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RESEARCH ARTICLE

Fruits and migrant health: Consequences of stopping over in exotic- vs. native-dominated shrublands on immune and antioxidant status of Swainson's Thrushes and Gray Catbirds

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ABSTRACT

Migration is a physiologically demanding activity. Recent studies suggest that migrating birds can improve their immune and antioxidant status during stopover, implying that variation in stopover habitat can affect migrants' health. We studied 2 species that are strongly frugivorous during fall migration, Swainson's Thrushes (*Catharus ustulatus*) and Gray Catbirds (*Dumetella carolinensis*). We asked whether birds that used shrubland dominated by exotic plants experienced differences in mass change, immune function, and antioxidant status relative to conspecifics in native-dominated shrubland during fall stopover in Michigan, USA, in 2012–2013. We found no habitat-related differences in any of the measured health parameters for Swainson's Thrushes. However, Gray Catbirds using native-dominated shrubland retained mass while those in exotic-dominated shrubland lost mass in 2013. Gray Catbirds in exotic-dominated habitat that year also had poorer immune status (elevated granulocyte:lymphocyte ratio and reduced hemagglutination and haptoglobin) and had lower plasma total carotenoids (immunostimulatory antioxidants) relative to conspecifics in native-dominated shrubland. While these findings were not replicated in 2012, circulating antioxidant capacity was lower in both years in Gray Catbirds captured in exotic habitat. Habitat-specific estimates of dietary energy, carotenoid, and antioxidant content per unit mass of fruit were similar between habitat types for Swainson's Thrushes. For Gray Catbirds, however, the fruit diet was lower in carotenoids and antioxidant capacity, but not in energy, in exotic habitat. Our results provide evidence that differential use of stopover habitats may affect the immune and antioxidant status of migrating landbirds. Furthermore, our results suggest that habitat may affect health status based on the complex relationships among quality and abundance of food, food preferences, and refueling performance, which vary among species. We suggest that future studies measure immune and antioxidant metrics in addition to refueling performance to better understand the effects of stopover habitat use on landbird migrant health. This information may improve assessment of habitat quality for migrants.

Keywords: Ecoimmunology, conservation physiology, habitat quality, invasive shrub, constitutive immunity, carotenoids, antioxidants, bird migration

Frutas y salud de los migrantes: consecuencias de las paradas en matorrales dominados por nativas vs. exóticas sobre el estado inmunológico y antioxidante de *Catharus ustulatus* y *Dumetella carolinensis*

RESUMEN

La migración es una actividad fisiológicamente demandante. Estudios recientes sugieren que las aves que migran pueden mejorar su estado inmunológico y antioxidante durante las paradas, sugiriendo que la variación en el hábitat de las paradas puede afectar la salud de los migrantes. Estudiamos dos especies que son fuertemente frugívoras durante la migración de otoño, *Catharus ustulatus* y *Dumetella carolinensis*. Nos preguntamos si las aves que usan matorrales dominados por plantas exóticas experimentan diferencias en cambio de masa, función inmunológica y estado antioxidante en relación a individuos de las mismas especies en matorrales dominados por plantas nativas, durante sus paradas de otoño de 2012–2013 en Michigan, EEUU. No encontramos diferencias relacionadas con el hábitat en ninguno de los parámetros medidos de salud en *C. ustulatus*. Sin embargo, los individuos de *D. carolinensis* en el hábitat dominado por nativas se reabastecieron mejor que las aves en el hábitat dominado por exóticas en 2013. En los hábitats dominados por exóticas, *D. carolinensis* perdió masa, tuvo un estado inmunológico más pobre (elevada relación granulocito:linfocito y hemaglutinación y haptoglobina reducida) y presentó carotenoides globales en plasma más bajos (antioxidantes inmunoestimulantes) con relación a los individuos de la misma especie presentes en el matorral dominados por nativas. Mientras que estos resultados no se replicaron en 2012, en ambos años la capacidad de antioxidante circulante fue más baja en los individuos de *D. carolinensis* capturados en el hábitat exótico. Las

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estimaciones específicas para cada hábitat de energía en la dieta y contenido de carotenoide y antioxidante por unidad de masa de fruta fueron similares para *C. ustulatus* entre los tipos de hábitat. Para *D. carolinensis*, sin embargo, la dieta de fruta fue más baja en carotenoides y capacidad antioxidante, pero no en energía, en el hábitat exótico. Nuestros resultados brindan evidencia de que el uso diferencial del hábitat de parada puede impactar el estado inmunológico y antioxidante de las aves terrestres migratorias. Más aún, nuestros resultados sugieren que el hábitat puede afectar en base a las relaciones complejas entre calidad y abundancia de alimento, preferencia de alimento y desempeño de reabastecimiento, lo que varía entre especies. Sugerimos que futuros estudios midan métricas inmunológicas y antioxidantes además del desempeño de reabastecimiento, para entender mejor los efectos del uso del hábitat de parada en la salud de las aves terrestres migrantes. Esta información puede mejorar la evaluación de la calidad de hábitat para los migrantes.

Palabras calve: antioxidantes, arbusto invasivo, calidad de hábitat, carotenoides, ecoinmunología, fisiología de la conservación, inmunidad constitutiva, migración de aves

INTRODUCTION

Migration involves major modulations of physiological status, and migrating birds may make tradeoffs between immune defense and the high energy demands of endurance flight (reviewed by Piersma 1997, Altizer et al. 2011). During migration, the spleen, a principal immune organ in birds, shrinks in size, and circulating leukocyte counts are reduced relative to levels during the breeding season (Fänge and Silverin 1985, Deerenberg et al. 2002, Owen and Moore 2006). Downregulation of immune activity and reactivation of latent infection have also been found in individuals with experimentally induced migratory disposition (Gylfe et al. 2000, Owen and Moore 2008b). While migration or prolonged flight may not affect the strength of induced immune responses or the level of baseline acquired immunity, constitutive (noninduced) immunity may become suppressed (Hasselquist et al. 2007, Hegemann et al. 2012, Nebel et al. 2012, 2013, Eikenaar and Hegemann 2016). Migrating birds in poor energetic condition (i.e. low fat storage) may experience a greater risk of infection (Owen and Moore 2008a). Furthermore, large quantities of tissue-damaging reactive oxygen species are produced as a consequence of strenuous flight and hyperphagia (i.e. refueling), and insufficient protection by antioxidants could lead to oxidative stress (Costantini et al. 2007, Jenni-Eiermann et al. 2014, Skrip et al. 2015, Eikenaar et al. 2016, 2017). Migrants may upregulate their enzymatic antioxidants and retain catabolically produced uric acid for defense, but acquisition of dietary antioxidants during stopover is likely important as well (Jenni-Eiermann et al. 2014, Skrip et al. 2015, Eikenaar et al. 2016, 2017). It has also been proposed that migratory stopover may involve recovery or upregulation of immune function (Owen 2004, Owen and Moore 2008a, Buehler et al. 2010, Nebel et al. 2012). The sole study to test this idea using repeated samples in the field reported concurrent increases in body mass and leukocyte counts during stopover following crossing of the Gulf of Mexico (Owen 2004). A migrant's ability to locate high-quality stopover sites has clear fitness consequences (Smith and Moore 2003, Moore et al. 2005). Understanding the factors that influence migrating birds' health, including immune and antioxidant status, as they face anthropogenic landscape changes has been identified as a research priority for avian ecophysiologists (Klaassen et al. 2012).

Habitat selection may have an effect on health when dietary quality differs between habitats. Here, we focus on the fruit quality of exotic and native shrubs as part of a larger study to evaluate the effect of the proportion of exotic vs. native shrubs on stopover habitat quality. The presence of exotic shrubs resulting from human land-use change is a significant conservation concern for migrating landbirds (Catling 2005, Ewert et al. 2015). For example, fruits of exotic plants common in the Midwestern and Northeastern USA, such as autumn olive (*Elaeagnus umbellata*), common buckthorn (*Rhamnus cathartica*), and honeysuckle (*Lonicera* spp.), have higher sugar and water content but lower fat, and consequently lower energy content, compared with their native counterparts such as northern spicebush (*Lindera benzoin*), gray dogwood (*Cornus racemosa*), and Virginia creeper (*Parthenocissus quinquefolia*; Appendix Table 3; White 1989, Smith et al. 2007, 2013). Recent work by Smith et al. (2015) showed poorer refueling in exotic- compared with native-dominated shrubland using plasma metabolite profiling. However, refueling is only one aspect contributing to health, and we know little about the effect of consuming exotic fruits on the immune and antioxidant status of migrants.

