailed comparisons with leukocyte data.
*Coefficient adjusted for year (second-order correlation coefficient).
†Significant based on $\alpha = 0.007$.

sponse and a redistribution of leukocytes, but rather reflects a suppressed immune system among birds unable to meet the energetic demands of migration.

A significant, positive relationship between eosinophil count and condition was found only in Wood Thrush, although a similar trend was observed in Veerys and Gray-cheeked Thrush. The relationship between total leukocyte count and condition in Wood Thrush is probably a function of variation in both eosinophils and lymphocytes. Although the function of the avian eosinophil is not well understood, its role may be similar to the mammalian eosinophil (Campbell 1995), including phagocytosis and parasiticidal activity. Unpublished data indicate that birds captured during the breeding season have higher eosinophil counts than non-

Table 4. The relationship between total leukocyte count and condition score while controlling for the four different types of leukocytes in Swainson's Thrush (*Catharus ustulatus*), Wood Thrush (*Hylocichla mustelina*), Veery (*Catharus fuscescens*), and Gray-cheeked Thrush (*Catharus minimus*) captured during spring migration in Johnson Bayou, Louisiana, 1999–2001.

	Swainson's Thrush		Veery		Wood Thrush		Gray-cheeked Thrush	
Control variable	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Zero correlation	0.31	0.01	0.45	<0.001	0.34	<0.001	0.44	<0.001
Lymphocytes	0.06	0.64*	0.16	0.16*	–0.03	0.76*	–0.06	0.63*
Heterophils	0.27	0.03	0.37	0.001	0.32	0.001	0.40	0.001
Eosinophils	0.26	0.04	0.38	<0.001	0.18	0.06*	0.31	0.01
Monocytes	0.17	0.29*	0.39	0.004	0.39	<0.001	0.47	0.002

*Significant based on $\alpha = 0.05$

Fig. 1. Cell-mediated immune response as measured by phytohemagglutinin injection as a function of change in mass and stopover length for Swainson's Thrushes (*Catharus ustulatus*) captured at a spring stopover site in southwest Louisiana, 2000–2001. Cell-mediated immune response positively and significantly increased with both an increase in mass and stopover length. The multiple linear regression equation was $y = 0.71$ (SE 0.09) + 0.05 (SE 0.01) × mass + 0.14 (SE 0.05) × days.

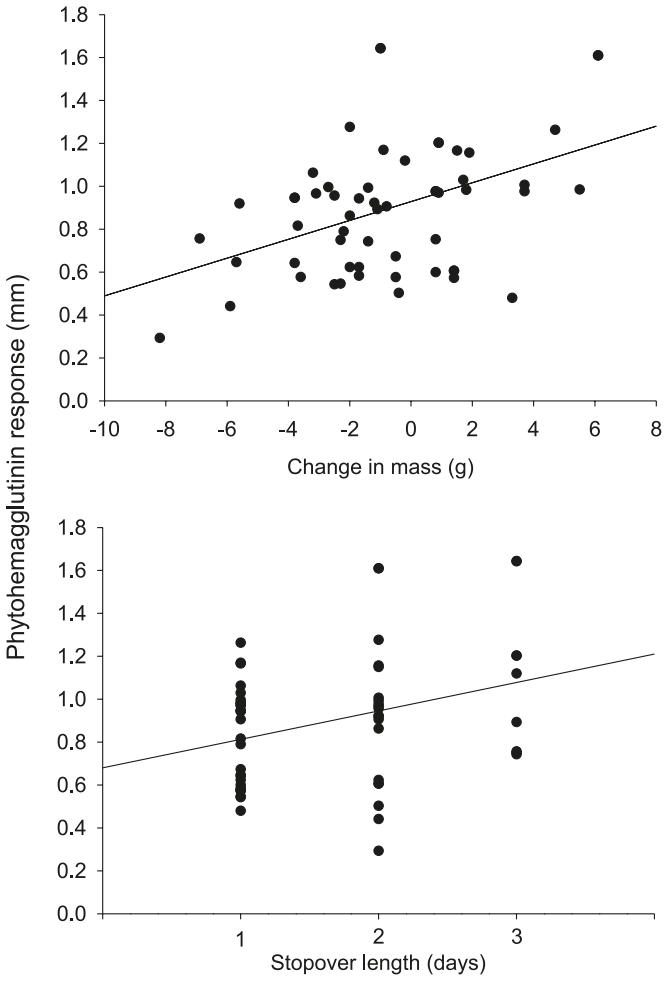
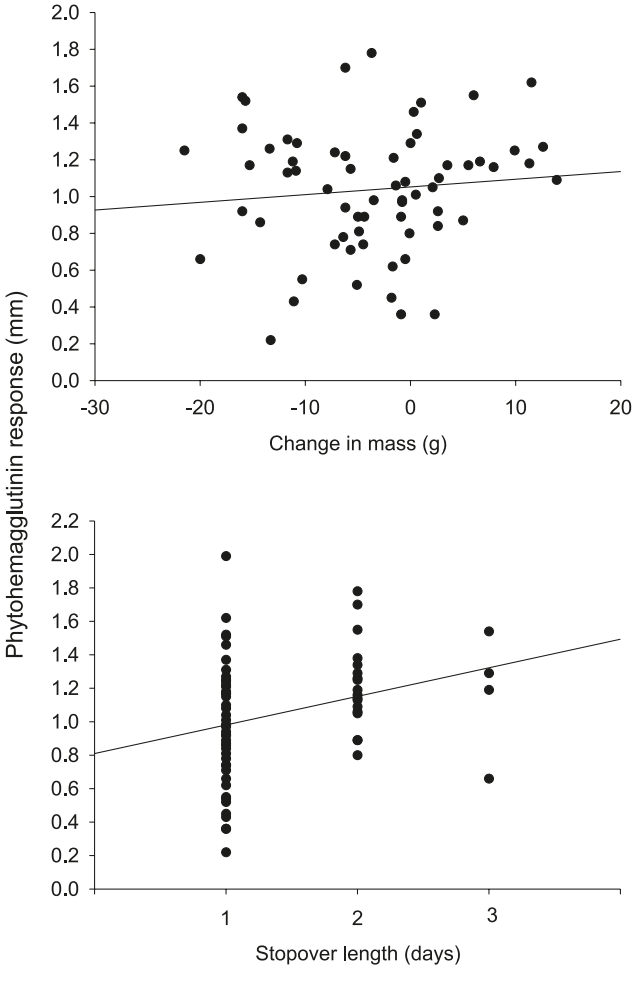


