DIFFERENTIAL STOPOVER HABITAT USE AND ITS HEALTH CONSEQUENCES IN FALL MIGRATING LANDBIRDS

By

Yushi Oguchi

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ABSTRACT

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Migration is a physiologically-demanding activity, and the ability to locate high-quality stopover habitat for refueling has major fitness implications for migrating landbirds. However, stopover habitat is being altered by human land-use changes such as introductions of exotic shrubs, and knowledge is lacking on whether or how differences in stopover habitat could impact migrating landbirds health. We first tested whether indices of fall migrant use differed between exoticdominated and native-dominated shrublands at a stopover site in mid-Michigan through banding and fecal analyses in 2012 and 2013, focusing on gray catbirds (Dumetella carolinensis) and Swainson's thrushes (Catharus ustulatus) (Ch. 1). Capture rate was lower in exotic shrubland than in native shrubland, and habitat switchover rate from exotic to native shrubland was greater than vice versa. Fecal analyses revealed that catbirds and thrushes selected certain native fruits. We then tested whether health status of catbirds and thrushes differed by habitat using blood collected from a subset of captured birds (Ch. 2). Plasma indices of refueling performance (fat metabolites) did not differ by habitat in either species or year, but 3 catbirds using exotic shrub had lower measures of constitutive innate immunity (only in 2013) and antioxidant capacity than conspecifics in native shrubland. Fruit nutrient and fecal data suggested that these health differences may have resulted from differential fruit consumption. This study thus provides the first evidence that differential use of stopover habitat could impact health status of migrating birds and that evaluation of stopover habitat quality using an ecophysiology approach should include immune and antioxidant measures in addition to indices of refueling performance.

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CHAPTER 1:

Exotic- and native-dominated shrubland habitat use and fruit consumption

in fall migrating landbirds at a stopover site in mid-Michigan

ABSTRACT

The ability to locate high-quality stopover habitat has major implications for fitness for migrating landbirds, but human land-use change has altered stopover habitats, including introductions of non-native shrubs. However, relatively little is known about the value of exotic fruits for fall migrants, and few studies have compared fall migrant use of exotic-dominated shrublands to native-dominated counterparts. We hypothesized that native-dominated shrubland represented superior-quality stopover habitat relative to exotic-dominated shrubland, and predicted that indices of bird use would be greater in the native shrubs and that certain native fruits would be selectively consumed by fall migrating landbirds. We indexed habitat structure, composition of shrubs and fruits, and abundance arthropods in exotic- and native-dominated shrublands and compared capture rates and habitat switchover rates in gray catbirds (Dumetella *carolinensis*) and Swainson's thrushes (*Catharus ustulatus*) between these habitats during fall migration of 2012 and 2013. Fecal samples collected from these birds were assayed to determine patterns of fruit consumption. Habitat structure and total fruit and arthropod abundance were similar between shrublands, with major differences occurring only in shrub and fruit compositions. Capture rate was significantly greater in the native shrubs in both years, and birds captured in exotic shrubs were more likely to switch to and stay in native shrubs than vice versa in 2013. The native common spicebush (*Lindera benzoin*) was disproportionately consumed, whereas the exotic autumn olive (Euglenas umbellata) and European buckthorn (Rhamnus *cathartica*) showed evidence of being selected against. Our results suggest that exotic shrubland may be of limited importance for fall frugivorous migrants in the presence of adjacent native shrubland, and that L. benzoin is the single most important shrub for habitat management aiming to improve habitat quality for fall frugivorous migrants in mid-Michigan.

Introduction

Conservation of migratory landbirds requires habitat management throughout the entire annual cycle, but relatively little is known about how birds use habitat during migration and the fitness consequence of that use (Moore et al. 2005; Smith et al. 2013). Migration is a demanding activity accompanied by high mortality (Sillett and Holmes 2002), and because migrating landbirds must stop during migration to rest and refuel, their ability to locate high-quality stopover sites undoubtedly has fitness consequences (Moore et al. 1995; Dierschke 2003; Smith and Moore 2003; Moore et al. 2005; Newton 2006). During fall migration, fruit is a major food source for many landbird species (Parrish 1997; Smith et al. 2007; Smith et al. 2013), and the quality and quantity of the fruit offered are likely a key factor determining stopover habitat quality. Unfortunately, stopover habitat is being altered by human land-use changes (Wilcove and Wikelski 2008; Klaassen et al. 2012) including the introduction of exotic shrub species (Suthers et al. 2000; Catling 2005; Smith et al. 2007; Craves 2009; Smith et al. 2013). In the Midwestern United States, exotics such as autumn olive (*Elaeagnus umbellata*), honeysuckle (*Lonicera* spp.), multiflora rose (Rosa multiflora), and European buckthorn (Rhamnus cathartica) have become common and widespread, possibly owing to their ability to thrive in disturbed landscapes and to inhibit native plant growth by changing the soil chemistry (Woods 1993; Collier et al. 2002; Orr et al. 2005). These shrub species bear fruits that are consumed by native landbird migrants (Suthers et al. 2000; Smith et al. 2007; Gleditsch and Carlo 2011; Smith et al. 2013), but more work is needed on the relative value of these exotic shrubs compared to the native counterparts (Smith et al. 2013).

Studies on exotic and native fruits (White and Stiles 1992; Stiles 1993; Smith et al. 2007; Smith et al. 2013) indicate that native fruits tend to have greater energetic potential and fat

content, whereas exotic fruits tend to contain more sugar and water. Based on energetic potentials of the fruits, it has been estimated that birds may need to consume around twice the amount of exotic fruits (e.g., *E. umbellata*) in wet mass than the native counterparts (e.g., common winterberry, *Ilex verticillata*), to obtain the same amount of energy (Smith et al. 2007). In addition, certain fruits rich in fat may also contain greater antioxidant capacity (Alan et al. 2013), and dietary antioxidants are beneficial for migrants because they control tissue-damaging reactive oxygen species produced as a result of strenuous flight (Costantini et al. 2007; Bolser et al. 2013; Jenni-Eiermann et al. 2014). Therefore, it is conceivable that consuming native fruits high in energetic potential also have significant physiological benefits for landbird migrants.

However, the relative value of exotic fruits for birds during fall migration is likely context-specific and mixed results have been reported. In general, energetically-rich native fruits appear to be consumed first by migrants (White and Stiles 1992; Stiles 1993; Smith et al. 2013), but in one feeding experiment (Drummond 2005), energetically poorer *Lonicera* fruits was preferentially consumed over cranberrybush (*Viburnum opulus*). Furthermore, in a matrix of forest, urban, and agricultural land in Pennsylvania, the abundance of birds was shown to be positively correlated with the abundance of *Lonicera* fruits (Gleditsch and Carlo 2011). At a mid-Michigan stopover site, long-term capture-recapture data (Craves 2009) revealed that frugivorous migrants that arrived late in the fall were still able to gain fat and mass even after most native fruits had been already depleted. These previous studies have evaluated use of exotic fruits within a habitat matrix composed of mixed exotic and native shrubs or compared exotic shrubland use to non-shrub habitats. Few studies, on the other hand, have compared migrant use between shrubland habitats that are predominantly exotic and predominantly native.

We compared indices of use in exotic-dominated shrubland (hereafter exotic shrubland)

and native-dominated shrubland (hereafter native shrubland) and the consumption patterns of these fruits as part of a larger study to evaluate relative stopover habitat quality. Based on knowledge on fruit quality, patterns of fruit consumption and stopover biology of landbird migrants, we hypothesized that native shrubland would represent superior-quality habitat than exotic shrubland. We predicted that: 1) overall bird capture rate would be greater in native shrubland during fall migration, and that primarily frugivorous fall migrants would exhibit 2) greater bias in capture rate toward native shrubs than less frugivorous species, 3) greater rates of habitat switching from exotic to native shrubland than vice versa, and 4) selection for certain native fruits.

Methods

Study Site

We conducted fieldwork on a State-managed wildlife area near East Lansing, MI, USA (Rose Lake Wildlife Research Area, N 42.811972°, W 84.384917°). The site (170 ha) was largely set within an agricultural and suburban landscape. Exotic and native shrublands existed in similar longitudes and in distinct patches adjacent to oak-maple woods and farmlands. Due to both the orientation and proximity of the two habitats we assumed that migrants using these shrublands were from the same population. The most common shrubs in the exotic shrubland were, as determined via vegetation sampling (see below and Table 1.1), *E. umbellata, Lonicera* spp. (*L. tartarica, L. morrowii*, and *L. x bella [morrowii x tatarica]*), *R. cathartica*, and *R. multiflora*, while the native shrubland was predominantly gray dogwood (*Cornus racemosa*), common spicebush (*Lindera benzoin*), *I. verticillata*, and poison sumac (*Toxicodendron vernix*).

Study Species

While all landbird species were used for analyses of capture rates, we focused on the habitat use and fruit consumption of gray catbirds (*Dumetella carolinensis*) and Swainson's thrushes (*Catharus ustulatus*) due to their high levels of frugivory during fall (Mack and Yong 2000; Smith et al. 2011) and large sample size at our study site. In addition, we selected the primarily arthropodivorous magnolia warblers (*Dendroica magnolia*) (Dunn and Hall 2010) to evaluate whether habitat-related differences (if any) in capture rate and movement (rate of habitat switchover) may be more pronounced in fall frugivorous species.

Characterizing Habitats and Resource Attributes

We collected data on attributes of shrub composition to validate our habitat characterization as either native-dominated or exotic-dominated. To further assess habitat differences in cover and fruit resources we compared overall habitat structure, fruit abundance and composition, and flying arthropod abundance between exotic and native shrublands.

Shrub Species Composition and Habitat Structure

Plot-based vegetation sampling (James and Shugart Jr 1970) was performed in the spring of 2013. Sample circles (0.04 ha, radius 11.3 m) were established around each mist net (midpoint at the net center) in both native (n = 14) and exotic (n = 13) shrublands. When two nets were close enough that the circles overlapped, half-circles were sampled around the second net to avoid duplication. The half-circles were treated as one independent sampling unit in the analysis. Every tree of diameter at breast height (dbh, 1.4 m) \geq 8 cm within a plot was identified, counted, and assigned to a size class category (A: 8-15 cm, B: 15-23, C: 23-38, D: 38-53, E: 53-69, F: 69-84). Next, the number of woody stems with dbh < 8 cm intersecting two-arm-length transect across

the circle in each cardinal direction was recorded by species. Saplings of trees were included as shrubs for the purpose of structural analysis but were eliminated for shrub compositional analysis. On the same transect presence or absence of ground cover, shrub cover, and canopy cover were recorded by sightings through an ocular tube for 20 locations (taken every 0.56 m). Heights of the three tallest trees within each plot and three tallest shrubs were estimated using an optical clinometer and triangulation and the means were recorded as the canopy and shrub heights (m) respectively. We then estimated tree density (stems/ha) and basal area (m²/ha) by size class, shrub stem density (stems/ha), as well as percent ground, shrub, and canopy cover. All estimates were calculated on a per-plot basis for statistical comparisons.

Fruit Abundance

We performed a net-based, direct fruit count (Blake et al. 1990) to provide a baseline index of fruit abundance in mid-September, 2012. The number of all fruits for each shrub species was counted within the 0.04 ha circle vegetation sampling plots using hand-held counters. When direct counting was not possible due to large quantity of fruits (>1,000), we counted all fruits on a single branch and multiplied the count by the number of similar-sized branches. Immature fruits were counted but removed from the dataset for subsequent analysis. Estimates of fruit abundance per plot were calculated as number of fruits per ha for each species.

Arthropod Abundance

Flying arthropod biomass was indexed using four Malaise traps (Smith and Hatch 2008) during the fall migration of 2012 and 2013. Two traps were placed at permanent locations in each habitat type. Arthropods were collected and traps reset every three to seven days. The arthropods were stored in 70% ethanol and later dried until a constant mass was achieved. The flying arthropod biomass is presented as dry mass collected per day.

Indexing Bird Use

We passively captured birds in 15 mist nets in each habitat type (30 nets total; 30 mm-mesh, 12 $m \times 2.6 m$) from August 13th and extending through October. The beginning of fall migration was marked by influx of species that have no local breeding record (J. Owen, personal observation), and field season ended with deer gun hunting season. Mist nets were operated daily during fall for 4-5 hours beginning 30 min before sunrise and were checked at 45 minute intervals. Captured birds were held in individual cloth bags or bird boxes until processing, and were banded with uniquely-numbered U.S. Geological Survey aluminum bands, aged as hatch-year (HY) or after hatch-year (AHY) based on plumage characteristics or skull ossification (Pyle 1997), and sexed where possible. We measured unflattened wing chord to the nearest 1 mm and mass to the nearest 0.1 g. Fat scores determined based on furcular and abdominal fat storage (0-6 scale, 0.5 increments) (modified from Helms and Drury 1960).