Feeding on exotic fruits may be suboptimal for migrants if doing so confers only limited refueling success. Poor energetic condition during stopover has been linked to weakened immune function and circulating antioxidant capacity (Costantini et al. 2007, Owen and Moore 2008a, Buehler et al. 2010, Skrip et al. 2015). But at least some exotic fruits have high antioxidant content, including high carotenoids, which are important immune stimulators in birds (McGraw and Ardia 2003, McGraw et al. 2006, Simons et al. 2012, Alan et al. 2013); hence, some exotic fruits may be important carotenoid sources for landbird

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FIGURE 1. Representative images of stopover habitats used by fall landbird migrants near East Lansing, Michigan, USA: (**A**) shrubland dominated by exotic plants (exotic shrubland), and (**B**) shrubland dominated by native plants (native shrubland).

migrants. Because birds select fruits based on various characteristics, including fat, energy, and antioxidant content (Alan et al. 2013, Bolser et al. 2013, Pierce and McWilliams 2014), multiple physiological parameters are necessary to assess the overall health effects of exotic habitat use.

We investigated whether the health status of 2 migrating bird species, the Swainson's Thrush (*Catharus ustulatus*) and the Gray Catbird (*Dumetella carolinensis*), differed depending on their use of exotic- vs. native-dominated shrubland. If health status is limited by migratory flight and changes in response to refueling in stopover habitat, a habitat in which birds gain more mass and have greater immune and antioxidant status could be considered to be of superior quality. Additionally, we tested whether the diets of birds using exotic- vs. native-dominated habitats differed in energy, carotenoids, and antioxidant capacity.

METHODS

Study Site and Shrubland Habitats

We conducted fieldwork during the fall migrations of 2012 and 2013 on a state-managed wildlife area (Rose Lake State Wildlife Research Area, 42.81°N, 84.38°W) near East Lansing, Michigan, USA, where exotic- and native-dominated shrublands occurred as distinct patches within an agricultural and suburban landscape (Oguchi 2015). The 2 shrubland types were largely separated by mature oak–maple (*Quercus–Acer*) woodland up to 250 m wide. Owing to the proximity of the 2 habitat types, it is unlikely that individuals in these shrublands represented different populations. Hence, carryover effects from past stopover sites, if present, would have affected measured parameters similarly between individuals captured in exotic and native shrublands. Exotic stems comprised ~70% of all stems in exotic-dominated shrubland (hereafter exotic shrubland; Figure 1A), whereas ~80% of stems were native shrub species in the native-dominated shrubland (hereafter native shrubland; Figure 1B; Oguchi 2015). Exotic shrubland was composed largely of *E. umbellata* (stems: 23%), *Lonicera* spp. (22%), and *Rosa multiflora* (multiflora rose; 13%), all introduced from Eurasia. Native shrubland was primarily composed of *C. racemosa* (stems: 20%), *Ilex verticillata* (common winterberry; 17%), *L. benzoin* (12%), and *Toxicodendron vernix* (poison sumac; 9%).

Study Species and Known Patterns of Their Habitat Use

We selected Swainson's Thrushes and Gray Catbirds as focal species due to their abundance in both habitat types and because they include a high percentage of fruit in their diets during fall migration (Parrish 1997). Based on fecal samples, the diets of both species were composed of >90% fruit in our study site (Oguch 2015). Swainson's Thrushes are intercontinental migrants (Mack and Yong 2000) and use our study site only as a stopover site on their annual migrations (Y. Oguchi, and J. C. Owen personal observations). Gray Catbirds are intracontinental migrants (Smith et al. 2011), and some individuals breed locally while others stop at our site during migration (Y. Oguchi and J. C. Owen personal observations). To avoid sampling catbirds of local origin, we excluded individuals undergoing extensive prebasic molt or those still retaining juvenal undertail coverts. We also conducted banding activities ~4 days per week throughout summer using the same net sites; all catbirds banded during these operations were excluded from the present study.

Given the proximity of the 2 shrublands, some birds may have switched sites, confounding assignment to shrubland type. In a radio-telemetry dataset of 141 Swainson's Thrush ($n = 12$ birds) and 203 Gray Catbird ($n = 13$ birds) locations in our study site, habitat switching occurred by 3 individuals of each species (9 times in thrushes; 8 times in catbirds), and switching within the

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same morning was detected only once per species. Durations of continuous use estimated from radiotelemetry data were ~2 days in exotic shrubland (both species) and ~3 days and ~5 days in native shrubland for thrushes and catbirds, respectively, with the use of either habitat type typically separated by use of the woodland separating the sites for ≤1 day. These lengths of use match the timescale at which the health parameters that we measured have been found to change. For example, ≤3 days were required for leukocyte counts and their profiles to change during stopover (Owen 2004), and ≤2 days were enough for other measures of constitutive immunity to vary between rested and exercised birds (Nebel et al. 2012). Circulating antioxidant levels can increase throughout the morning, likely in response to feeding (carotenoids; Hõrak et al. 2004). Because it is still possible that some birds had switched habitats just prior to sampling, we consider any habitat-related differences in health parameters to be conservatively estimated.

Mist-netting and Blood Collection

Birds were captured passively using 30 mist nets (15 per habitat; 30-mm mesh, 12.0 m × 2.6 m) from August 14 to September 30 in 2012 and from August 18 to September 30 in 2013. Nets in exotic and native shrublands were at least 250 m apart, divided by mature oak–maple woodland. Nets in both habitats were checked simultaneously at 45-min intervals beginning 30 min before sunrise for 4–5 hr daily. Capture time (±1 min) was recorded as the time that the bird was first detected in the net. We held birds in individual cloth bags or bird boxes (with paper liners for fecal sampling) until banding and blood collection. Birds were banded with U.S. Geological Survey aluminum bands and aged as hatch-year (HY) or after-hatch-year (AHY) using plumage or skull ossification (Pyle 1997). Both species are sexually monomorphic (Pyle 1997), so sex could not be determined. We measured unflattened wing chord (±1 mm) and mass (±0.1 g), and visually assigned fat scores (based on furcular and abdominal fat storage) on a scale of 0–6 in 0.5 increments (modified from Helms and Drury 1960) and pectoral muscle scores on a scale of 0–3 in 0.5 increments (modified from Bairlein 1995).

We collected ≤300 μL of blood (<1% of body mass, representing <10% of blood volume; see Owen 2011) from the brachial vein directly into heparinized capillary tubes. Upon blood collection, we made a single cell-layer slide which was air dried, fixed in absolute methanol, and later stained with Wright-Giemsa solution (Hema 3 22-122911, Fisher Scientific, Pittsburgh, Pennsylvania, USA). The remaining blood was stored over ice in the field until centrifuged, and plasma was stored at –80°C until use.

Handling time (the difference between the time of finding the bird in the net and sampling) was 81 ± 2 min in 2012 and 60 ± 2 min in 2013. While we recognize that delays in blood sampling and the uncertainty of the exact time of capture likely influenced our measured blood analyte values and introduced noise into our dataset, we still consider our habitat comparisons to be valid. First, net checks were synchronized in the 2 habitat types; thus, sampling was identical between habitats with respect to time in the net. Second, handling time did not differ between habitat types for any of our comparison groups (2-sample *t*-tests; Swainson's Thrush, 2012: $t_{154.29} = 0.8$, $P = 0.45$; 2013: $t_{226.80} = 0.7$, $P = 0.46$; Gray Catbird, 2012: $t_{72.25} = 0.0$, $P = 1.00$; 2013: $t_{85.77} = -1.3$, $P = 0.20$). Finally, most analytes that we assayed (see below) are little affected by delays in sampling or are known to change predictably. For instance, total leukocyte counts (Davis 2005) and haptoglobin level (Buehler et al. 2010) may decline steadily with handling stress, but the granulocyte:lymphocyte ratio may be robust up to at least 1 hr (Davis et al. 2008) and hemolysis and hemagglutination for at least 2 hr (Buehler et al. 2008). Antioxidant capacity measured by the OXY-adsorbent test would show no handling effect within the first 30 min (sequential sampling; Costantini et al. 2007) or between 20 and 162 min (regression; Skrip et al. 2015). Less is known about circulating carotenoids, but, given that their antioxidant property contributes to the antioxidant capacity measured by the OXY-adsorbent test (Skrip and McWilliams 2016), we would not anticipate that their levels would vary wildly in response to handling. We included known handling time in all full models of analytes to control for its potentially confounding effects.

Indexing Constitutive Innate Immunity

We focused on the cellular (leukocytes) and humoral (plasma protein) components of the constitutive innate immune branch because they represent the first lines of defense for migrants encountering novel pathogens and are deemed most relevant to short stopovers during which acquired immunity is too slow to respond (Schmid-Hempel and Ebert 2003, Buehler et al. 2010). All measures that we used have been proposed to recover or be upregulated during stopover by at least one previous study (Owen 2004, Owen and Moore 2008a, Buehler et al. 2010, Nebel et al. 2012).