Fig. 2. Cell-mediated immune response as measured by phytohemagglutinin injection as a function of change in mass and stopover length for Wood Thrushes (*Hylocichla mustelina*) captured at a spring stopover site in southwest Louisiana, 2000–2001. Cell-mediated immune response positively and significantly increased with both an increase in mass and stopover length. The multiple linear regression equation was $y = 0.80$ (SE 0.10) + 0.005 (SE 0.005) × mass + 0.18 (SE 0.07) × days.



migratory conspecifics (J.C. Owen, unpublished data). Among the four species studied, Wood Thrush are closest to their breeding grounds, which may explain the higher eosinophil count among birds in better condition.

Swainson's Thrush monocyte count was positively correlated with condition, but it does not appear to be a driving factor in the relationship between leukocyte count and condition. Monocytes are involved in both the innate and ac-

Table 5. Standard multiple regression analysis summary for phytohemagglutinin response as the criterion and change in mass and time in captivity as predictors for Swainson's Thrushes (*Catharus ustulatus*) and Wood Thrushes (*Hylocichla mustelina*) captured during spring migration in Johnson Bayou, Louisiana, 1999–2001.

Variables	*B* (SE)
Swainson's Thrush (n = 62, R^2 = 0.55, intercept (SE) = 0.71 (0.09))	
Change in mass (g)	0.05 (0.01)*
Time in captivity (days)	0.14 (0.05)*
Wood Thrush (n = 67, R^2 = 0.335, intercept (SE) = 0.80 (0.10))	
Change in mass (g)	0.005 (0.005)
Time in captivity (days)	0.18 (0.07)*

Note: *B*, slope of the sample regression line.
*$p < 0.01$.

Table 6. Means and standard deviations of PHA response for two species of intercontinental migrants (Wood Thrushes (*Hylocichla mustelina*) and Swainson's Thrushes (*Catharus ustulatus*)) held in captivity for 1–3 days.

		PHA response	
Days in captivity	*n*	Mean	SD
Wood Thrush			
1	45	0.97	0.37
2	18	1.22	0.26
3	4	1.17	0.37
Swainson's Thrush			
1	31	0.82	0.26
2	21	0.89	0.36
3	10	1.13	0.34

quired immune system, and like eosinophils, are rare in the circulation relative to the more common heterophils and lymphocytes. Typically an increase in monocyte numbers reflects a chronic infection and frequently coincides with an increase in heterophils (Campbell 1995). Note that birds captured during the breeding season have higher eosinophil counts than migrating individuals (J.C. Owen, unpublished data).