Indexing Fruit Consumption

We assessed diet of gray catbirds and Swainson's thrushes using the fecal samples. Captured birds waiting to be processed were stored in boxes lined with white sheets of paper to opportunistically collect fecal samples. Collected fecal samples were stored at -20 °C until analysis. Fruits were identified by a combination of seed, color, and texture of the pulp and skin (Parrish 1997). We smeared known fruits collected in the field on the same paper liner and stored under the same conditions to use as guide for identification. Arthropod consumption was identified by the presence of exoskeleton remains. Because we were interested in consumption proportions of fruit and arthropods in our sampling population and volumetric estimations in fecal samples were difficult (Parrish 1997), we counted samples multiple times when more than

one fruit species or when both fruit and arthropods co-occurred. All fruit were positively identified by a single observer blind to which habitat type the bird was captured in.

Statistical Analyses

We used Sigmaplot Version 11.0 (Systat Software 2008) to analyze habitat switchover rates. All other statistical computations were performed using R Version 3.1.2 (R Development Core Team 2014). All tests were performed two-tailed and outputs with P < 0.05 deemed as significant and 0.05 < P < 0.1 as a trend unless specified otherwise. Values are presented as means \pm SE throughout.

Characteristics of Habitats and Resource Attributes

We tested the expectation that exotic and native shrublands differed in shrub species composition (native-dominant vs. exotic dominant) but not with respect to structural attributes. We performed permutational multivariate analysis of variance (PerMANOVA) using function adonis in R package vegan (Cohen et al. 2012; Oksanen et al. 2015). PerMANOVA uses distance matrices and obviates the necessity for distributional assumptions that are rarely met in ecological community datasets (Anderson 2001). We compared shrub composition between habitats for two multivariate responses: (1) stem density estimates of all shrub species (8 exotic and 26 native) to detect species-level difference and (2) summarized total exotic stem density and total native stem density to confirm our habitat categorization of "exotic" and "native" shrublands. Habitat structural metrics of shrub density, shrub height, canopy height, percent ground cover, percent shrub cover, percent canopy cover, total tree density, and total tree basal area were entered as one multivariate response to test for difference in overall habitat structure. To test for variation in tree size class (A-F) between habitat types (potential confounder of shrubland habitat

categorization), we further ran PerMANOVAs on tree density by size class and basal area by size class.

Total fruit abundance was compared with two-sample *t*-tests after square root transformation. We then used PerMANOVA to test whether fruit composition differed between habitats. To compare indices of arthropod abundance by habitat in each year, we used linear mixed models fit by REML (R package nlme, function lme) with habitat type as the fixed factor and Julian date (continuous random effect) representing pseudoreplication within each trap. Arthropod biomass per day was ln-transformed to meet the normality assumption.

Indices of Bird Use

We used capture rate as an index for density (Smith and Hatch 2008; Akresh et al. 2009), which is generally positively correlated with habitat quality (reviewed in Johnson 2007). We calculated capture rates as numbers of newly-banded birds per net-hour and compared between exotic and native shrublands. Capture rates of gray catbirds, Swainson's thrushes, and magnolia warblers were similarly compared between habitat types. We used compound Poisson linear mixed model with log link function fit by Laplace approximation using function cpglmm in R package cplm (Zhang 2013) with habitat type specified as fixed factor and Julian date (continuous random effect) representing pseudoreplication within each individual net. We then performed single-term deletion χ^2 -based tests to determine the significance of the habitat effect.

In addition, we tested whether age ratio, size, fat mass, and rate of fat gain differed by habitat type in gray catbirds and Swainson's thrushes using their initial capture records. We compared age ratio using Fisher's exact tests and wing chord length using two-sample *t*-tests. Size-corrected fat mass for individuals were estimated using a series of regressions (Owen and Moore 2006; 2008). For each species, individuals of the same wing chord length class (1 mm

increments) were grouped (data compiled from records at the same site during fall migration in 2009-2014). Within each wing chord group, mass was regressed against fat score to obtain the intercept, which is the estimate for size-specific fat free mass (fat score = 0). In the second regression, the size-specific fat-free mass was then regressed against wing chord length to derive a linear model that predicts size-specific fat-free mass for a given wing chord length. Based on this model, fat-free mass of individuals were estimated and subsequently subtracted from actual mass to derive size-corrected fat mass (g) of individuals. Habitat differences in fat mass and fat gain were detected by multiple linear regressions (modified from Dunn 2000). For each species and year, we fitted models explaining fat mass by time after sunrise (proxy of foraging time) and habitat type as well as their interactions after controlling for the confounding effect of Julian date (positively related to fat mass in all comparisons, P < 0.05). A significant interaction between habitat type and time after sunrise indicates different refueling performance between habitats. Non-significant terms (P > 0.05) were sequentially dropped to test whether fat mass differed by habitat type (test for condition-based habitat partitioning; Tietz and Johnson 2007). Fat mass of Swainson's thrushes were ln-plus-5 transformed to attain normality.

Using birds with a recapture record we compared the proportion of individual birds that switched from one habitat type to the other (Akresh et al. 2009). Migrants during stopover tend to move into habitats where food resources are more abundant or contain more shelter (Moore et al. 1995; Cohen et al. 2012). Thus, a net emigration from poorer-quality habitat and a net immigration into better-quality habitat would be expected from capture-recapture data. We defined a bird as having switched habitat when the individual was recaptured in the other habitat relative to the one in which it was originally captured and was not recaptured again in the habitat where it was first captured. Because we detected few birds that transferred from one habitat to

another multiple times over the recapture history (2012: n = 2; 2013: n = 3), these individuals were excluded from further analyses. The proportion of birds that switched was calculated for all species combined and for gray catbirds, Swainson's thrushes, and magnolia warblers. We used χ^2 contingency tests to compare the proportion of individuals that switched vs. stayed in the exotic and native habitats and Fisher's exact tests for individual species.

Patterns of Fruit Consumption

Fecal samples with identified diet from 2012 (gray catbirds: n = 63, Swainson's thrush: n = 171) and 2013 (gray catbird: n = 42, Swainson's thrush: n = 39) were pooled to increase sample size after confirming that the consumption ranks of fruits (counts of identified fruits found in fecal samples) across habitats were consistent between years (Friedman's rank sum test for year with fruit species as block, P > 0.05). We used Fisher's exact tests to compare consumption proportions of fruits vs. arthropods and of fruit species between habitats. In order to assess whether birds consumed certain fruits disproportionate to their abundance within the entire study site, we pooled fruit abundance estimates and fecal samples from both habitats and compared these proportions using Fisher's exact test. When multinomial tests showed significant difference, we performed post-hoc exact binomial tests for each of the categories against the sum of all other categories with Bonferroni corrections in which critical $\alpha = 0.05$ was divided by the number of categories ($\alpha = 0.0031$, trend at 0.0031 < P < 0.0063) (McDonald 2014).

Results

Characteristics of Habitats and Resource Attributes

Estimated density of stems showed that the compositions of shrub species were significantly different between exotic and native shrublands (pseudo- $F_{1, 25} = 20.14$, P < 0.001; Table 1.1).

Summarized total exotic and native shrub density also differed significantly between the habitat types (pseudo- $F_{1, 25} = 53.97$, P < 0.001). Exotic stems represented 70% of the shrub stems in exotic shrubland but comprised only 12% in the native shrubland. On the other hand, overall habitat structure was similar in these shrublands (pseudo- $F_{1, 25} = 0.38$, P = 0.57), as well as tree density (pseudo- $F_{1, 25} = 0.43$, P = 0.84) and basal area (pseudo- $F_{1, 25} = 1.06$, P = 0.36) by size class.

Total fruit abundance were similar in both habitats ($t_{15.94} = -1.54$, P = 0.14). However, fruit composition differed significantly in the two habitat types (pseudo- $F_{1, 23} = 8.73$, P < 0.001; Figure 1.1). Summarized total exotic and native fruits also differed significantly (pseudo- $F_{1, 23} = 6.57$, P < 0.001). Exotic fruit represented 50% and 95% of the available fruit in the native and exotic shrublands, respectively. However, *R. cathartica*, an exotic species, was the most abundant fruit in the native habitat (Figure 1.1). Flying arthropod abundance did not differ by habitat in either 2012 ($F_{1, 2} = 4.89$, P = 0.16) or 2013 ($F_{1, 2} = 1.19$, P = 0.39; Figure 1.2).

Indices of Bird Use

We banded a total of 2006 birds including 398 gray catbirds and 576 Swainson's thrushes on 45 mornings in 2012 and 2182 birds including 314 gray catbirds and 360 Swainson's thrushes on 40 mornings in 2013.

Overall capture rates (all species) were greater in native shrubs as compared to exotic shrubs by 71% in 2012 ($\chi^2_1 = 30.73$, P < 0.001; Figure 1.3A) and 92% in 2013 ($\chi^2_1 = 17.74$, P < 0.001; Figure 1.3B). Fall frugivorous gray catbirds were 259% (2012: $\chi^2_1 = 70.67$, P < 0.001) and 340% (2013: $\chi^2_1 = 118.42$, P < 0.001) more likely to be captured in native shrubland than in exotic shrubland. Capture rate of Swainson's thrushes differed by habitat in the same direction by 21% (2012: $\chi^2_1 = 66.19$, P < 0.001) and 29% (2013: $\chi^2_1 = 9.82$, P = 0.002). We found no difference in capture rate by habitat type in primarily arthropodivorous magnolia warblers in 2012 ($\chi^2_1 = 2.63$, P = 0.11) and a non-significant trend toward greater capture rate in native shrubs (by 44%) in 2013 ($\chi^2_1 = 2.82$, P = 0.09).

In both years, over 90% of gray catbirds and over 85% of Swainson's thrushes captured were HY birds. Gray catbirds did not show habitat differences in age ratio (2012: P = 0.19; 2013: P = 0.63) or wing chord length (2012: $t_{140.50} = 1.14$, P = 0.26; 2013: $t_{86.52} = 0.55$, P = 0.59). Similarly, we did not find age (2012: P = 0.61; 2013: P = 1.0) or wing chord differences (2012: $t_{513.28} = -1.10$, P = 0.27; 2013: $t_{341.60} = -1.62$, P = 0.11) in Swainson's thrushes.

Based on regression-estimated fat mass, gray catbirds in 2012 gained fat with similar rates (by 0.17 g/hr; $F_{1,421} = 3.92$, P = 0.05) between habitat types (time after sunrise × habitat: $F_{1,420} = 0.04$, P = 0.83). In 2013 however, fat gain rates of gray catbirds differed significantly by habitat type ($F_{1,303} = 4.42$, P = 0.04) in that individuals using native shrubs gained fat by 0.25 g/hr ($t_{303} = 2.257$, P = 0.02) while no fat was gained in exotic shrubland ($t_{303} = -1.33$, P = 0.18). The main effect of habitat on fat mass was not found in either 2012 ($F_{1,421} = 1.87$, P = 0.17) or 2013 ($F_{1,303} = 0.10$, P = 0.75). No habitat-related difference in fat mass change in Swainson's thrushes were found in either year (2012: $F_{1,578} = 0.79$, P = 0.37; 2013: $F_{1,351} = 0.30$, P = 0.58); there was no evidence of fat gain in these years (2012: $F_{1,579} = 2.44$, P = 0.12; 2013: $F_{1,352} =$ 0.71, P = 0.40). Fat mass of Swainson's thrushes did not differ significantly by habitat in 2012 ($F_{1,580} = 2.43$, P = 0.1195) or 2013 ($F_{1,353} = 1.47$, P = 0.23).