Total leukocyte count and granulocyte:lymphocyte ratio. Total leukocyte count has been widely used as a measure of immunological readiness (e.g., Campbell and Dein 1984, Kilgas et al. 2006a, Owen and Moore 2006, Buehler et al. 2010), and various stressors (Davis et al. 2008), including migration (Owen and Moore 2006, 2008a), have been linked to elevated heterophil:lymphocyte (H:L) or granulocyte:lymphocyte (G:L) ratios. We performed differential leukocyte counts on blood smears by observing 100 fields of view (each containing ~200 erythrocytes) at 1,000× magnification (Campbell and Dein 1984, Owen and Moore 2006). Total leukocyte count was

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obtained as the tally of all leukocyte types (heterophil, eosinophil, basophil, lymphocyte, and monocyte) divided by 2 and is hence expressed as the number of leukocytes per 10,000 erythrocytes. The G:L ratio was calculated by dividing the number of granulocytes (heterophils, eosinophils, and basophils) by the number of lymphocytes.

Hemolysis and hemagglutination activities. We modified the hemolysis–hemagglutination assay of Matson et al. (2005) to index the ability of natural antibodies to agglutinate foreign erythrocytes and to activate the complement system, which results in lysis and hence visible clearing of the blood cell suspension. Briefly, 10 μL of plasma in column 2 of a round-bottomed, 96-well plate (Corning Coster 3795, Corning, New York, USA) was serially diluted (1:2) in 10 μL of 0.01 M phosphate buffered saline (PBS; Sigma-Aldrich P3744, St. Louis, Missouri, USA) through column 12 (2–2,048-fold dilution). A 1% rabbit erythrocyte suspension (10 μL; prepared from HemoStat Laboratories RBA050, Dixon, California, USA) was added to each well. Following incubation at 37°C for 90 min, plates were tilted at a 60° angle at room temperature for 20 min and scanned twice (once for hemagglutination scoring, and a second time for hemolysis after an additional 70 min) using a flatbed scanner (300 dpi). Scores were assigned as the last well (highest dilution) exhibiting the respective activity ($\log_2$-transformed measures of strength). PBS (10 μL) in column 1 served as a negative control, and chicken serum in row 1 of each plate served as a quality-control standard.

Haptoglobin. Haptoglobin is an acute phase protein that binds to freely circulating heme (iron) preventing it from becoming a nutrient to pathogens (Delers et al. 1988), and its baseline level generally predicts readiness of an acute phase (fever) response (Matson et al. 2012). In addition, because heme induces free radical formation, the role of haptoglobin as an antioxidant (Miller et al. 1997) may be important during migration. We assayed haptoglobin using a commercially available kit (Tri-Delta Diagnostics TP801, Morris Plains, New Jersey, USA) following directions provided by the manufacturer, with minor modifications: We halved the volumes of all samples and reagents so that the assay could be run using only 3.75 μL of plasma, and took background absorbance to correct for initial variation in plasma color.

Indexing Circulating Antioxidants

Because frugivorous fall migrants may preferentially consume antioxidant-rich fruits during stopover (Bolser et al. 2013), it is reasonable to expect dietary differences between habitats to be reflected in their antioxidant status. Antioxidants are also related to immunity, in that they play a preventative role against immunopathology resulting from oxygen radicals produced by inflammation (reviewed by Costantini 2008).

Plasma total carotenoids. Carotenoids deserve special attention because they are entirely driven by diet and have strong immunostimulatory effects (Simons et al. 2012), although their role as circulatory antioxidants is likely minor (Skrip and McWilliams 2016). We used a spectrophotometric assay to measure plasma total carotenoid concentration (Tella et al. 1998, Ninni et al. 2004, McGraw et al. 2008). Absolute ethanol (90 μL) was added to plasma (10 μL; 1:10 dilution), vortexed (10 sec), and centrifuged (1 min, 16,800× *g*). The supernatant (70 μL) was pipetted into a flat-bottomed, 96-well plate (Thermo Scientific 475094, Waltham, Massachusetts, USA) and read at $\lambda = 450$ nm. Standard curves were generated from 70 μL of xanthophyll in absolute ethanol (0–20 μg mL^{-1}; Sigma-Aldrich X6250, St. Louis, Missouri, USA), and measurements were multiplied by 10 to correct for the dilution.

Circulating antioxidant capacity. We used the OXY-adsorbent test (Diacron International MC435, Grosseto, Italy) to measure the ability of plasma samples to neutralize a pathologically relevant oxidant (hypochlorous acid: HOCl). This functional assay reflects the combined activity of various nonenzymatic antioxidants (e.g., vitamins C and E, carotenoids, and thiols) unconfounded by catabolically produced uric acid (Costantini 2011, Skrip and McWilliams 2016). Briefly, 5 μL of plasma was diluted 1:100 with distilled water (495 μL), 2.5 μL of which was incubated at 37°C for 10 min with titered HOCl (100 μL). A chromogenic mixture (2 μL) was then added and read immediately at $\lambda = 540$ nm. The intensity of the color resulting from residual HOCl is inversely related to antioxidant capacity. A calibrator serum (350 mmol L^{-1} HOCl neutralized, diluted 1:100) and distilled water (blank) were used as control references, and calculations were performed as per the manufacturer's directions.

Energy, Carotenoids, and Antioxidant Capacity of Fruits

Energy density. We used published values of energy density (kJ g^{-1} dry mass) of commonly occurring fruits at our site (see Appendix Table 3). For consistency, we relied primarily on a single comprehensive database (White 1989) that included wet fruit mass (mg), seed load (% wet seed mass), and pulp water content (%). The energy density of wet fruit (kJ g^{-1} wet fruit), the unit actually consumed, was derived as follows: We estimated wet pulp mass (g) as fruit mass (g) × proportional mass of wet pulp (i.e. [100 – % seed mass]/100), total dry pulp mass (g) as wet pulp mass (g) × proportional mass of dry pulp (i.e. [100 – % water in pulp]/100), and energy contained in total dry pulp (kJ) as energy density of dry pulp (kJ g^{-1} dry fruit) × total dry pulp mass (g). Dividing the energy of total dry pulp by the wet pulp mass (g) yielded kJ g^{-1} wet fruit. We were unable to find values for nannyberry (*Viburnum lentago*),

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TABLE 1. Summary of variables tested in generalized linear models (GLMs) with backward selection ($P < 0.1$). Retained variables were subsequently included as covariates in GLMs testing the effect of habitat on health parameters of Swainson's Thrushes and Gray Catbirds near East Lansing, Michigan, USA, in 2012 and 2013. A = age, D = ordinal date, F = fat score, H = handling time, M = muscle score, N = no covariates, T = time after sunrise, and W = wing chord. Plus and minus signs denote the direction of effect. Variables in parentheses indicate those that were removed ($P > 0.05$) from the final, minimal model that included habitat.

Parameter	Swainson's Thrush, 2012	Swainson's Thrush, 2013	Gray Catbird, 2012	Gray Catbird, 2013
Total leukocytes	(+A)	+D, −H, −F	−D, −H, (+A)	(+W)
Granulocyte:lymphocyte ratio	+D, +H	−W	(+A)	(+D), −M
Hemolysis	+D, (−H), −F, (−A)	(+H)	+D, (+A)	(+D), (−A)
Hemagglutination	+D	+D, (+W)	(+D)	(+D), +T, (−H), −A
Haptoglobin	−D, +H	−H	−D, (−T), +M	N
Carotenoids	+D, +F	+D, +F, (+W), −A	+F	+D, +F
Antioxidant capacity (OXY)	+T, (+H)	(+D), +F	(−D), +H, +F, (+M), +W	+D, (−A)

so data from the closely related *V. prunifolium* (Winkworth and Donoghue 2005) were used as substitutes.

Carotenoids and antioxidant capacity. We applied the same assays used for plasma to total carotenoids (μg g^{-1} wet fruit) and antioxidant capacity (mmol HOCl neutralized kg^{-1} wet fruit) of exotic and native fruits (all mature, late September 2013; see Appendix Table 3 for species). A minimum of 5 fruits per species (enough to capture individual variation; see Smith et al. 2015) were collected; for fruits found in both exotic and native shrubland (*E. umbellata*, *Lonicera* spp., *R. multiflora*, *R. cathartica*, *Viburnum opulus* [European cranberrybush], and *L. benzoin*), we collected a minimum of 5 fruits per species per habitat type. Fruit samples were stored at −80°C until assay.

We extracted fruit carotenoids in ethanol for spectrophotometric assay (Rodriguez-Amaya 2001, Alan et al. 2013). After seed removal, 100 mg of wet fruit pulp (±0.01 mg) was placed in 1 mL (±1 μL) of absolute ethanol (1:10 dilution), vortexed (2 min), and sonicated (5 min). The mixture was then centrifuged (5 min, 16,800× *g*), after which the supernatant and xanthophyll standards (0–50 μg mL^{-1}) were processed as described earlier for plasma carotenoids. Data from *T. vernix* were unobtainable owing to the supernatant being a turbid sol.