IgG titer, an indirect measure of acquired, humoral immunity, was not related to energetic condition, which is not surprising (cf. Lochmiller et al. 1993; Møller and Petrie 2002). The half-life of IgG is approximately 20 days, so IgG titers may reflect circumstances encountered weeks earlier rather than the current state of the individual. Migrants sampled in our study were captured soon after a long, nonstop flight across an ecological barrier and their energetic state upon capture is almost surely a reflection of that experience.

The immune response to a PHA challenge was positively related to time and change in mass over time among Wood Thrush, and change in mass alone among Swainson's Thrush (i.e., birds gaining more mass had higher PHA responses). Lifjeld et al. (2002) also reported fluctuations in PHA response with short-term changes in body mass, consistent with the notion that birds became immunosuppressed following periods of food deprivation and associated mass loss. Both Lochmiller et al. (1993) and Gonzalez et al. (1999) found that birds maintained on protein-restricted diets had reduced cell-mediated immune responses to PHA. Likewise, Møller and Petrie (2002) demonstrated that body condition, expressed as residual body mass, was positively correlated with cell-mediated immune response in Common Peafowls (*Pavo cristatus* L., 1758). Our results point to the importance of rest on the immune system, an area of research needing further attention.

Our finding that migrating thrushes in poor energetic condition have reduced immunocompetence (i.e., low leukocyte and lymphocyte counts) is contrary to that of Merila and Svensson (1995), who found no relationship between an index of leukocyte count (buffy coat layer) and subcutaneous fat stores in migrating Goldcrests (*Regulus regulus* (L., 1758)). Aside from the fact that Goldcrest are intracontinental migrants and do not engage in long distance, nonstop flights over ecological barriers, we would argue that a better indicator of body condition includes both fat stores and muscle mass (see our condition score). Depletion of fat stores during migration is not necessarily indicative of poor condition, rather reflects a natural consequence of energy consumption. Depleted muscle, however, is indicative of protein catabolism (Biebach 1998). During extreme periods of fasting, proteins from skeletal muscle and organs may be broken down as an energy source. Production of new proteins is energetically demanding and requires a significant input of amino acids. Therefore, the availability of amino acids may be a limiting factor in synthesis of proteins for the immune system (Lochmiller and Deerenberg 2000).

Although migrating birds in good condition have high leukocyte and lymphocyte counts, we do not necessarily know if they are better at resisting disease than individuals with depressed counts. To our knowledge, no studies have examined the functional significance (see Viney et al. 2005) of variation in leukocyte profiles. A few studies have examined the relationship of leukocyte numbers and a bird's infection status (Ferris and Bacha 1986; Davis et al. 2004). For instance, House Finches (*Carpodacus mexicanus* (Statius Muller, 1776)) infected with *Mycoplasma gallisepticum* have higher H/L ratios, leukocyte, heterophil, and monocyte counts than uninfected individuals (Davis et al. 2004). Yet, these studies do not provide information on whether birds with a particular number of circulating leukocytes are at an advantage, immunologically, than an individual with lower counts. Hõrak et al. (1998) found that Great Tit (*Parus major* L., 1758) nestlings with higher PHA response and higher lymphocyte counts had higher survival (but see Dubiec et al. 2005). This question about functional significance takes on added significance in light of the array of parasites and pathogens to which long-distance, intercontinental migrants like our study species are exposed during their annual cycle.

The four species in the present study each have different migration strategies that may account for the few differences observed in the relationships between condition and variables of immune function. However, until we know more about the origin and destination of the migratory birds stopping over following trans-Gulf migration, we cannot say with any certainty how far they have travelled from their wintering areas or the distance left to travel before they reach their breeding grounds in relation to energetic condition and immunocompetence. We do argue that, when re-

sources are limited and an individual is unable to compensate for increased demands, a trade-off between immune defense and another activity may ensue (e.g., Bachman 2003). A migratory bird able to cross the Gulf of Mexico without depleting lipid and protein stores is better able to sustain its immune function during the migratory period than a migrant that has consumed fat stores and catabolized protein reserves. Moreover, a bird that departs with fat stores insufficient to meet energetic demands may arrive immunocompromised. The opportunity to rest and refuel during stopover is not only necessary for a successful migration vis-à-vis energetic demand, but also impacts a migrating bird's ability to defend itself against novel pathogens and parasites.

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