We found that birds (data from all species) initially captured in the exotic shrubs were more likely to be recaptured in native shrubs (58% switched) than vice versa (32%) during migratory stopover in 2012 ($\chi^2_1 = 11.79$, P < 0.001; Figure 1.2A), and similar results were obtained from 2013 (60% switch from exotic to native, 24% switch from native to exotic; $\chi^2_1 = 26.85$, P < 0.001; Figure 1.4B). Examining species-specific movement in 2012, gray catbirds trended toward a greater switchover rate from exotic to native shrubs (57%, compared to native-to-exotic switch of 19%; P = 0.051; Figure 1.5A), but Swainson's thrushes (P = 0.27; Figure 1.5C) did not show habitat-related differences. In 2013, habitat switchover from exotic to native was significantly greater than vice versa in both gray catbirds (71%, compared to 24% switch from native to exotic; P = 0.02; Figure 1.5B) and Swainson's thrushes (71%, compared to 30% switch from native to exotic; P = 0.02; Figure 1.5D). Magnolia warblers did not show difference in switching by habitat in 2012 (P = 1.0; Figure 1.5E) or 2013 (P = 0.23; Figure 1.5F).

Patterns of Fruit Consumption

Gray catbird and Swainson's thrush fecal samples mostly contained fruit; arthropod remains were only represented in 6% and 14% of the samples in these species respectively. These fruitto-arthropod ratios did not differ by habitat in either species (P = 1.0). Comparing estimated fruit abundance and fecal samples within the entire study site, we found evidence of non-random selection of certain fruits in both gray catbirds and Swainson's thrushes (P < 0.001; Figures 1.2 and 1.6). Post-hoc tests (see Statistical Analyses on Bonferroni-corrected P-values) revealed that the native *L. benzoin* was disproportionately consumed by gray catbirds (319% greater than expected; P < 0.0031) and Swainson's thrushes (243% greater than expected; P < 0.0031), while the exotic *E. umbellata* was selected against (86% less than expected in gray catbirds and by 88% in Swainson's thrushes; both P < 0.0031). Swainson's thrushes also showed evidence of selecting against the exotic *R. cathartica* (consumed 31% less than expected; P < 0.0031) and the native *I. verticillata* (54% less than expected; P < 0.0031). Similar trends were found in gray catbirds (40% less than expected consumption of *R. cathartica* and 69% less of *I. verticillata*; both P = 0.0044). In addition, Swainson's thrushes disproportionately consumed the native common elderberry (*Sambucus canadensis*, by 431%), and exotic bittersweet nightshade (*Solanum dulcamara*, by 268%), whereas *R. multiflora* was consumed significantly less (by 89%) than expected (all P < 0.0031).

Discussion

We found that indices of bird use, particularly those of gray catbirds and Swainson's thrushes, were significantly greater in native shrubland than in exotic shrubland. Based on the consumption patterns of fruit species, we believe these differences are the result of fall frugivorous birds selecting for certain native fruits, and that native shrubland represent higherquality stopover habitat in comparison to exotic shrubland.

Characteristics of Habitats and Resource Attributes

We found that vegetation structure and flying arthropod abundance were similar in exotic and that native shrublands. The importance of shelter from predators or weather (Moore et al. 1995; Moore et al. 2005; Ktitorov et al. 2008) and arthropod diet (Smith et al. 2007; Smith et al. 2013) during migratory stopover has been well established, and our results suggest that the quality of exotic shrublands as stopover habitat would be similar to native counterparts with respect to these attributes. Similarly, we did not find difference in the total abundance of fruits. On the other hand, these shrublands were significantly different in shrub and fruit species composition and we confirmed that exotic shrubland was indeed exotic-dominated and native shrubland and native-dominated. Therefore, we consider the main difference between exotic shrubland and

native shrubland to be in fruit species composition.

Indices of Bird Use

We predicted that bird use during fall migration would be greater in native shrubs than exotic shrubs, particularly in fall frugivorous species. Consistent to our prediction we found greater capture rate in native shrubland relative to exotic shrubland and greater overall switchover rate from exotic to native shrubland during fall migration of 2012 and 2013. Capture rates in spring and summer at the same study site did not differ between habitat types (Y. Oguchi and J. Owen personal observation) suggesting that the difference may be attributed to exotic vs. native fruits available. Indeed, fall frugivorous gray catbirds and Swainson's thrushes showed greater capture rates in native compared to exotic shrubland in both years and greater habitat switchover into native shrubland from exotic shrubland in 2013, whereas the primarily arthropodivorous magnolia warbler did not show differences in these measures. Because greater indices of density (e.g., capture rate) in a habitat (Johnson 2007; Smith and Hatch 2008; Akresh et al. 2009) and net influx into a habitat from adjacent ones (Johnson 2007; Akresh et al. 2009; Cohen et al. 2012) generally represent superior habitat quality, our results provide evidence for greater quality of native shrublands compared to exotic ones.

While the majority of gray catbirds and Swainson's thrushes captured were HY birds, we found no age- or size-related differences in habitat use. Thus, we do not consider age- or size-related dominance played a significant role in the distribution of these birds. We also found no evidence for condition-based habitat partitioning (sensu Tietz and Johnson 2007) based on our fat mass estimates. Additionally, we did not find evidence of differential refueling success (i.e. rates of fat mass gain) during morning foraging in any comparison groups except gray catbirds in

2013, which showed fat gain only in native shrubland. Earlier work in Michigan (Craves 2009) also suggested that Swainson's thrushes were able to gain mass at a stopover site composed of mixed exotic and native shrubs after depletion of high-energy native fruits. It is possible that birds could compensate for the lower energetic potential in exotic fruits by increased consumption, but more detailed work will be necessary to compare relative effort and energy gain of consuming exotic vs. native fruits.

Patterns of Fruit Consumption

Based on theories of optimal foraging (Emlen 1966; MacArthur and Pianka 1966) and optimal migration (Alerstam and Lindström 1990; Alerstam 2011), birds during stopover should prefer food that permits greater energy gain with the same handling effort to maximize refueling performance and minimize predation risk (see also Sih and Christensen 2001; Shochat et al. 2002). Because native fruits are generally more energy-dense (White 1989; Smith et al. 2007; Smith et al. 2013), we predicted that certain native fruits would be selectively consumed. Despite the inherent difficulties of detection of diet items using fecal samples (Blake and Loiselle 1992; Parrish 1997), our results, along with previous work based on fruit removal (Smith et al. 2013), suggest that high-energy native fruits are selected for by landbird migrants. In particular, we found that both gray catbirds and Swainson's thrushes disproportionately consumed L. benzoin, a native fruit very rich in fat and energy (White 1989; Smith et al. 2013). As expected, we found evidence for birds selecting against the energy-poor exotic fruits of E. umbellata, R. cathartica (non-significant trend in gray catbirds), and R. multiflora (only in Swainson's thrushes). I. *verticillata* was the only native fruit that was selected against by gray catbirds (trend) and Swainson's thrushes. Because *I. verticillata* was among the least energy-dense of the native

fruits found at our site (based on White 1989), birds were likely consuming other abundant native fruits that were higher in energy content. The shrub and fruit composition data showed that the highly-selected *L. benzoin* was only abundant in native shrubland, and fruits found in exotic shrubland were predominantly those selected against (*E. umbellata*, *R. cathartica*, and *R. multiflora*). Thus, selection of certain fruits explains the pattern of high native shrubland use by gray catbirds and Swainson's thrushes.

Based on the observation that birds select against some of the most common exotic fruits within the overall study site, there are two possible explanations for the use of exotic shrubland by refueling landbirds: optimal foraging and insufficient information about relative habitat quality. Optimal foraging would result in the ideal free distribution in which some individuals occupy lower-quality habitat to avoid competition and attain equal fitness to those using higher-quality habitat (Emlen 1966; MacArthur and Pianka 1966; Shochat et al. 2002). But this is based on the assumption that birds are familiar with all available habitat, which is not the case during migratory stopover for most landbirds (Moore et al. 1995; Shochat et al. 2002). Hence, the birds' lack of information about relative habitat quality (perceptual limitation) could result in use of non-optimal habitats (Abrahams 1986; Shochat et al. 2002). Perceptual limitation is also related to ecological traps in that birds are drawn to sub-optimal habitat as a result of mismatching between cues of habitat quality and actual quality of habitats (Weldon and Haddad 2005).

Our data suggest that both models could partially explain the observed habitat occupancy. Consistent with optimal foraging, bird density was greater in native shrubs, and avoiding that density by foraging in exotic shrubs did not appear to compromise refueling success with the exception of gray catbirds in 2013. Evidence for perceptual limitation comes from the pattern of greater habitat switchover rate from exotic to native compared to vice versa. Furthermore, from a

subset of gray catbirds with blood samples, we found that several measures of health status (antioxidant capacity and immunity) were lower in individuals using exotic shrubland than conspecifics captured in native shrubland (Y. Oguchi and J. Owen personal observation). Future studies should quantitatively test optimal foraging vs. perceptual limitation as explanations for exotic shrubland use.

Conclusions and Management Implications

Our results suggest that native-dominated shrubland is more important to fall migrating landbirds relative to exotic-dominated shrubland, and that this importance derives from the value of certain native fruits such as *L. benzoin*. While primarily arthropodivorous migrants such as magnolia warblers may be less impacted by invasions of exotic shrubs, our results suggest that fall frugivorous species such as gray catbirds and Swainson's thrushes would select against exotic shrubs and their abundant fruits, moving out into native shrublands if available.

These findings should encourage managers to conserve and restore *L. benzoin* across its native range in North America, spanning eastward from Ontario and Texas to the Atlantic coast (USDA and NRCS 2015). This lipid-rich fruit (White 1989; Smith et al. 2013) was disproportionately consumed relative to its abundance in both exotic and native shrublands. We also found *L. benzoin* fruits to be among the richest in antioxidant capacity among the 14 different fruits assayed in our study site, with moderate concentration of immunostimulatory carotenoids (Y. Oguchi, personal observation). Thus, we consider *L. benzoin* to be the single most important fruit for fall frugivorous migrating landbirds in mid-Michigan.

The relative value of exotic fruits for migrating landbirds would depend on what other food resources are present at a stopover site. Exotic fruits may be of high importance when a

stopover site is set in a landscape where few other fruiting species are present (Gleditsch and Carlo 2011) or for late migrant arriving after native fruits have been depleted (White and Stiles 1992; Craves 2009). In our study site, the possibility remains, therefore, that native fruits of high value could have been depleted after the termination of field season at the end of October, in which case the relative importance of exotic shrubland might have increased. Nevertheless, our results support the view that exotic shrubs are of limited value to fall frugivorous migrants in the presence of native counterparts (Smith et al. 2013), and our site-wide fruit abundance and consumption data suggests that these birds can select for certain high-energy native fruits even though exotic fruits such as *R. cathartica* and *E. umbellata* are more abundant. We thus recommend efforts on preventing encroachment of exotic shrubs into native-dominated shrubland and partially supplanting exotic shrubs in a predominantly exotic shrubland by native shrubs such as *L. benzoin* if management priority is to improve habitat quality for fall frugivorous migrants.

We conclude that native shrublands represent superior quality habitat compared to exotic counterparts and that this finding is limited to fall frugivorous migrants. Evaluation of exotic shrub impact on native migrating landbirds is complex and may be contingent on phenology over the annual cycle in addition to from inter-species and local variation in other food resources as described earlier (reviewed in Rodewald 2012). For example, recent evidence indicates that the exotic shrublands dominated by *Lonicera* spp. may function as an ecological trap for breeding birds (Rodewald 2009; Rodewald et al. 2010; Rodewald et al. 2011), while these shrubs have been shown to produce sufficient arthropod abundance that permit successful refueling in spring (Smith 2006; Smith and Hatch 2008). Studies that associate seasonally-dependent nutritional opportunities in exotic and native shrubs with food and habitat selection over the entire annual

cycle and will likely enhance our understanding of the impact of these shrubs on landbird populations and communities.

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Table 1.1. Estimated shrub stem densities (stems/ha) in exotic- and native-dominated shrublands near East Lansing, MI, USA. Data (mean \pm SE) are presented in decreasing order in exotic shrubland and in increasing order in native shrubland, showing profound difference in composition between the two shrublands.