We modified the OXY-adsorbent test described for bird dietary analysis, which captures the activities of both hydrophilic and lipophilic antioxidants in dietary samples (Costantini 2010). While it is uncertain how antioxidant capacity measured by the OXY-adsorbent test is related to specific fruit antioxidants (e.g., polyphenols; Bolser et al. 2013), we chose this assay so that the fruit data would be more relatable to the bird plasma data (various antioxidants are disparately reflected among assay types; see Skrip and McWilliams 2016). Wet fruit pulp (100.00 ± 0.01 mg) was placed in 1 mL of distilled water (±1 μL; 1:10 dilution), vortexed (2 min), and sonicated (5 min). This emulsion (100 μL) was further diluted in 900 μL of distilled water (total dilution of 1:100) and vortexed, and 2.5 μL was then plated to be processed similarly to the equivalent plasma assay. We took initial background absorbance ($\lambda = 540$) to correct for variation in fruit coloration and turbidity.

Fruit consumption. We used pooled 2012–2013 fecal counts (Swainson's Thrush, exotic: $n = 89$, native: $n = 166$; Gray Catbird, exotic: $n = 22$, native: $n = 116$) representing fruit from our study species collected as part of a concomitant study on habitat use (Oguchi 2015) to derive energy, carotenoids, and antioxidant capacity in the fruit diet in each habitat as weighted means (see below). Samples that contained multiple food items were partitioned by visually assigned volumetric proportions (10% increments). Arthropod proportions were similar between habitat types ($P > 0.05$) and thus were excluded from the analysis (~7% and ~5% of items in Swainson's Thrush and Gray Catbird feces, respectively).

Statistical Analyses

All analyses were performed using R 3.1.2 (R Core Team 2014). All tests were 2-tailed with critical $\alpha = 0.05$. Analyses were performed separately for each species–year group due to differing significant covariates among these groups (Table 1) and insufficient degrees of freedom to test for many levels of multiway (covariate × year × species × habitat) interactions. Within species–year groups, we tested for a habitat effect on the potential confounding variables of age ratio (χ^2 contingency test) and structural size (i.e. wing chord; 2-sample *t*-test).

Health parameters. We used generalized linear models (GLMs; gamma family with an identity link) to test whether the habitat-specific change in mass (g hr^{-1}) differed between exotic and native shrublands during the morning foraging hours for each species in each year (Dunn 2000, Guglielmo et al. 2005). After controlling for ordinal date and wing chord (linearly and positively related to mass in all comparison groups, $P < 0.05$), mass was regressed against time after sunrise including an interaction term with habitat type. Habitat-related difference in mass change was tested on the significance of this interaction term (slope difference), and generalized linear

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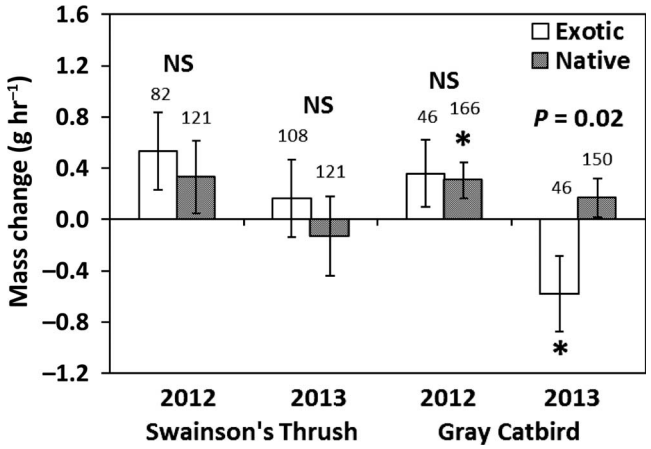


FIGURE 2. Habitat-specific changes in mass (± SE) during morning foraging hours of Swainson's Thrushes and Gray Catbirds refueling in exotic- or native-dominated shrublands near East Lansing, Michigan, USA, in 2012 and 2013. Mass changes were estimated as regression slopes of mass against time after sunrise after controlling for ordinal date and wing chord length. An asterisk indicates a significant difference from 0 g hr^{-1}. *P*-values are for habitat effects for species by year combinations. Sample sizes are given above each bar.

hypothesis tests (GLHTs) were used to test whether the slope differed from 0 in each habitat type. Handling time and age were unrelated to mass ($P > 0.05$), and thus were not included in any model.

We similarly used GLMs to test for a habitat effect on each blood analyte. We fitted Gaussian GLMs with an identity link for hemolysis scores, hemagglutination scores of Gray Catbirds, haptoglobin (except Gray Catbirds in 2012), and antioxidant capacity. Other parameters showed evidence of nonnormal error distributions and heteroscedasticity, so the following GLMs were used: negative binomial with a log link for total leukocyte counts, compound Poisson with a log link (function cpglm in R package cplm; Zhang 2013) for the G:L ratio, and gamma with a log link for carotenoids, hemagglutination of Swainson's Thrushes, and haptoglobin of Gray Catbirds in 2012. We first fitted a full model explaining each health parameter with ordinal date, time after sunrise at capture, handling time, fat score, muscle score, wing chord, and age as predictors. Important predictors were selected by stepwise backward selection with critical $\alpha = 0.1$ (Guglielmo et al. 2005). Mass was not included in the model due to its redundancy with fat score ($r^2 = 0.17$–0.54, $P < 0.001$) and being a poorer predictor of health parameters than the latter. Habitat and habitat × covariate (each retained predictor) interaction terms were added to the reduced model, and stepwise backward selection ($P < 0.05$) was again used to produce the minimum model that included habitat (Guglielmo et al. 2005). No final models showed multicollinearity (variance inflation factors < 10), and assumptions were verified using residual plots. Because no interaction terms remained in any of the final models, we present all results as least square means (LSMEANS ± SE) of habitat type. Graphical presentation of G:L ratios relied on LSMEANS obtained through refitting the final models as Markov chain Monte Carlo GLMs (function MCMCglmm in R package MCMCglmm; Hadfield 2010) due to R package incompatibility.

Fruit energy, carotenoids, and antioxidant capacity. Fruit total carotenoids and antioxidant capacity were compared among species using one-way ANOVA with post hoc Tukey's HSD multiple comparisons. None of the 6 fruit species collected from both shrublands showed habitat differences in either analyte ($P > 0.05$), so data were pooled by species.

We then tested whether the average fruit diet in the 2 habitats differed in energy, carotenoid, and antioxidant contents using weighted 2-sample *t*-tests (function wtd.t.test in R package weights; Pasek 2016). For each bird species in each habitat, the mean energy density of fruits (kJ g^{-1} wet fruit) = ∑(energy density of wet mass of each fruit species × its consumption proportion), with weights (i.e. consumption proportion) coded as raw counts of fecal samples containing that fruit (see Appendix Table 3). This approach was also taken for carotenoids (μg g^{-1} wet fruit) and antioxidant capacity (mmol HOCl neutralized kg^{-1} wet fruit), but, to reflect the measured within-species variation (from varying assay sample sizes; $n = 5$–11), the analyte measurement for each fruit was entered as the sampling unit with its weight standardized by dividing the fecal count of that fruit species by the assay sample size.

RESULTS

We sampled 203 Swainson's Thrushes in 2012 (exotic shrubland: $n = 82$, native shrubland: $n = 121$) and 229 Swainson's Thrushes in 2013 (exotic shrubland: $n = 108$, native shrubland: $n = 121$). We sampled 212 Gray Catbirds in 2012 (exotic shrubland: $n = 46$, native shrubland: $n = 166$) and 196 Gray Catbirds in 2013 (exotic shrubland: $n = 46$, native shrubland: $n = 150$). Sample sizes varied for each assay due to individual variation in the blood volume that we were able to collect (Figures 2–4). Median capture dates of thrushes were September 12, 2012, and September 14, 2013, slightly later than those of catbirds (September 5, 2012, and September 9, 2013). The majority of our samples represented HY birds (Swainson's Thrush, HY:AHY = 151:52 in 2012, 205:24 in 2013; Gray Catbird, HY:AHY = 190:22 in 2012, 186:10 in 2013), but age ratio did not differ by habitat type for either thrushes (χ^2 contingency tests, 2012: $\chi^2_1 = 1.0$, $P = 0.32$; 2013: $\chi^2_1 = 0.1$, $P = 0.77$) or catbirds (2012: $\chi^2_1 = 0.2$, $P = 0.67$; 2013: $\chi^2_1 = 1.1$, $P = 0.30$). Likewise, wing chord did not differ between habitat