Scientific name	Common name	U.S.	Exotic	Native
		status	shrubland	shrubland
Elaeagnus umbellata	Autumn Olive	Exotic	1575.8 ± 221.5	30.0 ± 22.3
<i>Lonicera</i> spp.	Honeysuckle	Exotic	1459.8 ± 324.6	0
Rosa multiflora	Multiflora Rose	Exotic	902.9 ± 299.7	409.5 ± 115.6
Vitis riparia	Riverbank Grape	Native	722.3 ± 250.1	157.08 ± 42.7
Akebia quinata	Five-leaf Akebia	Exotic	600.7 ± 455.6	0
Rhamnus cathartica	European Buckthorn	Exotic	357.4 ± 83.9	171.2 ± 70.2
Rubus occidentalis	Black Raspberry	Native	146.4 ± 96.3	7.1 ± 4.8
Parthenocissus quinquefolia	Virginia Creeper	Native	114.0 ± 24.2	75.89 ± 26.7
Rubus allegheniensis	Common Blackberry	Native	57.0 ± 53.0	0
Rhamnus alnifolia	Alderleaf Buckthorn	Native	51.3 ± 33.9	1.8 ± 1.8
Rhus typhina	Staghorn Sumac	Native	34.2 ± 34.2	0
Cornus alternifolia	Alternate-leaved Dogwood	Native	34.2 ± 19.9	0
Malus spp.	Crabapple	Native	32.3 ± 19.7	1.8 ± 1.8
Toxicodendron radicans	Poison Ivy	Native	26.6 ± 15.2	14.1 ± 9.2
Viburnum trilobum	American Cranberrybush	Native	26.6 ± 26.6	35.3 ± 31.5
Rubus idaeus	American Red Raspberry	Native	22.8 ± 22.8	47.7 ± 20.6
Viburnum opulus	European Cranberrybush	Exotic	17.1 ± 10.2	42.4 ± 20.5
Rubus flagellaris	Northern Dewberry	Native	13.3 ± 13.3	0
Sambucus canadensis	Common Elderberry	Native	7.6 ± 7.6	455.4 ± 240.2
Amelanchier spp.	Serviceberry	Native	5.7 ± 5.7	28.2 ± 19.9
Vaccinium angustifolium	Lowbush Blueberry	Native	0	1.8 ± 1.8
Prunus virginiana	Chokecherry	Native	0	10.6 ± 7.6
Betula pumila	Bog Birch	Native	0	12.4 ± 12.4
Crataegus spp.	Hawthorn	Native	0	21.2 ± 21.2
Berberis thunbergii	Japanese Barberry	Exotic	0	30.0 ± 30.0
Physocarpus opulifolius	Common Ninebark	Native	0	130.6 ± 74.2
Solanum dulcamara	Bittersweet Nightshade	Exotic	0	169.4 ± 58.8
Cornus sericea	Red Osier Dogwood	Native	0	176.5 ± 161.9
Viburnum lentago	Nannyberry	Native	0	194.2 ± 82.8
Corylus Americana	American Hazelnut	Native	0	356.5 ± 130.2
Table 1.1 (cont'd)

Scientific name	Common name	U.S.	Exotic	Native
		status	shrubland	shrubland
Toxicodendron vernix	Poison Sumac	Native	0	656.6 ± 121.9
Lindera benzoin	Common Spicebush	Native	0	806.6 ± 167.1
Ilex verticillata	Common Winterberry	Native	0	1163.2 ± 284.4
Cornus racemosa	Gray Dogwood	Native	19.0 ± 19.0	1350.3 ± 422.6



Figure 1.1. Estimated fruit abundance in exotic- and native-dominated shrublands near East Lansing, MI, USA during fall migration of 2012. Data (mean \pm SE) are presented in decreasing order in exotic shrubland and in increasing order in native shrubland. Asterisk indicates exotic species. Permutational MANOVA revealed significant difference in fruit composition, as well as composition of total exotic vs. total native fruits between shrublands.



Figure 1.2. Flying arthropod abundance in exotic- and native-dominated shrublands near East Lansing, MI, USA during fall migration of 2012 and 2013. Arthropods were collected using two Malaise traps in each habitat and data are presented as dry biomass (\pm SE). Linear mixed models on ln-transformed data showed no significant habitat difference in arthropod abundance in either year.



Figure 1.3. Capture rates of newly-banded landbirds (\pm SE) in exotic- and nativedominated shrublands near East Lansing, MI, USA during fall migration of 2012 and 2013. Birds were passively captured in 15 mist nets in each habitat. Compound Poisson linear mixed models revealed significantly greater capture rate in native shrubs as compared to exotic counterparts in both years.



Figure 1.4. Movement of banded landbirds between exotic- and native-dominated shrublands near East Lasing, MI, USA during fall migratory stopover in 2012 and 2013. Significantly greater switchover rate from exotic- to native-dominated shrubland compared to vice versa was found in both years.



Figure 1.5. Movement of banded gray catbirds, Swainson's thrushes, and magnolia warblers between exotic- and native-dominated shrublands near East Lasing, MI, USA during fall migratory stopover in 2012 and 2013. Significantly greater switchover rate from exotic- to native-dominated shrubland compared to vice versa was found in gray catbirds (B) and Swainson's thrushes (D) in 2013, and a similar non-significant trend in gray catbirds in 2012 (A). All other comparison groups (C, E, F) did not show difference in switchover rate.





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CHAPTER 2:

Health consequences of differential stopover habitat use and fruit consumption

in fall migrating landbirds

ABSTRACT

Migration is a physiologically-demanding activity, and recent studies suggest the importance of migrating birds being able to restore immune and antioxidant status during stopover. However, knowledge is lacking on whether or how differential use of stopover habitat impacts the health status of migrating landbirds. We tested whether frugivorous fall migrating landbirds using exotic-dominated shrubland in central Michigan, USA experience difference in health relative to conspecifics captured in native-dominated shrubland. We assayed blood from migrating gray catbirds (Dumetella carolinensis) and Swainson's thrushes (Catharus ustulatus) captured in exotic or native shrubland in the fall of 2012 and 2013 for indices of refueling performance, constitutive innate immunity, total circulating carotenoids and antioxidant capacity. We did not find habitat difference in plasma triglyceride levels (refueling index) in either species or year, but three immune indices (granulocyte:lymphocyte ratio, hemagglutination activity and haptoglobin) and plasma total carotenoids were significantly lower in gray catbirds using exotic shrubs than in conspecifics captured in native shrubs in 2013. Gray catbirds in exotic shrubland also showed lower antioxidant capacity in both years. No such differences were found in Swainson's thrushes. Based on the carotenoids and antioxidant capacity of fruits and patterns of consumption, we found limited evidence that differential consumption of exotic and native fruits might have been reflected in the health status of these birds. These results provide the first evidence that differential stopover habitat use may have important health consequences for migrating landbirds and suggest the value of including measures of immunity and antioxidant status in ecophysiology studies evaluating stopover habitat quality, particularly when nutritional difference is suspected between habitats.

Introduction

Migration involves major modulations of physiological status and trading off immune defense with high energy demands of endurance flight may occur in migrating birds (reviewed in Piersma 1997; Altizer et al. 2011). Evidence shows that during migration the spleen, a principal immune organ in birds (Fänge and Silverin 1985), shrinks in size (Deerenberg et al. 2002), and circulating leukocyte counts (Owen and Moore 2006) are lower in migrating birds than in conspecifics during breeding. Downregulation of immune activity has also been found in individuals with experimentally-induced migratory disposition (Owen and Moore 2008b). Furthermore, prolonged flight (Nebel et al. 2012; Nebel et al. 2013) and limited food supply (Buehler et al. 2009) were shown to result in reduced measures of constitutive (non-induced) immunity (but see Hasselquist et al. 2007; Hegemann et al. 2012 for induced response), and migrating birds in poor energetic condition may experience greater risk of infection (Owen and Moore 2008a). Migratory flight is also accompanied by oxidative stress resulting from production of large quantities of tissue-damaging reactive oxygen species (Costantini et al. 2007; Costantini 2008; Jenni-Eiermann et al. 2014) and depletion of antioxidants (Costantini 2008; Costantini et al. 2008). A reduction in immune status may play a role in the high mortality that occurs during migration (see Sillett and Holmes 2002), and a migrant's inability to locate highquality stopover sites to rest and refuel may further exacerbate their condition and have major fitness consequences (Moore et al. 1995; Smith and Moore 2003; Moore et al. 2005).

Recently, physiological parameters related to individual performance have been identified as valuable tools to evaluate habitat quality (Albano 2012). The analyses of plasma fat metabolites, whose profiles change within hours of foraging (Jenni and Jenni-Eiermann 1992; Jenni-Eiermann and Jenni 1994), have gained popularity in comparing individual refueling

performance between different habitat types (e.g., Guglielmo et al. 2005; Cerasale and Guglielmo 2010; Smith and McWilliams 2010; Evans Ogden et al. 2013; Liu and Swanson 2014). However, refueling success is only one aspect of performance, and evaluations of immune and antioxidant status are also likely germane to habitat assessment (Albano 2012). Indeed, the need to understand factors influencing health status of migratory birds facing anthropogenic landscape changes has been identified as a research priority for avian ecophysiologists (Klaassen et al. 2012). Limited evidence indicates that successful stopover involves recovery or upregulation of immune function (Owen and Moore 2008a; Buehler et al. 2010; Nebel et al. 2012) and circulating antioxidants (Costantini et al. 2007; Alan et al. 2013) that were reduced during flight. However, to our knowledge it is unknown whether stopover habitat quality has any consequences on immune and antioxidant status.

It is conceivable that differential stopover habitat use manifests as differences in health status, particularly when dietary quality differs between habitats. Here, we focused on the difference in the fruit quality between exotic and native shrubs as part of a larger study to evaluate stopover habitat quality between predominantly exotic and predominantly native shrublands. Introductions of exotic shrubs due to human land-use change has been identified as a potential conservation concern for migrating landbirds (Suthers et al. 2000; Catling 2005; Craves 2009), particularly because exotic fruits common in Midwestern and Northeastern United States such as autumn olive (*Elaeagnus umbellata*), European buckthorn (*Rhamnus cathartica*), and honeysuckle (*Lonicera* spp.) generally have higher sugar contents and lower energetic potential compared to the native counterparts (White 1989; Smith et al. 2007a; Smith et al. 2013). Thus, consuming the high-fat, high-energy native fruits such as common spicebush (*Lindera benzoin*), gray dogwood (*Cornus racemosa*), and Virginia creeper (*Parthenocissus quinquefolia*) (White

1989; Smith et al. 2013) could be advantageous for refueling (Smith et al. 2007a; Smith et al. 2013). These nutritional differences and observed selection of certain native fruits has led previous work (Smith et al. 2013) to conclude that exotic shrubs may represent poorer stopover habitat than native counterparts during fall migration.

Refueling performance may also impact recovery of overall health status because superior energetic condition has been linked to stronger immune function (Owen and Moore 2008a; Buehler et al. 2010) and antioxidant capacity (Costantini et al. 2007) during stopover. But exotic and native fruits also vary by species in their antioxidant capacity and carotenoid contents (Alan et al. 2013), and carotenoids are well-known for their strong immunostimulatory effects in birds (McGraw and Ardia 2003; McGraw et al. 2006; McGraw et al. 2011; Simons et al. 2012). Because birds select fruits based on a combination of different characteristics including fat and antioxidant contents (Stiles 1993; Alan et al. 2013; Bolser et al. 2013; Smith et al. 2013; Pierce and McWilliams 2014), the combined effect of fruit quality and consumption likely impacts a migrant's health status. However, the health consequences of consuming exotic fruits relative to consuming native counterparts are largely unknown.

In the present study, we investigated whether health status of fall migrating landbirds using exotic-dominated shrubland differed from conspecifics using native-dominated counterpart. Because migrants need to refuel and restore their health during stopover (sensu Costantini et al. 2007; Costantini et al. 2008; Owen and Moore 2008a; Buehler et al. 2010; Nebel et al. 2013), a stopover habitat in which individuals exhibit greater indices of fat gain, immune function, and antioxidant capacity could be considered superior-quality. We assessed (1) refueling performance, (2) cellular (leukocytes) and humoral (plasma protein) constitutive innate immunity, (3) plasma total carotenoids, and (4) plasma antioxidant capacity of two species of fall

frugivorous migrants. As indices of immunity we focused on the constitutive innate branch because it represent the first line of defense for birds encountering novel pathogens during migration and deemed most relevant during short stopover when acquired immunity is too slow to respond (Schmid-Hempel and Ebert 2003; Buehler et al. 2010). The immune parameters selected for this study (Davis 2005; Sepp et al. 2010; van de Crommenacker et al. 2010; Nebel et al. 2012), as well as circulating carotenoids (Hõrak et al. 2004) and antioxidant capacity (van de Crommenacker et al. 2010) appear to change within a range of several hours to several days, well within the stopover lengths of fall migrating landbirds (Woodrey and Moore 1997; Craves 2009).