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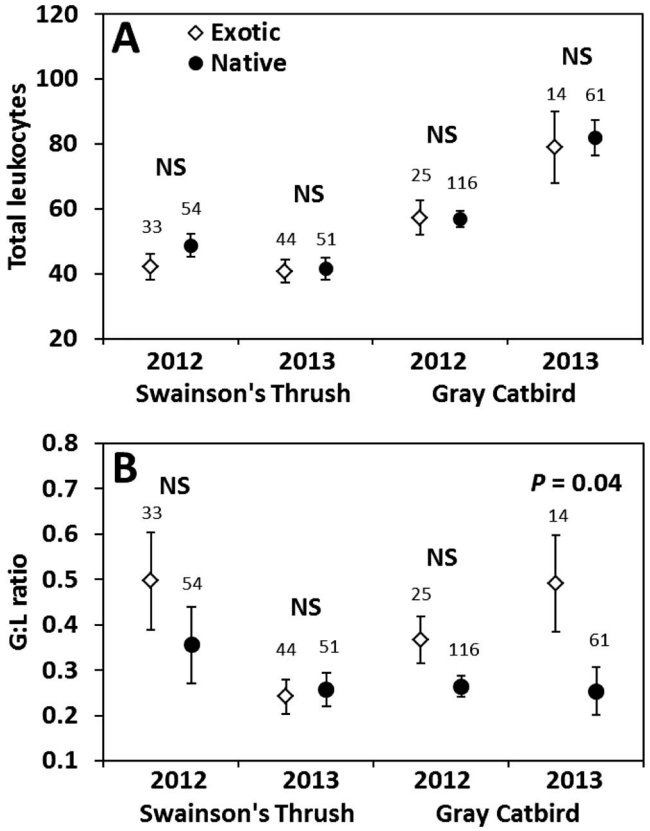


FIGURE 3. Least square means (± SE) of (**A**) total leukocyte count per 10,000 erythrocytes and (**B**) granulocyte:lymphocyte (G:L) ratio of Swainson's Thrushes and Gray Catbirds refueling in exotic- or native-dominated shrublands near East Lansing, Michigan, USA, in 2012 and 2013. Least square means were obtained by controlling for ordinal date, time after sunrise, handling time, fat score, muscle score, wing chord, and age as necessary (see Table 1). *P*-values and sample sizes are given above bars.

types (2-sample *t*-tests; Swainson's Thrush, 2012: $t_{148.28} = -1.5$, $P = 0.13$; 2013: $t_{218.18} = -1.3$, $P = 0.20$; Gray Catbird, 2012: $t_{71.27} = 1.0$, $P = 0.30$; 2013: $t_{69.55} = 1.1$, $P = 0.29$).

Refueling Performance

Size- and date-corrected mass did not differ by habitat type ($P > 0.05$), except for Swainson's Thrushes in 2012 (GLM; greater by 1.6 g in native shrubland, $F_{1,198} = 10.6$, $P = 0.001$). We did not find a habitat-related difference in mass change in most comparison groups (GLMs; Swainson's Thrush, 2012: $F_{1,197} = 0.2$, $P = 0.63$; 2013: $F_{1,223} = 0.5$, $P = 0.48$; Gray Catbird, 2012: $F_{1,206} = 0.0$, $P = 0.86$; Figure 2). Gray Catbirds in 2013 were the only group to exhibit a habitat-related difference in mass change (GLM; mean difference of 0.8 g hr^{-1}, $F_{1,190} = 5.3$, $P = 0.02$); individuals that foraged in exotic shrubland experienced mass loss (GLHT; –0.6 g hr^{-1}, $z = -2.0$, $P = 0.05$), whereas conspecifics in native habitat maintained, but did not gain, mass ($z = 1.1$, $P = 0.26$; Figure 2). We detected mass gain in

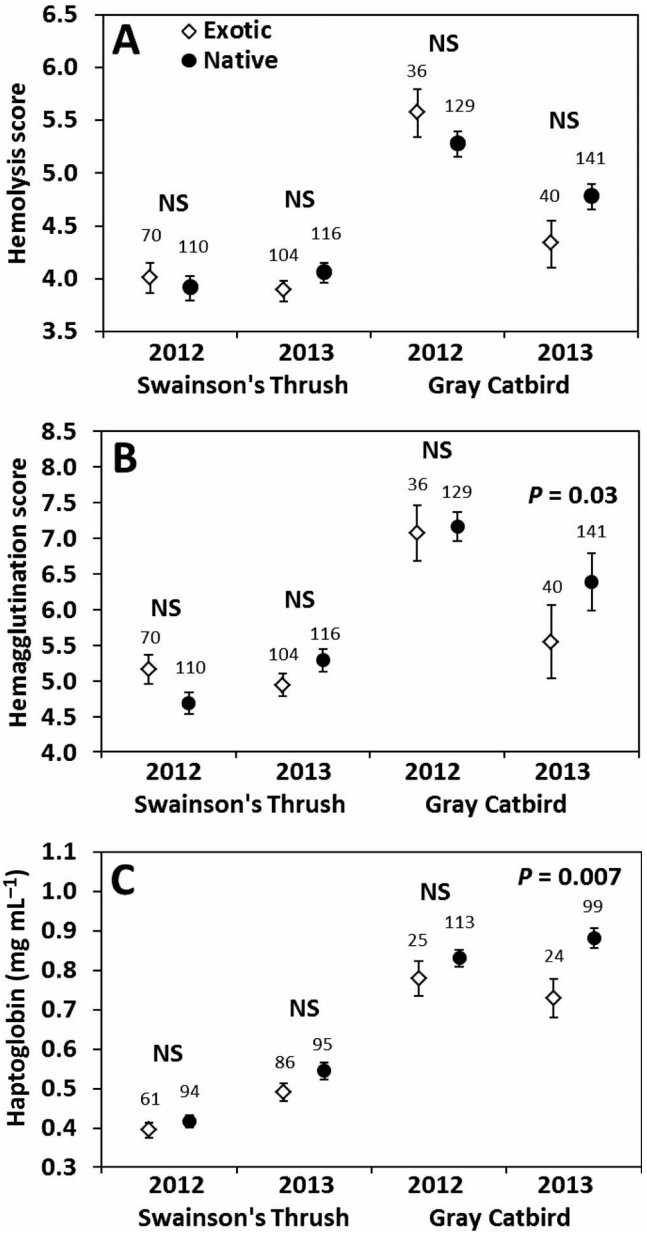


FIGURE 4. Least square means (± SE) of (**A**) hemolysis score, (**B**) hemagglutination score, and (**C**) haptoglobin concentration of Swainson's Thrushes and Gray Catbirds refueling in exotic- or native-dominated shrublands near East Lansing, Michigan, USA, during fall migration in 2012 and 2013. Least square means were obtained by controlling for ordinal date, time after sunrise, handling time, fat score, muscle score, wing chord, and age as necessary (see Table 1). *P*-values and sample sizes are given above bars.

catbirds in 2012 in native (GLHT, $z = 2.2$, $P = 0.03$) but not in exotic shrubland ($z = 1.4$, $P = 0.17$), and did not detect mass gain in thrushes captured in either year regardless of habitat type (2012 exotic: $z = 1.8$, $P = 0.08$; 2012 native: $z = 1.2$, $P = 0.24$; 2013 exotic: $z = 0.6$, $P = 0.58$; 2013 native: $z = -0.4$, $P = 0.67$; Figure 2).

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Constitutive Innate Immune Function

Total leukocyte counts did not differ by habitat type in Swainson's Thrushes (GLMs; 2012: likelihood ratio [LR] $\chi^2_1 = 1.4$, $P = 0.23$; 2013: LR $\chi^2_1 = 0.0$, $P = 0.87$) or Gray Catbirds (2012: LR $\chi^2_1 = 0.0$, $P = 0.93$; 2013: LR $\chi^2_1 = 0.1$, $P = 0.81$) in either year (Figure 3A). Handling time, whenever significant, negatively affected total leukocyte counts (Table 1). We also did not find a habitat-related difference in the G:L ratios of thrushes in either year (GLMs; 2012: $F_{1,83} = 1.3$, $P = 0.25$; 2013: $F_{1,92} = 0.1$, $P = 0.81$) or of catbirds in 2012 ($F_{1,139} = 3.0$, $P = 0.08$); however, catbird G:L ratio was 95% higher in exotic compared with native habitat in 2013 ($F_{1,72} = 4.4$, $P = 0.04$; Figure 3B). No covariate consistently explained variation in the G:L ratio among species and years (Table 1).

Hemolysis scores of Swainson's Thrushes did not differ by habitat type in 2012 (GLM, $F_{1,176} = 0.3$, $P = 0.61$) or 2013 ($F_{1,218} = 1.7$, $P = 0.20$; Figure 4A). Likewise, hemolysis scores of Gray Catbirds were similar between habitats in both years (GLMs; 2012: $F_{1,162} = 1.3$, $P = 0.26$; 2013: $F_{1,179} = 3.2$, $P = 0.08$; Figure 4A). Ordinal date, whenever significant, had a positive effect on hemolysis scores (Table 1). Hemagglutination scores of thrushes did not differ by habitat type in either year (GLMs; 2012: $F_{1,177} = 3.5$, $P = 0.06$; 2013: $F_{1,217} = 2.4$, $P = 0.12$), and results were similar for catbirds in 2012 ($F_{1,163} = 0.0$, $P = 0.83$; Figure 4B). However, in 2013, catbirds captured in exotic shrubland had lower scores than conspecifics in native shrubland (GLM, mean score difference: 0.84; $F_{1,177} = 4.5$, $P = 0.03$; Figure 4B), representing an average 44% reduction in hemagglutination activity in exotic compared with native habitat (back-transforming $\log_2$ scores to activity). Time after sunrise had a significantly positive effect on hemagglutination in this comparison group (Table 1). Plasma levels of haptoglobin were similar between habitat types in Swainson's Thrushes in both years (GLMs; 2012: $F_{1,151} = 0.8$, $P = 0.37$; 2013: $F_{1,178} = 2.9$, $P = 0.09$) and in Gray Catbirds in 2012 ($F_{1,134} = 1.0$, $P = 0.32$), but in 2013 we found 17% lower haptoglobin levels in catbirds using exotic shrubland compared with conspecifics captured in native shrubland (mean difference: 0.15 mg mL^{-1}; $F_{1,121} = 7.6$, $P = 0.007$; Figure 4C). No covariate was consistently related to haptoglobin across species and years (Table 1).

Circulating Antioxidants

We did not find differences by habitat type in plasma total carotenoids of Swainson's Thrushes in either year (GLMs; 2012: $F_{1,177} = 0.7$, $P = 0.41$; 2013: $F_{1,211} = 1.6$, $P = 0.21$; Figure 5A) or of Gray Catbirds in 2012 ($F_{1,160} = 1.0$, $P = 0.30$). However, in 2013, circulating carotenoids of catbirds in exotic shrubland were 20% lower than those of catbirds in native shrubland (GLM, mean difference: 3.85 μg mL^{-1}; $F_{1,165} = 6.1$, $P = 0.01$; Figure 5A). Fat score was positively related to carotenoids in all comparison groups (Table 1).

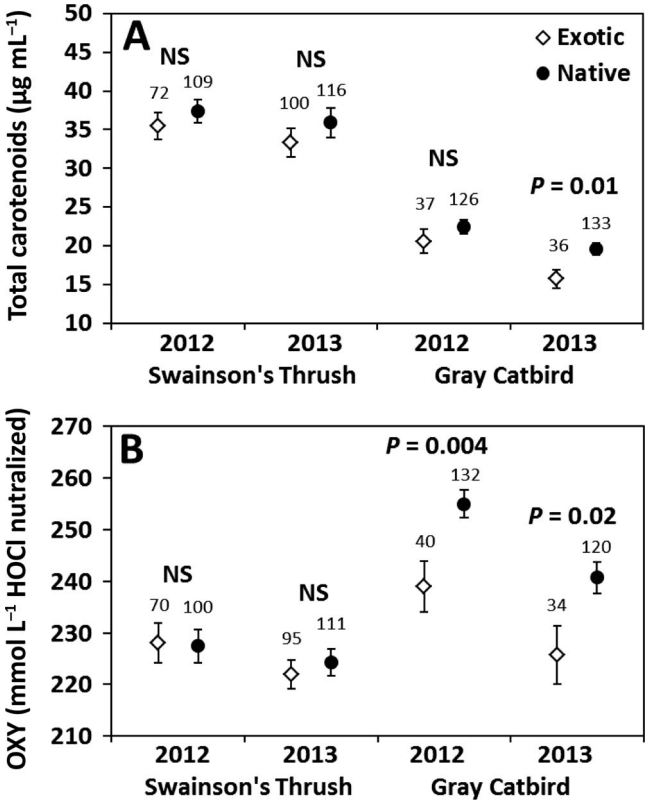


FIGURE 5. Least square means (± SE) of (**A**) plasma total carotenoids and (**B**) circulating antioxidant capacity of Swainson's Thrushes and Gray Catbirds refueling in exotic- or native-dominated shrublands near East Lansing, Michigan, USA, during fall migration in 2012 and 2013. Least square means were obtained by controlling for ordinal date, time after sunrise, handling time, fat score, muscle score, wing chord, and age as necessary (see Table 1). *P*-values and sample sizes are given above bars.

No habitat effect on circulating antioxidant capacity was found for Swainson's Thrushes in either year (GLMs; 2012: $F_{1,167} = 0.0$, $P = 0.91$; 2013: $F_{1,203} = 0.4$, $P = 0.55$; Figure 5B). In contrast, Gray Catbirds that foraged in exotic shrubland exhibited lower antioxidant capacity than those in native shrubland by 6% in 2012 (GLM, mean difference: 15.95 mmol L^{-1} HOCl neutralized; $F_{1,167} = 8.3$, $P = 0.004$) and by 7% in 2013 (mean difference: 14.94 mmol L^{-1} HOCl neutralized; $F_{1,151} = 5.5$, $P = 0.02$; Figure 5B). No covariate was universally related to antioxidant capacity across species and year (Table 1).

Fruit Energy, Carotenoid, and Antioxidant Contents

Fruit carotenoids varied among species (one-way ANOVA, $F_{12,77} = 88.06$, $P < 0.001$), and were highest in the fruits of exotic *R. multiflora*, *Solanum dulcamara* (climbing nightshade), and *Lonicera* spp. (Figure 6A). The fruits of the different species also varied in their antioxidant capacity ($F_{13,91} = 37.73$, $P < 0.001$), with fruits of the

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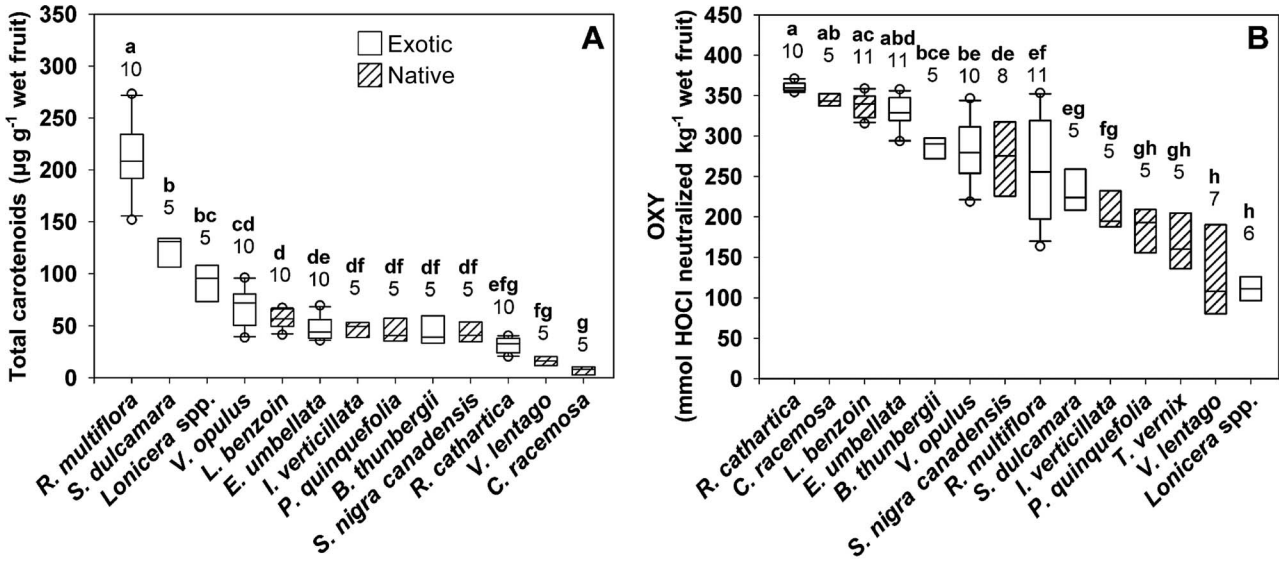


FIGURE 6. Box and whisker plots of (**A**) Total carotenoids and (**B**) antioxidant capacity (OXY) of fruits found in shrublands used by migrating landbirds near East Lansing, Michigan, USA. Exotic fruits are shown as white boxes and native fruits are barred. Fruit species are ordered from highest to lowest means. Boxes indicate the medians and the first and third quartiles, whiskers indicate lower and upper 1.5× interquartile range, and circles indicate outliers. Differences in letters above boxes represent significance ($P < 0.05$), and numbers below the letters denote sample sizes. See Appendix Table 3 for full species names.

exotic *R. cathartica* and *E. umbellata* and the native *C. racemosa* and *L. benzoin* showing the highest levels (Figure 6B).

We did not find differences in the weighted means of dietary energy density by habitat type for either species (Table 2). Swainson's Thrush diets also lacked habitat-related differences in carotenoid content and antioxidant capacity, but the values of these parameters were 17% and 11% lower, respectively, in Gray Catbird diets in exotic compared with native habitat (Table 2).