Additionally, we investigated whether differential fruit quality and consumption were reflected as habitat-related differences in health parameters. We measured total carotenoid concentration and antioxidant capacity in exotic and native fruits found in our study site and combined these data with consumption proportions as found in fecal samples to test for habitat differences in expected dietary means of carotenoids and antioxidant capacity. This integrative approach provides a more comprehensive picture of how differential stopover habitat use may have important health consequences for migrating landbirds.

Methods

Study Site and Habitats

We conducted fieldwork during fall migration of 2012 and 2013 on a state-managed wildlife area near East Lansing, MI, USA (Rose Lake State Wildlife Research Area, N 42°48'43.1," W 84°23'05.7"). The site was set largely within an agricultural and suburban landscape but no significant geographical barrier was present in surrounding areas. Both exotic- and nativedominated shrublands were at similar longitudes and occurred as distinct patches divided by

mature oak-maple woodland around 250 m wide.

Woody vegetation structure was similar between shrublands (Y. Oguchi and J. Owen, unpublished data). Native-dominated shrubland (hereafter native shrubland) was primarily composed of *C. racemosa*, *L. benzoin*, common winterberry (*Ilex verticillata*), poison sumac (*Toxicodendron vernix*), common elderberry (*Sambucus canadensis*), and various saplings of canopy species. Exotic-dominated shrubland (hereafter exotic shrubland) was composed largely of *E. umbellata*, honeysuckles (*Lonicera tartarica*, *L. morrowii*, and *L. x bella* [*morrowii* x *tatarica*]), and *R. multiflora*, all introduced from East Asia. Although multiflora rose and the exotic *R. cathartica*) occurred in similar frequencies in both habitats, greater than 70% of the shrub stems in exotic shrubland were exotic, whereas the exotic stem frequency was only 12% in native shrubland (Y. Oguchi and J. Owen, unpublished data).

Study Species, Capture, and Blood Collection

We selected the gray catbird (*Dumetella carolinensis*) and the Swainson's thrush (*Catharus ustulatus*) as focal species due to their presence and abundance in both shrublands and their high consumption of fruits during fall migration (fruits present in >93% and >85% in gray catbird and Swainson's thrush fecal samples respectively, Y. Oguchi and J. Owen unpublished data; also see Parrish 1997). Birds were captured passively using 30 mist nets (30 mm-mesh, 12m x 2.6m), 15 in each habitat type, from August 13 to September 30 in 2012 and 2013. Nets were checked at 45-min intervals beginning 30 min before sunrise for 4-5 h daily. Capture time was recorded (± 1 min) as the time the bird was first detected in the net. We held birds in individual cloth bags or bird boxes until banding and blood collection. Bird boxes were lined with a sheet of paper to collect fecal samples for fruit consumption analysis. 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. Unflattened wing chord was measured to the nearest mm and mass to the nearest 0.1 g. We visually assigned fat scores based on furcular and abdominal fat storage (0-6 scale, 0.5 increments) (modified from Helms and Drury 1960) and pectoral muscle scores (0-3 scale, 0.5 increments) (modified from Bairlein et al. 1995).

We collected \leq 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. Time of blood sampling (±1 min) was recorded and handling time was calculated by subtracting the capture time (see above) from the time of bleeding. Upon collection we made a single-cell layer slide which was air dried, fixed in 100% methanol, and later stained with Wright-Giemsa solution (Fisher Scientific Hema3 #22-122-911, Hampton, NH, USA). The remaining blood was stored over ice in the field until centrifuged. After centrifugation, plasma was collected and stored in -80°C freezer until use.

Capture rates of study species were lower in exotic compared to native shrubland (Y. Oguchi and J. Owen, unpublished data), and in total, we sampled 212 (exotic shrubland: 46; native shrubland: 166) and 196 (exotic shrubland: 46; native shrubland: 150) gray catbirds in 2012 and 2013 respectively, and 203 (exotic shrubland: 82, native shrubland: 121) and 229 (exotic shrubland: 108; native shrubland: 121) Swainson's thrushes in 2012 and 2013 respectively. Sample sizes varied for each assay due to inter-individual variation in the blood volume we were able to collect (see means ± SE and sample size for health parameters in Table 2.1). All work was performed under the following permits: USGS Master Banding (#23629), USFWS Scientific Collection (#MB19470), MI Scientific Collection (#SC1386), Rose Lake

Special Use, and Michigan State University IACUC protocol (#07/11-145-00).

Indices of Refueling Performance

Size-corrected Fat Mass

Size-corrected fat mass (Owen and Moore 2006; 2008a), regressed against time after sunrise, can be used to detect habitat differences in fat gain or loss (see Statistical Analyses and Dunn 2000). We used this method in addition to plasma metabolites (see below) because it provides estimates of actual rate of fat mass change and because plasma metabolites can be sensitive to capture effects (Jenni and Jenni-Eiermann 1992; Guglielmo et al. 2002) and may be affected by dietary differences in carbohydrates and fats (Seaman et al. 2005; Smith et al. 2007b; Smith and McWilliams 2009). To estimate size-corrected fat mass, we first grouped conspecific individuals by 1-mm-increment wing chord class and regressed body mass on fat score using data of the study species captured at our site during fall of 2009-2014. The intercept (fat score = 0) was taken as the fat-free body mass for that wing chord class. We then regressed size-specific fat-free mass on wing chord to produce a linear equation, which was used to calculate size specific fatfree mass of each individual. The size-corrected fat mass was calculated by subtracting the fatfree mass from the actual mass.

Plasma Metabolites

Plasma triglycerides (triacylglycerol: TRIG) and β-hydroxybutyrate (BUTY) levels are commonly applied to index stopover habitat quality (e.g., Cerasale and Guglielmo 2010; Evans Ogden et al. 2013). In general, high levels of TRIG represent greater fattening rates within a few hours prior to capture whereas higher BUTY levels represent greater rates of fat catabolism (Jenni and Jenni-Eiermann 1992; Jenni-Eiermann and Jenni 1994; 1996; 1998). While plasma

metabolites do not translate to actual fat mass being gained, the advantage of this approach is that these metabolites are very precise and time-sensitive (Guglielmo et al. 2005). Previous studies (e.g., Guglielmo et al. 2002; Guglielmo et al. 2005; Smith and McWilliams 2010; Evans Ogden et al. 2013) suggest that TRIG is the most reliable and informative plasma lipid metabolite for comparing habitat differences in refueling performance, while some doubt has been placed on the utility of BUTY for this purpose (see Acevedo Seaman et al. 2006; Smith and McWilliams 2010; Evans Ogden et al. 2013).

We used commercially-available reagents to measure both plasma TRIG and BUTY concentrations (mmol/l) as previously described (Guglielmo et al. 2002; Guglielmo et al. 2005). Plasma samples (10 µl) were serially diluted (1:2 and 1:4) in 10 µl of 0.9% NaCl solution. Twofold and four-fold dilutions were used for TRIG and BUTY respectively, which yielded concentrations that fell within the range of the standard curves. TRIG was assayed sequentially using an endpoint assay. Briefly, 2.5 µl of diluted plasma were pipetted into a flat-bottom 96well assay plate (Thermo Nunc #475094, Waltham, MA, USA) with 120 µl of glycerol reagent (Sigma, # F6428, St. Louis, MO, USA) and incubated for 10 min at 37°C. After taking an initial reading with a microplate reader (BioTek #ELx800, Winooski, VT, USA) at $\lambda = 540$ nm, 30 µl of triglyceride reagent (converts TRIG to glycerol, Sigma # T2449, St. Louis, MO, USA) were added, incubated, and read under the same conditions. The concentration TRIG was obtained by subtracting the concentration of free glycerol (first reading) from total glycerol (second reading). Values were multiplied by 2 to correct for the dilution. Standard curves were obtained by serially diluting (5.64, 2.82, 1.41, 0.705, 0.3535, and 0 mmol/l) the glycerol standard (Sigma, #G7793, St. Louis, MO, USA). Assays were run in duplicate.

BUTY assays were performed using a kinetic endpoint assay (R-Biopharm,

#10907979035, Marshall, MI, USA). Briefly, 5 μ l of diluted plasma were pipetted into a flatbottom 96-well plate with 200 μ l of working solution of reagents prepared as per manufacturer's directions. After 2 min of incubation at room temperature, 4 μ l of β -hydroxybutyrate dehydrogenase suspension were added. Absorbance was read (λ = 490nm) at 0, 30, and 40 min, and three times the change in absorbance between 30-40 min (background) was subtracted from the change between 0-40 min. This change, linearly related to BUTY concentration, was calculated against the change in standards of 4, 2, 1, 0.5, 0.25, and 0 mmol/l of BUTY (Sigma #166898, St. Louis, MO, USA). The measures were multiplied by 4 to correct for the dilution. Assays were run in duplicate.

Indices of Constitutive Innate Immune Function

Total Leukocyte Count and Granulocyte:lymphocyte Ratio

Total leukocyte count have 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 (Maxwell 1993; Davis et al. 2008) including migration (Owen and Moore 2006; 2008a) are 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 under ×1,000 magnification (oil-immersion) representing an average of 200 erythrocytes (Campbell and Dein 1984; Owen and Moore 2006; Owen 2011; Owen et al. 2013). Total leukocyte count was obtained as the tally of all leukocyte types (heterophil, eosinophil, lymphocyte, monocyte, and basophil) divided by 2 and is hence expressed as the number of leukocytes per 10,000 red blood cells. G:L ratio was calculated by dividing the number of granulocytes (tally of heterophils, eosinophils, and basophils) by the number of lymphocytes.

Hemolysis and Hemagglutination Activities

We modified the hemolysis-hemagglutination assay (Matson et al. 2005) to index activities of natural antibodies and complement in the plasma. This technique characterizes the ability of natural antibodies to agglutinate foreign red blood cells and to activate the complement system, which results in lysis and visible clearing of the blood cell suspension. Briefly, 10 μ l of plasma were pipetted into column 2 of a round-bottom 96-well plate (Corning Coaster #3795, Corning, NY, USA). Then, 10 µl of 0.01 M Phosphate buffered saline (PBS, Sigma #P3744, St. Louis, MO, USA) were pipetted into columns 2-12, and samples were serially diluted (1:2) through column 12 using a multi-channel pipette. Because both gray catbird and Swainson's thrush showed hemolysis at 1:2 dilution of plasma, 10 μ l of PBS were pipetted into column 1 as negative control (instead of undiluted plasma in the original protocol). A 1% rabbit red blood cell suspension was prepared from a commercially available source (HemoStat Laboratories #RBA050, Dixon, CA) and 10 µl were added to each well. Chicken serum was assayed in row 1 of each plate as a quality control standard. Plates were gently shaken, covered with an acetate plate sealer (Thermo #3501, Milford, MA), and then incubated in a 37 °C water bath for 90 min. After removal from the bath, plates were tilted at a 60-degree angle for 20 min at room temperature to enhance the visualization of hemagglutination. Plates were scanned at 300 dpi using Epson Perfection 4180 Photo Scanner for hemagglutination scoring. A hemolysis scan was then taken after an additional 70 min of incubation at room temperature. From the scans hemolysis and hemagglutination were scored blindly by a single observer as the last well (highest dilution) exhibiting the respective activity. Thus in principle, scores represent log-2 transformed (1:2 serial dilutions) measures of hemolysis or hemagglutination activity.

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 response (Matson et al. 2012a). In addition, because heme induces oxidative stress during endurance exercise (Cooper et al. 2002), the role of haptoglobin as an antioxidant (Gutteridge 1987) may play an important role during migration. We assayed haptoglobin using a commercially-available kit (Tridelta Development #TP801, Maynooth, County Kildare, Ireland). Plasma samples remaining from previous assays were used because one freeze-thaw cycle does not measurably degrade haptoglobin (Brinc et al. 2012). Following the manual method described by the manufacturer, 50 μ l of Reagent 1 were added to 3.75 μ l of plasma samples in a 96-well flat-bottom assay plate. After taking a background absorbance at $\lambda =$ 630 nm, 70 µl of Reagent 2 was added and incubated at room temperature for 5 min. Then, the plate was immediately read at the same absorbance. To correct for the initial variation in plasma color or turbidity, the background was subtracted from the final reading. The standard curve was generated from serial dilutions (2.5, 1.25, 0.625, 0.312, and 0 mg/ml) of the calibrator serum. Concentrations of samples are reported as mg/ml.

Plasma Total Carotenoids

Carotenoids are dietary-derived pigments that play a minor role as antioxidants (Costantini and Møller 2008; Simons et al. 2012) and have strong stimulatory effects on various branches of immunity (McGraw and Ardia 2003; McGraw et al. 2006; McGraw et al. 2011; Simons et al. 2012). We used a spectrophotometric assay to measure plasma total carotenoid concentration (Tella et al. 1998; Ninni et al. 2004; Hõrak et al. 2006; McGraw et al. 2008). Plasma samples

remaining from previous assays (one prior freeze-thaw cycle) were used (Nierenberg 1985; Comstock et al. 2001). Briefly, 90 µl of absolute ethanol were added to 10 µl of plasma (1:10 dilution) to extract carotenoids and precipitate proteins. The mixture was vortexed for 10 sec and centrifuged for 1 min under 16,800×g. Then, 70 µl of the supernatant was pipetted into a flatbottom 96-well assay plate. Clear plastic tape was placed over each row to prevent evaporation of ethanol until reading at λ = 450 nm. A standard curve was generated from 70 µl of xanthophyll standard (Sigma #X6250, St. Louis, MO, USA) serially diluted (20, 10, 5, 2.5, 1.25, and 0 µg/ml) in absolute ethanol. Measurements of plasma total carotenoids were multiplied by 100 to correct for the dilution and were expressed as µg/ml.

Plasma Total Antioxidant Capacity

Antioxidants protect migrating birds against oxidative damage resulting from large quantities of reactive oxygen species produced during strenuous flight (Costantini et al. 2007; Costantini et al. 2008; Jenni-Eiermann et al. 2014). Because those oxygen free radicals are also generated by phagocytes during inflammation, antioxidants play a role in preventing immunopathology (reviewed in Matés and Sánchez-Jiménez 1999; Costantini 2008). We used the OXY-Adsorbent test (Diacron International # MC435, Grosseto, Italy) to measure the plasma total antioxidant capacity (Costantini et al. 2006; Saino et al. 2011). This functional assay measures the ability of plasma samples to neutralize hypochlorous acid (HOC1), a powerful and pathologically-relevant oxidant in biological systems. Briefly, 5 µl of plasma were diluted 1:100 with distilled water (495 µl). Then, 2.5 µl of diluted plasma were incubated with a 100 µl of titered HOCl solution for 10 min at 37°C. Following incubation, 2 µl of N,N-diethyl-p-phenylenediamine solubilized in a chromogenic mixture were rapidly added, and after gently shaken the plates were immediately

read at $\lambda = 540$. The intensity of the pink coloration produced as the chromogen reacts with residual HOCl is inversely related to the plasma total antioxidant capacity. A calibrator serum of known antioxidant capacity (350 mmol/l of HOCl neutralized, diluted 1:100) and distilled water (blank) were used as control reference, and calculations were performed as per directions by the manufacturer. Measurements were obtained as mmol/l of HOCl neutralized.

Dietary Carotenoids and Antioxidant Capacity

Measuring Carotenoids and Antioxidant Capacity of Fruits

For dietary carotenoid and antioxidant capacity assays we sampled both non-native and native fruits in late September 2013. Exotic fruits collected were *E. umbellata*, *Lonicera* spp., *R. multiflora*, *Rhamnus cathartica*, European cranberrybush (*Viburnum opulus*), Japanese barberry (*Berberis thunbergii*), and bittersweet nightshade (*Solanum dulcamara*). Native fruits collected were *C. racemosa*, *I. verticillata*, *L. benzoin*, *T. vernix*, *S. canadensis*, nannyberry (*Viburnum lentago*), and Virginia creeper (*Parthenocissus quinquefolia*). One fruit per shrub (minimum of 5 fruits per species) was collected, and for fruit species found in both exotic and native shrubland (*E. umbellata*, *Lonicera* spp., *R. multiflora*, *R. cathartica*, *V. opulus*, and *L. benzoin*), a minimum of 5 fruits per species were collected from each habitat type. Fruit samples were stored in -80°C freezer until laboratory assays on a later date.

We extracted fruit carotenoids in ethanol for spectrophotometric assay (Rodriguez-Amaya 2001; Alan et al. 2013). After removing the seeds, approximately 100 mg of wet fruit, weighed to the nearest 0.01 mg, was placed in 1 ml absolute ethanol (1:10 dilution). Samples were vortexed for 2 min and sonicated for 5 min (Fisher Scientific Sonic Dismembrator Model 100, Hampton, NH, USA). The mixture was then centrifuged for 5 min under 16,800×g, and 70 μ l of the supernatant was placed in a flat-bottom 96-well assay plate to be read at λ = 450 nm. We generated a standard curve from 70 μ l of serially diluted (50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, and 0 μ g/ml) xanthophyll standard. Carotenoid concentrations were obtained as μ g/g of wet fruit. We were unable to quantify carotenoids in poison sumac because the supernatant obtained was a turbid, waxy gel.

We modified the OXY-Adsorbent test described for bird dietary analysis (Costantini 2010). After removal of seeds, approximately 100 mg of wet fruits (weighed to the nearest 0.01 mg) were placed in around1 ml of distilled water (measured to the nearest μ l) to attain 1:10 dilution, vortexed for 2 min, and sonicated for 5 min. This emulsion (100 μ l) was further diluted in 400 μ l of distilled water (total dilution of 1:100) and vortexed. Then 2.5 μ l of this dilution were placed in a flat-bottom 96-well assay plate with 100 μ l of HOCl. Because the coloration and turbidity of this mixture differed among fruit, initial background absorbance was immediately read with a spectrophotometer at $\lambda = 540$. The final reading was taken under the same setting after 5 min incubation at 37 °C. The background was subtracted from the final absorbance and the difference used to calculate antioxidant capacity. Using the calibrator serum provided in the kit, the antioxidant5 capacity was obtained as mmol of HOCl neutralized per kg of wet fruit.

Indices of Fruit Consumption

We used fecal samples collected from birds held in bird boxes to identify fruits consumed. Identification of fruits relied on a combination of seed, color, and texture of the pulp (Parrish 1997). We smeared fruits collected in the field on the same paper liner and used them as reference. Fruit remains were identified by a single observer blind to which habitat type the bird was captured in, and samples that did not allow positive identification of fruits were removed

from further analysis. Because we were interested in relative consumption of fruit species, for each bird species, the number of occurrence of fruits species in the fecal samples was tallied by habitat (i.e., samples with two fruit species were counted twice to represent both fruit species). In total, 63 (exotic shrubland: 6, native shrubland: 57) fecal samples from gray catbirds and 171 (exotic shrubland: 56, native shrubland: 115) samples from Swainson's thrushes in 2012. In 2013, 42 (exotic shrubland: 6, native shrubland: 34) samples from gray catbirds and 39 (exotic shrubland: 21, native shrubland: 18) samples from Swainson's thrushes allowed identification.

Statistical Analyses

All statistical analyses were performed using R Version 3.1.2 (R Development Core Team 2014). Significance level was set as P < 0.05 for all tests with 0.05 < P < 0.1 deemed as a trend unless otherwise specified. All tests were performed two-tailed and data were transformed as necessary to meet normality assumption. For graphical purposes, least square means (± SE) are presented. Prior to analyses of health parameters, we tested for habitat-related differences in age ratio (χ^2 contingency test) and structural size (i.e. wing chord; two-sample *t*-test) in gray catbirds and Swainson's thrushes in each year to evaluate whether variations in these potential confounders may have affected our results.

Health Parameters

We performed multiple linear regressions to test whether the rate of change in size-corrected fat mass differed between the two habitat types during the morning foraging hours in each species and year (Dunn 2000; Guglielmo et al. 2005). Specifically, after controlling for ordinal date (positively related to fat mass in all comparison groups, P < 0.05), habitat-related difference in rate of fat mass change was detected as a significant interaction between habitat and time after

sunrise. Fat mass of Swainson's thrushes were ln-plus-5 transformed to attain normality and handling time was not significantly related to fat mass (P > 0.05) and thus not included in any model. When the interaction was non-significant, then we removed the interaction to test if birds gained or lost mass within the overall study site (main effect of time after sunrise) or whether fat mass differed by habitat type.

We then tested whether each of the health parameters differed by habitat in gray catbirds and Swainson's thrushes in each year. We used ANCOVAs to test habitat-related differences in the health parameters and to calculate least square means for graphing. The distributions for hemolysis scores, hemagglutination scores in gray catbirds, haptoglobin (except in 2012 gray catbirds), and antioxidant capacity were normal and no transformations were necessary. We transformed TRIG (ln-plus-1), BUTY (sqrt), total leukocytes (ln), hemagglutination scores of Swainson's thrushes (ln), haptoglobin of 2012 gray catbirds (ln), and total carotenoids (sqrt) to bring the distributions to normal, but for graphical purposes hemagglutination and haptoglobin data are shown untransformed. Because no transformation was appropriate for G:L ratios, we fitted compound Poisson GLMs (function cpglm, package cplm) with log link functions (Zhang 2013). We first fitted a multiple linear model explaining each health parameter by ordinal date, time after sunrise at capture, handling time, fat score, muscle score, wing chord, and age as predictors. We kept fat and muscle scores as separate covariates because many parameters were only related to either fat or muscle (see Table 2.2). Backward selection with critical $\alpha = 0.1$ was used to select predictor variables (summarized in Table 2.2; Guglielmo et al. 2005; Liu and Swanson 2014). These variables were retained as covariates in ANCOVA models explicitly testing habitat effect along with covariate \times habitat interaction terms. In particular, significant interaction between time after sunrise and habitat in TRIG, similarly to fat mass, is considered an indicator of differential refueling performance between habitats (Evans Ogden et al. 2013). To produce the minimum model that included habitat, we eliminated all non-significant covariates (P > 0.05) with backward selection. All procedures were similar in the compound Poisson GLMs on G:L ratios, but least square means for graphing was obtained by re-fitting the final models as Markov chain Monte Carlo GLMs (function MCMCglmm, package MCMCglmm) due to incompatibility of R packages of cplm and lsmeans (Hadfield 2014; Lenth and Hervé 2015). Multicollinearity was not detected in any of the final models (vif < 10).

Dietary Carotenoids and Antioxidant Capacity

Fruit total carotenoids and antioxidant capacity were analyzed using one-way ANOVA to test whether there were differences among fruit species. Post-hoc Tukey's HSD multiple comparisons analyses were used to compare these measures between each pair of fruit species. None of the 6 fruit species collected from both exotic and native shrublands showed significant differences in carotenoids or antioxidant capacity by habitat type (P > 0.05) so these data were pooled by species. We also pooled fecal data from in 2012 and 2013 by bird and fruit species to increase sample size (see Table 2.3) after confirming that consumption ranks of fruit species within habitat types were similar between years in both bird species (Friedman's rank sum test for year with fruit species as block, P > 0.05).

We then evaluated whether habitat-related variation in carotenoids and antioxidant capacity in birds could be attributable to differential fruit quality and consumption. We calculated expected means of carotenoids and antioxidant capacity in a unit mass of fruit consumed in each habitat type by combining the fruit assay fecal data. Specifically, the expected values were derived as mean carotenoids or antioxidant capacity weighted by consumption proportion in each habitat. Conceptually, for each bird species in each habitat, the expected mean

dietary carotenoids ($\mu g/g$ of wet fruit) = Σ (carotenoid contents of each fruit \times its consumption proportion). Similar approach was taken for the expected mean dietary antioxidant capacity (mmol HOCl neutralized/kg of wet fruit). These habitat-based dietary means could be then statistically compared using weighted two-sample *t*-tests (function wtd.t.test in package weights in R), with weights as fecal counts rather than proportions (Pasek et al. 2014). But because data for carotenoid contents and antioxidant capacity were obtained from multiple fruits with varying sample sizes (range: n = 5-11), weight for each fruit required standardization by the sample size of these assays. For example, gray catbirds captured in native shrubland had 63 fecal samples containing *L. benzoin*, with 10 samples assayed for carotenoids; the weight for each *L. benzoin* fruit in native shrubland was entered as 63/10 = 6.3.