DISCUSSION

Habitat Effect on Refueling Performance

We found no effect of habitat on mass changes of Swainson's Thrushes in either year nor Gray Catbirds in 2012. Analyses that included birds from which we did not take blood samples yielded similar results (Oguchi 2015), suggesting that sample size did not compromise our ability to detect refueling differences. Native fruits generally have higher fat and energy content than their exotic counterparts (see Appendix Table 3 and White 1989, Smith et al. 2007, 2013), but, contrary to what has previously been found near the southern shoreline of Lake Ontario, USA (Smith et al. 2015), our results suggest that foraging in exotic-dominated shrublands may not result in poorer refueling relative to doing so in native shrublands.

Estimates of the average energy density of wet fruit consumed by Swainson's Thrushes and Gray Catbirds also did not differ by habitat type. Fruits that were relatively dry (<50% water; *T. vernix* and *R. multiflora*) were rarely consumed, even though their energy densities were highest on a wet mass basis. Of the fruits with >50% water content, the exotic *R. cathartica* and the native *L. benzoin*

TABLE 2. Estimated habitat-specific means (± SE) of energy (kJ g^{-1} wet fruit), total carotenoids (µg g^{-1} wet fruit), and antioxidant capacity (OXY: mmol HOCl neutralized kg^{-1} wet fruit) of a fruit diet of Swainson's Thrushes and Gray Catbirds refueling in exotic- or native-dominated shrublands near East Lansing, Michigan, USA, during fall stopover (2012–2013). Estimates were derived as the value of fruit weighted by its consumption proportion (fecal counts) by each bird species in the respective habitat type. An asterisk indicates a significant difference at $\alpha = 0.05$.

Parameter	Species	Exotic	Native	*t*	df	*P*
Energy	Swainson's Thrush	6.24 ± 0.77	8.04 ± 0.71	−1.7	25.9	0.10
	Gray Catbird	6.22 ± 0.74	8.10 ± 0.72	−1.8	26.0	0.08
Carotenoids	Swainson's Thrush	54.98 ± 4.16	54.21 ± 2.15	0.2	118.2	0.87
	Gray Catbird	44.74 ± 2.11	53.63 ± 2.27	−2.9	127.4	0.005*
OXY	Swainson's Thrush	319.27 ± 6.27	319.46 ± 5.86	−0.0	177.2	0.98
	Gray Catbird	290.57 ± 9.59	325.45 ± 5.69	−3.1	117.2	0.002*

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were more energy-dense than most other fruits within their respective habitat types, and thrushes and catbirds predominantly consumed these fruits. In particular, *L. benzoin* was commonly consumed by birds in exotic shrubland, despite its low abundance (see Appendix Table 3 and Oguchi 2015), suggesting that birds in exotic habitat may be able to attain similar mass gain to those in native habitat through selectively consuming rare but energy-dense native fruits. Alternatively, because birds have an exceptionally high capacity for de novo synthesis of fatty acids from carbohydrates (Klasing 1998), a sugary diet (i.e. exotic fruits) may serve just as well as a fatty one (i.e. native fruits) for refueling if birds can compensate for lower energy density by increased feeding.

A notable exception, however, was found in 2013 for Gray Catbirds. Individuals captured in exotic shrubland exhibited poorer refueling performance compared with conspecifics in native shrubland. These birds represented the only comparison group to show a similar pattern to that reported previously for fall migrants foraging in exotic- vs. native-dominated shrublands (Smith et al. 2015). Catbirds using exotic shrubland in our site in 2012 lost mass by 0.6 g hr^{-1}, and this poor refueling performance was accompanied by a relative reduction in immune status (see below). Thus, possible reasons for interspecific and interannual differences in refueling with respect to habitat use should be considered.

Local food availability at the time of arrival is a major factor determining refueling performance (reviewed by Klaassen et al. 2012). Differential refueling in exotic- vs. native-dominated shrubland previously reported by Smith et al. (2015) was attributed to ~60% lower fruit yield in the former, quite apart from fruit quality differences. Crude fruit counts performed at our site in 2012 showed no differences in total fruit yield between exotic and native habitats (Oguchi 2015), but fruit yield by shrub species likely varied between 2012 and 2013. For example, it is known that the fruit yield, timing, and nutritional quality of shrub species are differentially affected by weather (Gallinat et al. 2015, Smith et al. 2015, Sood 2015), and, at our site, cumulative summer precipitation in 2013 was approximately twice that of 2012 (379.1 mm vs. 190.2 mm; NOAA 2013). Interannual variation in the yield of fruit species and its timing would vary in its effect on bird species based on species-specific fruit preferences, irrespective of local fruit abundance (Herrera 1998, Mudrzynski and Norment 2013). Swainson's Thrushes, being later migrants, have been shown to readily consume exotic fruits and refuel successfully even after most native fruits have been depleted by earlier migrants in Michigan (Craves 2009). Earlier-arriving Gray Catbirds, on the other hand, seem to consume proportionally more native fruits; in this study, they showed a >3-fold higher capture rate in native shrubland, and, even in exotic shrubland, fruits consumed by catbirds were 53% native, compared with 33% for thrushes (Appendix Table 3). While this observation may reflect the benefit of migrating early while high-quality native fruits are still available, an alternative explanation could be that catbirds may rely more heavily on certain native fruits than thrushes. If so, catbirds using exotic habitat may expend relatively more time and energy searching for scarce native fruits than thrushes, making them more vulnerable to refueling failure in years of poor native fruit yield in predominantly exotic habitats. Provided that thrushes and catbirds show otherwise similar patterns in their degree of frugivory and use of shrublands (Oguchi 2015), it is likely that variation in preferred fruit abundance or quality lead to interspecies and interannual difference in refueling performance.

Habitat Effect on Immune and Antioxidant Status

In 2013, Gray Catbirds captured in exotic habitat had a higher G:L ratio and lower hemagglutination activity, haptoglobin, and total carotenoids compared with conspecifics in native shrubland. These results were consistent with the finding that, in the same year, as a group, they lost mass throughout the morning; no other comparison group showed habitat-related differences in any of these measures. Limited access to food has been found to raise the G:L or H:L ratio (stress response; Maxwell et al. 1992) and depress levels of circulating carotenoids (McGraw et al. 2005), which are important immune stimulators (McGraw and Ardia 2003, McGraw et al. 2006, Simons et al. 2012). Demonstrating causal relationships between carotenoids and immunity is difficult in the field because plasma carotenoid levels can fluctuate rapidly in response to feeding bouts (see Hõrak et al. 2004), whereas sustained high levels in circulation may be required to enhance immunity (see experimental designs in McGraw and Ardia 2003, McGraw et al. 2006). Nevertheless, the consistency in the direction of the habitat effect on mass change, carotenoids, and 3 out of 5 immune parameters in catbirds in 2013, together with the lack of a habitat effect in any other group whose mass change was comparable between habitat types, suggests that immune status was at least partly dependent on refueling performance. If so, our result is consistent with the view that migrants recover or upregulate immune defense during stopover in a condition-dependent manner (Owen 2004, Owen and Moore 2008a, Buehler et al. 2010), rather than making a tradeoff between immunity and the need to restore fat reserves (Klaassen et al. 2012).

The circulating antioxidant capacity of Swainson's Thrushes did not differ by habitat type, but the antioxidant capacity of Gray Catbirds using exotic shrubland was consistently lower than that of conspecifics in native shrubland in both sampling years. This observation may reflect the need of catbirds in native shrubs to better

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counter the risk of lipid peroxidation due to their high-fat diet (Eikenaar et al. 2016, 2017). However, habitat-related differences in food availability have also been linked to oxidative status; birds that expended more energy to obtain food in breeding habitats with lower arthropod abundance experienced greater oxidative damage (van de Crommenacker et al. 2011). Catbirds exhibited a habitat-related difference in antioxidant status seemingly independent of refueling performance (as seen in 2012). However, given their relatively high consumption of rare native fruits in predominantly exotic shrubland (see above), catbirds may have had to work harder during foraging, causing increased oxidative cost. Differences in work load, combined with the antioxidant capacity of consumed fruits (see below), may have resulted in the consistently poorer antioxidant status of catbirds in exotic vs. native shrubland.

Birds captured in exotic shrublands were more likely to switch habitats (Oguchi 2015). Perhaps the poorer oxidative status of Gray Catbirds in exotic habitat resulted from the fact that, on average, a bird in exotic habitat had arrived more recently. Newly arrived birds may have poorer refueling performance due to reduced gut size and digestive capacity (reviewed by McWilliams and Karasov 2005), and have been hypothesized to have lower immune and antioxidant status than birds that have stayed longer (Owen 2004, Owen and Moore 2008a, Buehler et al. 2010, Nebel et al. 2012, Skrip et al. 2015). However, habitat preferences and movement patterns were similar between the 2 species, so these factors do not explain why only catbirds showed habitat-related difference in health status. Perhaps thrushes, as long-distance migrants, are physiologically less sensitive to flight and stopover. Results from earlier work, however, suggest otherwise. Short-distance migrants that typically do not fast for more than a day may have spare digestive capacity that could allow rapid food intake and mass gain immediately (Lee et al. 2002). Then, one would expect thrushes to be more affected by digestive constraints than catbirds. Furthermore, species with different migratory flight modes have shown similar oxidative consequences at a stopover site (Costantini et al. 2007). It is also thought that a few days of continuous use would be enough for immune and antioxidant measures to reflect current health status and hence habitat associations (see Study Species and Known Patterns of Their Habitat Use). Thus, while the timing of habitat use over the course of stopover may partly account for the low health status of Gray Catbirds in exotic shrubland, it is unlikely to be the sole reason behind the observed pattern.

In addition, the average carotenoid and antioxidant content of a fruit diet (per unit wet mass) was estimated to be lower in exotic compared with native habitat only for Gray Catbirds, not Swainson's Thrushes. Fruits of *R. multiflora* had exceptionally high carotenoid content, as previously described by Alan et al. (2013), but contributed little to the birds' carotenoid acquisition because they were rarely consumed by either species, even in exotic habitat (Appendix Table 3). Rather, the bulk of the carotenoids and antioxidants in the diets of thrushes and catbirds were likely derived from the exotic *R. cathartica* and the native *L. benzoin* in their respective habitats, because these fruits contained moderate carotenoid levels and high antioxidant capacities (among the highest in the fruits that we assayed) and were consumed in high proportions. Yet, Swainson's Thrushes consumed proportionately more *R. cathartica* fruits in exotic compared with native habitat, whereas Gray Catbirds did not (Appendix Table 3). Instead, catbirds in exotic habitat seemed to consume proportionately more *V. lentago* than conspecifics in native habitat (Appendix Table 3), and these native fruits contained only low levels of carotenoids and antioxidant capacity. Taken together, some bird species (e.g., Swainson's Thrushes) seem to take advantage of exotic fruits as a source of carotenoids and other antioxidants, while others (e.g., Gray Catbirds) may not readily do so, acquiring lower quantities of these nutrients in exotic compared with native shrubland on a per-unit-mass basis. Nevertheless, our estimates of fruit quality and consumption alone do not fully explain the yearly variation in catbird health in relation to habitat use. An improved method using observed consumption per unit time would likely provide better estimates of consumption quantity.

The reduction in the health status of Gray Catbirds using exotic shrubland may have been ephemeral, given that the measured immune and antioxidant parameters can change within a few days and that birds may depart or move to other habitats within similar time frames (Hõrak et al. 2004, Sepp et al. 2010, van de Crommenacker et al. 2010, Nebel et al. 2012). Nonetheless, small differences in health status resulting from varying quality of stopover habitat may have major implications for disease resistance and survival (Klaassen et al. 2012). The importance of antioxidants to migrating birds is well established (Costantini et al. 2007, 2008, Jenni-Eiermann et al. 2014, Skrip et al. 2015), and having robust immunity during stopover is likely also adaptive in the defense against pathogens ingested during foraging (Buehler et al. 2010, Altizer et al. 2011). Constitutive innate immunity may be particularly important in immunologically naïve HY birds experiencing their first fall migration (see Ochsenbein and Zinkernagel 2000). In addition, baseline haptoglobin levels have been positively linked to future flight duration (Nebel et al. 2013), and carotenoids to rapidness of takeoff initiation and flight speed during escape (Blount and Matheson 2006). Indeed, higher measures of constitutive immunity (Kilgas et al. 2006b, Parejo and Silva 2009, Townsend et al. 2010) and antioxidant capacity (Saino et al. 2011) have been associated with improved survival in nestling and

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adult birds. These studies suggest a period of higher risk from infection, predation, or oxidative damage for catbirds stopping over in exotic shrubland.

Conclusions and Future Directions

Interest is growing in how human-induced land-use changes may impact the health and disease risk of migrating animals and humans, necessitating integrative approaches to understanding habitat use and its health consequences for avian migrants (Altizer et al. 2011, Klaassen et al. 2012). Our results provide evidence that differential use of stopover habitat may affect aspects of health such as the immune and antioxidant status of migrating landbirds. The effects that we detected, however, were not consistent between species or years, and varying outcomes could arise from complex relationships between food abundance, food quality, and species-specific dietary preferences. It is conceivable that birds that readily consume exotic fruits in predominantly exotic shrublands (e.g., Swainson's Thrushes) may be less susceptible to reductions in refueling resources or health measures compared with those that do not (e.g., Gray Catbirds). Experimental studies will be needed to test mechanistic links among the energy and nutritional contents of diets, refueling performance, immunity, and antioxidant capacity. We also emphasize the need for future studies to explicitly test whether health recovers during stopover and, if so, whether refueling performance and immune parameters covary. Such studies may be best performed in spring when the dietary quality among individuals is relatively consistent (i.e. arthropodivorous diet). Nevertheless, our results suggest that measuring immune and antioxidant condition in addition to refueling performance will better reveal habitat quality for migrating birds (Albano 2012, Klaassen et al. 2012).

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APPENDIX TABLE 3. Swainson's Thrush and Gray Catbird fecal counts containing fruit and the published values of nutrients and energy in those fruits used to derive habitat-specific mean energy density of consumed fruits weighted by consumption proportion. Data are presented in decreasing order of energy density of wet fruit.

Plant species	Common name	U.S. status [a]	Swainson's Thrush [b]		Gray Catbird [b]		% Fat [c]	% Carb [c]	% Protein [c]	% Water [d]	Energy (kJ g^{-1} dry) [e]	Energy (kJ g^{-1} wet) [f]	Reference
			Exotic	Native	Exotic	Native							
Toxicodendron vernix	Poison sumac	N	0	0	0	0	41.4	2.9	1.8	4.6	27.44	26.18	White (1989)
Rosa multiflora	Multiflora rose	E	2	0	0	0	1.5	55.1	7.6	41.6	17.52	10.23	White (1989)
Lindera benzoin [g]	Northern spicebush	N	22	101	5	74	48.0	12.0	11.9	66.1	28.68	9.74	White (1989), Smith et al. (2013)
Viburnum lentago [h]	Nannyberry	N	2	2	2	2	2.4	20.2	1.8	58.4	21.68	9.02	White (1989)
Cornus racemosa	Gray dogwood	N	0	0	0	0	33.5	22.1	3.6	67.0	26.55	8.76	White (1989)
Rhamnus cathartica	Common buckthorn	E	32	22	6	17	0.1	56.9	3.0	65.8	18.18	6.23	White (1989)
Parthenocissus quinquefolia	Virginia creeper	N	0	5	3	1	16.2	19.4	7.6	73.3	21.84	5.83	White (1989)
Ilex verticillata	Common winterberry	N	2	7	1	4	4.3	43.1	2.6	76.9	18.40	4.25	White (1989)
Berberis thunbergii	Japanese barberry	E	0	0	0	0	0.0	36.7	3.7	79.1	19.94	4.17	White (1989)
Lonicera tatarica [i]	Tatarian honeysuckle	E	0	1	0	0	0.6	90.3	2.3	78.7	16.66	3.55	White (1989)
Solanum dulcamara	Climbing nightshade	E	7	6	0	3	0.5	48.6	6.2	84.7	18.63	2.85	White (1989)
Elaeagnus umbellata	Autumn olive	E	5	2	2	2	1.4	62.7	4.7	84.1	17.34	2.76	White (1989)
Sambucus nigra canadensis	American black elderberry	N	3	6	1	4	4.9	43.9	9.1	87.8	19.12	2.33	White (1989)
Viburnum opulus [j]	European cranberrybush	E	8	4	2	4	0.9	UA	UA	86.3	16.73	2.29	Smith et al. (2013)
Arthropods	—	—	6	11	1	5	—	—	—	—	—	—	—
Total			89	166	22	116							

[a] Species classified as either exotic (E) or native (N).
[b] Samples containing multiple food items were partitioned by visually-assigned volumetric proportions (10% increments) but are rounded to whole numbers for presentation. Arthropod proportions were similar between habitat types ($P > 0.05$) and thus were excluded from analysis (~7% and ~5% of items in Swainson's Thrush and Gray Catbird feces, respectively, across habitat types).
[c] Percentage of fat, carbohydrate, and protein in dry fruit pulp as reported.
[d] Percentage of water in wet pulp as reported.
[e] Energy density of dry pulp as reported.
[f] Energy density of wet pulp derived from reported values.
[g] Fat, water, and energy data relied on Smith et al. (2013) due to improved drying technique (freeze drying) for oily fruits.
[h] Nutrient and energy values substituted from a sister species, blackhaw (*V. prunifolium*), due to data unavailability.
[i] Used due to data availability and *Lonicera* at our site being a combination of *L. tatarica*, *L. morrowii* (Morrow's honeysuckle), and their hybrids.
[j] Carbohydrate and protein data unavailable (UA); seed load (~12%) measured in this study ($n = 5$) to calculate energy of wet fruit.

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