Results

Age and Size by Habitat Type

The majority of our sampled birds represented HY birds in both in gray catbirds (HY: AHY = 190: 22 in 2012; 186: 10 in 2013) and in Swainson's thrushes (HY: AHY = 151: 52 in 2012; 205: 24 in 2013). We confirmed that age ratio did not differ by habitat type in gray catbirds (2012: $\chi^2_1 = 0.18$, P = 0.67; 2013: $\chi^2_1 = 1.06$, P = 0.30) or Swainson's thrushes (2012: $\chi^2_1 = 0.97$, P = 0.32; 2013: $\chi^2_1 = 0.08$, P = 0.77) in either year. Similarly, wing chord did not differ between habitats in either species or year (gray catbirds, 2012: $t_{71.27} = 1.04$, P = 0.30; 2013: $t_{69.55} = 1.08$, P = 0.29; Swainson's thrushes, 2012: $t_{148.28} = -1.54$, P = 0.13; 2013: $t_{218.18} = -1.30$, P = 0.20).

Indices of Refueling Performance

Based on size-corrected fat mass change during the morning foraging hours, gray catbirds in

2012 gained fat in both habitat types by 0.33 g/hr (time after sunrise: $F_{1, 207} = 7.16$, P = 0.008) but the rate of gain did not differ by habitat (time after sunrise × habitat: $F_{1, 208} = 0.115$, P = 0.735). Significant interaction between time after sunrise and habitat ($F_{1, 191} = 5.43$, P = 0.02) was found in 2013 gray catbirds (Figure 2.1): fat was lost by 0.60 g/hr in exotic shrubland ($t_{191} = -2.002$, P = 0.05) while no change in fat mass was found in native shrubland ($t_{191} = 1.206$, P = 0.23). Rate of fat mass change of Swainson's thrushes did not differ by habitat type in either year (2012: $F_{1, 198} = 0.080$, P = 0.78; 2013: $F_{1, 224} = 0.081$, P = 0.78). Additionally, in 2012 fat mass of Swainson's thrushes in native shrubland was greater by 1.20 g ($F_{1, 199} = 11.78$, P < 0.001) and fat was gained in both habitats by 1.05 g/hr ($F_{1, 225} = 0.889$, P = 0.35), and no gain or loss in fat was detected across both habitats ($F_{1, 225} = 0.32$, P = 0.57).

Plasma TRIG levels of both study species increased with time after sunrise in consistently in 2012 and 2013 (P < 0.05, Table 2.2), but we found no significant habitat effect in gray catbirds (2012: $F_{1, 172} = 0.30$, P = 0.59; 2013: $F_{1, 180} = 0.02$, P = 0.90) or Swainson's thrushes (2012: $F_{1, 159} = 0.002$, P = 0.96; 2013: $F_{1, 217} = 2.22$, P = 0.14) in either year (Figure 2.2A). Interactions between time after sunrise and habitat were also non-significant in all comparison groups (P > 0.05). Plasma BUTY decreased with time after sunrise in both species and years (P < 0.05, Table 2.2). Gray catbird BUTY in exotic shrubs in 2012 showed a lower trend (by 1.29 mmol/l) than in native shrubs ($F_{1, 174} = 3.21$, P = 0.07) without significant time after sunrise × habitat interactions (P > 0.05; Figure 2.2B). In 2013, BUTY of gray catbirds using exotic shrubland decreased more rapidly with time after sunrise than in conspecifics captured in native shrubs, as indicated by significant time after sunrise × habitat interaction ($F_{1, 179} = 4.52$, P = 0.03), although the main effect of habitat was non-significant ($F_{1, 179} = 0.29$, P =

0.59). This interaction effect was small, however, translating to BUTY decreasing by only an additional 0.039 mmol/l per hour in exotic compared to in native shrubland. A model without the interaction term also yielded minimal BIC (396.30, as opposed to 396.90 with interaction) so we graphically present least square means by habitat type based on this reduced model (BUTY lower by 1.39 mmol/l in exotic relative to native shrubland; $F_{1, 180} = 5.17$, P = 0.02; Figure 2.2B). Plasma BUTY of Swainson's thrushes did not differ by habitat in 2012 ($F_{1, 160} = 1.17$, P = 0.28) or 2013 ($F_{1, 218} = 1.95$, P = 0.16), with no significant interactions between time after sunrise and habitat (P > 0.05; Figure 2.2B). Apart from time after sunrise, fat score had a consistently positive effect on TRIG in both species and years (Table 2.2). Handling time, if significant, showed a consistent negative effect on TRIG and a positive effect on BUTY (Table 2.2).

Indices of Constitutive Innate Immune Function

Total leukocyte counts did not differ by habitat in gray catbirds (2012: $F_{1, 137} = 0.02$, P = 0.89; 2013: $F_{1, 73} = 0.03$, P = 0.86) or Swainson's thrushes (2012: $F_{1, 85} = 0.52$, P = 0.47; 2013: $F_{1, 92} = 0.08$, P = 0.78) in either year (Figure 2.3A). Handling time, whenever significant, negatively impacted total leukocyte counts (Table 2.2).

We found a trend toward higher G:L ratio in gray catbirds using exotic shrubland compared to those captured in native shrubland in 2012 ($F_{1, 139} = 3.02$, P = 0.08) and a significant difference in the same direction in 2013 ($F_{1, 72} = 4.36$, P = 0.04; Figure 2.3B). Swainson's thrushes did not show habitat-related difference in G:L ratio in either year (2012: $F_{1, 83} = 1.33$, P = 0.25; 2013: $F_{1, 92} = 0.06$, P = 0.81; Figure 2.3B). No covariate consistently explained variations in G:L ratio between species and years (Table 2.2).

Hemolysis scores of gray catbirds did not show habitat-related difference in 2012 ($F_{1, 162}$

= 1.29, P = 0.26), but in 2013, a trend toward lower hemolysis was observed in individuals captured in exotic shrubland ($F_{1, 179} = 3.17$, P = 0.08) compared to conspecifics in native shrubland (Figure 2.4A). This trend implies 27% lower hemolysis activity in gray catbirds using exotic shrubs than in conspecifics captured in native shrubs (back transforming log-2 scores to activity). No difference in hemolysis was found in Swainson's thrushes in 2012 ($F_{1, 176} = 0.27$, P= 0.61) or 2013 ($F_{1, 218} = 1.66$, P = 0.20; Figure 2.4A). Ordinal date, whenever significant, had a positive effect on hemolysis scores (Table 2.2).

Hemagglutination scores of gray catbirds did not differ by habitat in 2012 ($F_{1, 163} = 0.05$, P = 0.83), but was significantly lower in individuals captured in exotic shrubland than conspecifics in native shrubland in 2013 ($F_{1, 177} = 4.51$, P = 0.03; Figure 2.4B). This difference would translate to 44% reduction on average in hemagglutination activity in exotic shrubs from that in native shrubs (back transforming log-2 scores). Time after sunrise had a significantly positive effect on hemagglutination in this comparison group (Table 2.2). Significant habitat effect was absent in Swainson's thrushes in either year (2012: $F_{1, 117} = 2.01$, P = 0.16; 2013: $F_{1, 218} = 1.66$, P = 0.19; Figure 2.4B). Ordinal date either had a significantly positive effect or a positive trend on hemagglutination across species and years (Table 2.2).

Plasma haptoglobin levels of gray catbirds did not differ significantly by habitat in 2012 $(F_{1, 134} = 1.83, P = 0.18)$, but in 2013 we found 0.152 mg/ml or 17% lower haptoglobin levels in individuals using exotic shrubs relative to conspecifics captured in native shrubland $(F_{1, 121} = 7.56, P = 0.007;$ Figure 2.4C). No habitat-related differences were found in Swainson's thrushes in 2012 $(F_{1, 151} = 0.80, P = 0.37)$, but a non-significant trend toward lower haptoglobin in exotic shrubs (by 0.054 mg/ml or 10%) than in native shrubs was found in 2013 $(F_{1, 178} = 2.89, P = 0.09;$ Figure 2.4C). We did not find consistent patterns in significant covariates explaining
haptoglobin between species and years (Table 2.2).

Plasma Total Carotenoids

While we did not find significant differences by habitat in plasma total carotenoids of gray catbirds in 2012 ($F_{1, 160} = 0.58$, P = 0.45), in 2013 carotenoid concentrations of individuals in exotic shrubs were 4.55 µg/ml lower in exotic shrubs had significantly lower carotenoid concentrations than conspecifics in native shrubs ($F_{1, 165} = 9.03$, P = 0.003; Figure 2.5A). No such difference was found in Swainson's thrushes in 2012 ($F_{1, 177} = 0.99$, P = 0.32) or 2013 ($F_{1, 211} = 1.74$, P = 0.89; Figure 2.5A). Fat score had consistent positive effects on plasma total carotenoids (Table 2.2).

Plasma Total Antioxidant Capacity

We found that antioxidant capacity of gray catbirds was consistently lower in exotic shrubs than in native counterparts by 6% in both 2012 (mean difference: 16.0 mmol/l of HOCl neutralized; $F_{1, 167} = 8.32$, P = 0.004) and 2013 (mean difference: 14.9 mmol/l of HOCl neutralized; $F_{1, 151} =$ 5.48, P = 0.02; Figure 2.5B). But significant covariates differed between years in gray catbirds: in 2012 handling time, fat score, and wing chord length had significant positive effects on antioxidant capacity, whereas 2013 only ordinal date was positively related to antioxidant capacity (Table 2.2). No habitat effect was found in Swainson's thrushes in either year (2012: $F_{1, 167} = 0.01$, P = 0.91; 2013: $F_{1, 203} = 0.36$, P = 0.55; Figure 2.5B). Significant covariates also did not show consistent patterns in Swainson's thrushes between years (Table 2.2).

Dietary Carotenoids and Antioxidant Capacity

Our measures of fruit carotenoids showed substantial variation among species ($F_{12, 77} = 88.06$, P < 0.001; Figure 2.6A). Carotenoid concentration was highest in exotic fruits of *R. multiflora*, *S. dulcamara*, and *Lonicera* spp., with *L. benzoin* as the highest ranking native fruit (rank 5) (Figure 2.6A). We also found significant differences in the measure of antioxidant capacity among fruit species ($F_{13, 91} = 37.73$, P < 0.001; Figure 2.6B). The exotic *R. cathartica*, *E. umbellata* and the native *C. racemosa* and *L. benzoin* had the highest levels of antioxidant capacity (Figure 2.6B).

Based on the number of fruits identified in fecal samples (Table 2.3), the exotic *R*. *cathartica* was the most frequently consumed fruit in the exotic shrubland, accounting for 21% and 29% of the diet in gray catbirds and Swainson's thrushes respectively. In the native shrubland, the native *L. benzoin* ranked first and was found in 69% and 64% of fecal samples from gray catbirds and Swainson's thrushes respectively. In contrast, the carotenoid-rich exotic fruits of *Rosa multiflora* and *Lonicera* spp. were rarely consumed by either bird species in either habitat.

Integrating fruit quality and fecal data, the expected mean total carotenoids obtained through diet was significantly lower in gray catbirds using exotic shrubs relative to using native shrubs (by 10.0 µg/g wet fruit; $t_{19.84} = -2.21$, P = 0.04), but not in Swainson's thrushes ($t_{109.37} = -$ 0.07, P = 0.95; Figure 2.7A). We did not find significant difference in the expected mean dietary antioxidant capacity in gray catbirds ($t_{14.25} = -1.36$, P = 0.19) or Swainson's thrushes ($t_{165.37} =$ 0.36, P = 0.72; Figure 2.7B).

Discussion

We tested whether frugivorous landbirds during fall migration experience difference in energetic and immunological condition between individuals using exotic-dominated shrubland compared to conspecifics using native-dominated shrub habitat. Collectively our findings indicate that exotic habitat may be suboptimal for some species of fall migrating landbirds. Despite it being a complex story, our study provides a unique approach that highlights the value of using multiple health parameters as a way of assessing stopover habitat quality.

Effect of Habitat Use on Indices of Refueling Performance

Contrary to our expectations based on nutrient properties of exotic and native fruits, we did not find habitat differences in refueling performance in most comparison groups. Although native fruits generally have higher fat and energy contents than exotic counterparts (White 1989; Smith et al. 2007a; Smith et al. 2013), birds gained fat mass through the morning only occurred in 2012 and the rate did not differed by habitat. While we observed mass loss in gray catbirds captured in exotic habitat in 2013 this relationship was not replicated in SWTH, nor did it occur in 2012.

Likewise, our plasma metabolite results also do not indicate refueling differences between habitats for either species. Both species regardless of habitat and year showed a general pattern of TRIG increasing and BUTY decreasing during the morning, a pattern consistent to successful foraging (e.g., Jenni-Eiermann and Jenni 1994; Guglielmo et al. 2005; Smith and McWilliams 2010). Although in 2013 gray catbird BUTY decreased at faster rate in exotic shrubland than in native shrubland, this metabolite is considered less reliable and informative in inferring habitat differences refueling performance than TRIG (Acevedo Seaman et al. 2006; Smith and McWilliams 2010; Evans Ogden et al. 2013), which did not vary by habitat in this

comparison group. These results are consistent with previous work in mid-Michigan (Craves 2009) showing that Swainson's thrushes primarily consuming exotic fruits could still gain mass during stopover even after the depletion of native fruits. Taken together our findings on the estimated rates of fat mass change and plasma metabolites, we consider that refueling performance of birds using exotic shrub was equivalent to conspecifics foraging in native shrubland.

We found minor incongruence between refueling performance as inferred from rate of fat mass change and that based on plasma metabolites in 2013 gray catbirds. Fat was lost only in exotic habitat while we failed to detect habitat differences in TRIG. There are two potentially confounding factors that may explain this incongruence; they are (1) time between birds hitting the net and time of extraction and (2) effect of dietary macronutrient composition on TRIG and BUTY. Although we corrected for the effect of handling time on plasma metabolites, we do not know the precise time of birds hitting the net (birds extracted every 45 min). Because plasma metabolite levels, particularly BUTY, are sensitive to delay in blood sampling (Jenni and Jenni-Eiermann 1992; Guglielmo et al. 2002; Guglielmo et al. 2005; Smith and McWilliams 2010), variable extraction delay likely added noise. Differences in macronutrient composition in exotic (high sugar) and native (high fat) fruits might also explain the lack of differences in our TRIG results (but see Cerasale et al. 2006). In feeding experiments using controlled diet (Seaman et al. 2005), TRIG was found to be higher and BUTY lower in birds on low-fat diet than on high-fat diet, even though mass gain was similar. Similar results were found in experiments using isoenergetic diet that differed in these macronutrient composition (Smith and McWilliams 2009). Although not as energetically efficient as directly assimilating dietary fat, birds have exceptionally high capacity for *de novo* synthesis of fatty acids from carbohydrates (Stevens

1996; Klasing 1998), and thus a sugar-rich diet (i.e. exotic fruits) may serve just as well as fatrich diet (i.e. native fruits) for refueling. Therefore, we caution that macronutrient variation between habitats may well confound plasma metabolite data (Seaman et al. 2005; Smith and McWilliams 2009) and that sampling should be done as soon as possible post capture in the net (Guglielmo et al. 2002; Guglielmo et al. 2005; Smith and McWilliams 2010). Measurements of constitutive immunity (Buehler et al. 2008) and antioxidant capacity (Costantini et al. 2007) may provide a more robust approach for dealing with handling stress or sampling delay.

Effect of Habitat Use on Indices of Constitutive Innate Immune Function

Increased G:L or H:L ratio occurs as circulating heterophils increase and lymphocytes decrease in response to elevated glucocorticoid stress hormone levels (Maxwell 1993; Davis et al. 2008). We found that gray catbirds captured in exotic habitat had higher G:L ratios than conspecifics in native shrubs in 2013 with a similar, but non-significant trend in 2012. However, no habitat related differences were observed for total leukocyte counts for either species or G:L ratios in Swainson's thrushes in both years. The habitat-related difference in G:L ratio in 2013 gray catbirds could be interpreted as a sign of stress and/or flight-induced reduction of lymphocytes. Food restriction (Maxwell et al. 1992), migration (Owen and Moore 2006), and intense flight (Matson et al. 2012b; but see Nebel et al. 2012) have all been suggested to be important stressors based on G:L or H:L ratios. But elevated G:L ratio could be also due to reduced lymphocytes in circulation that may represent adaptive tradeoff between acquired immunity and flight (Owen and Moore 2006; 2008a). Because 2013 gray catbirds showed fat loss and higher G:L ratio without significant reduction in total leukocyte counts in exotic shrubs compared to conspecifics in native habitat, the difference in G:L ratio seems more consistent with higher stress in exotic shrubland, possibly resulting from suboptimal foraging opportunities.

Likewise, gray catbirds using exotic shrubs in 2013 had significantly lower hemagglutination activities and haptoglobin levels with a non-significant trend toward lower hemolysis activities compared to conspecifics in native shrubs. Habitat effects on any of those measures did not reach significance in Swainson's thrushes. Similarly to the lymphocyte responses to flight and stopover, recent experimental work on European starlings (*Sturnus vulgaris*) showed reduction in measures of hemolysis, hemagglutination, and haptoglobin as a result of prolonged flight; starlings rested and foraged for 2 d post flight exhibited greater hemolysis and trends toward greater hemagglutination and haptoglobin compared to individuals immediately sampled after flight (Nebel et al. 2012). Evidence of recovery or upregulation of hemolysis and haptoglobin, contingent on refueling success, has also been found in red knots (*Calidris canutus*) at a stopover site (Buehler et al. 2010). We therefore consider that habitatrelated differences in gray catbird immunity captured in 2013 may have reflected differential immune recovery.

Migration is thought to be the phase in the birds' annual cycle during which the greatest mortality occurs (Sillett and Holmes 2002), and small differences in immune status resulting from varying quality of stopover habitat may have significant implications for future disease resistance and survival (Klaassen et al. 2012). Low H:L ratio has been linked to greater resistance to bacterial infection (Al-Murrani et al. 2002; Krams et al. 2012) and high hemagglutination activity to lower ectoparasite load (Whiteman et al. 2006)). These or similar measures of constitutive immunity also appear to be positively related to survival in nestlings and adult birds (Kilgas et al. 2006b; Parejo and Silva 2009; Townsend et al. 2010). Having robust immunity during stopover is likely adaptive in the defense against pathogens ingested

during foraging (Buehler and Piersma 2008; Buehler et al. 2010; Altizer et al. 2011), and it is possible that birds leaving stopover with lower immunity may face greater risk of future infection (Owen and Moore 2008a). Constitutive innate immunity may be particularly important in immunologically naïve HY birds experiencing their first fall migration. In addition, baseline haptoglobin levels prior to flight have been positively linked to flight duration (Nebel et al. 2013). Taken together, it is conceivable that some species using exotic shrubs in certain years suffer a relative fitness disadvantage compared to conspecifics stopping over in native shrubs.

We are uncertain as to why these habitat differences in immunological condition were found in only one year (2013) in only species (gray catbirds). However, it was only this comparison group that showed fat loss and lower circulating carotenoids (see below) in exotic habitat. Furthermore, all immune parameters except total leukocyte counts in these gray catbirds showed habitat effect toward the same direction. We thus suspect that the measured immunological parameters are linked to energetic condition and/or carotenoids, and that loss of fat and/or lower carotenoids in 2013 gray catbirds captured in exotic shrubs may have contributed to their poorer immunological condition than in native shrubs. Future studies should further investigate under what nutritional conditions birds may manifest habitat differences in immunity, and whether these immune parameters vary in sync in migrating landbirds during stopover. At the same time, fruit abundance and consumption data by year will be necessary to elucidate inter-annual variations in these links.

Effect of Habitat Use on Plasma Total Carotenoids

We found significantly lower plasma total carotenoid concentrations in gray catbirds using exotic shrubland in 2013 than those in native shrubland, but no other comparison groups showed this

pattern. Carotenoids are immunostimulatory pigments entirely derived from diet (McGraw and Ardia 2003; McGraw et al. 2006; McGraw et al. 2011; Simons et al. 2012), thus may link habitat-dependent carotenoid acquisition to immune status (see below for detail). Because plasma carotenoids may fluctuate rapidly due to feeding and resting (see Hõrak et al. 2004), and sustained high levels of circulation may be required to impact immunity (see experimental designs in McGraw and Ardia 2003; McGraw et al. 2006), it is difficult to directly link plasma total carotenoids with immunity during the relatively short time scale of migratory stopover. However, the only comparison group that exhibited significant habitat effect in multiple measures of immunity (2013 gray catbirds) also differed in circulating carotenoid levels in the same direction. It is thus possible that these gray catbirds could attain sustained high levels of carotenoids in native shrubs and thereby could enhance their immune parameters.

Effect of Habitat Use on Plasma Total Antioxidant Capacity

Antioxidant capacity of gray catbirds using exotic shrubland was consistently lower than conspecifics in native shrubland in 2012 and 2013, although no such relationships were present in Swainson's thrushes. The consistent differences in antioxidant capacity by habitat in gray catbirds suggest that these differences reflect inherent differences in habitat quality for this species. The importance of antioxidants in birds subject to oxidative damage resulting from strenuous flight is well-established (Costantini et al. 2007; Costantini et al. 2008; Jenni-Eiermann et al. 2014). During stopover body condition was shown to be positively related to antioxidant capacity (Costantini et al. 2007), which may indicate recovery of antioxidant status during stopover. Recent findings that birds preferentially consume fruits with high antioxidant levels (Alan et al. 2013; Bolser et al. 2013) also suggest the need to restore antioxidant status during migratory stopover. Having a sufficient antioxidant barrier may be important for combatting infection, because antioxidants may be required to control reactive oxygen species produced during inflammatory response (Costantini 2008; Costantini and Møller 2009). Overall, the ability to successfully control oxidative damage likely has significant fitness implications. In a long-term study in barn swallows (*Hirundo rustica*), antioxidant capacity was found to be positively related to return rate, with an increase of 1 mmol/l of HOCl neutralized corresponding to 0.6% increase in survival (Saino et al. 2011). While their findings are not directly applicable to our study, the magnitude of habitat effect observed in gray catbirds (15 mmol/l of HOCl neutralized) suggests a relative fitness disadvantage of using exotic shrubland.

Dietary Carotenoids and Antioxidant Capacity

We found that both total carotenoids and antioxidant capacity of exotic and native fruits were highly variable among species. Unlike the pattern of fat and energy contents reported on exotic and native fruits (Smith et al. 2013), we did not find a clear pattern that suggests native fruits are greater in measures of carotenoids and antioxidant capacity. Instead, certain exotic fruits (e.g., *R. multiflora*) had exceptionally high carotenoid contents, similarly to previously described (Alan et al. 2013).

However, the fecal data show that birds rarely consumed the carotenoid-rich exotic fruits of *R. multiflora*, *S. dulcamara*, or *Lonicera* spp. (see also Suthers et al. 2000; Alan et al. 2013; Bolser et al. 2013; Smith et al. 2013). Abundant fruits of the exotic *E. umbellata* with moderately high carotenoid contents were also rare in fecal samples (see also Smith et al. 2007a; Smith et al. 2013). These exotic fruits, therefore, likely contributed minimally to carotenoid acquisition. The fruits frequently consumed in our study were the native *L. benzoin* and the exotic *R. cathartica*

within their respective shrublands. Fruits from these two species had very high antioxidants and moderate levels of carotenoids and were likely major contributors of dietary acquisition of these nutrients. In addition, *L. benzoin* fruits have very high fat and energy contents (White 1989; Smith et al. 2013) and was the single most consumed fruit across habitat types. As birds select fruits based on multiple factors including fat, energy, carotenoids, and antioxidant contents (Smith et al. 2007a; Alan et al. 2013; Bolser et al. 2013; Pierce and McWilliams 2014), *L. benzoin* may provide the best combination of nutrients compared to other fruits in our study site, and conserving this shrub appears important for fall frugivorous migrants.

Integrating fruit consumption and nutrient data of carotenoids and antioxidants, we found limited evidence that differential fruit consumption may have been reflected in the immune and antioxidant status for either species. Given the fecal data we would predict that gray catbirds using exotic shrubland could have lower carotenoid levels in their average fruit diet (per unit mass) than in native shrubland, with no such pattern in Swainson's thrushes. We were not able to detect significant habitat differences in the expected dietary means of antioxidant capacity in either species; small fecal sample size (only 14 samples from exotic shrubland) along with relatively large intra- and inter-species variation in fruit antioxidant capacity likely hampered detection.

While we present these data with caution due to limitations of fecal sample size and inherent difficulties of dietary analyses based on fecal sampling (Blake and Loiselle 1992; Parrish 1997), we consider it is plausible that differential fruit consumption between habitats impacted health status in gray catbirds. Simultaneous intake of fat has been suggested to improve absorption of carotenoids and other antioxidants (Pietta 2000; van het Hof et al. 2000; Surai et al. 2001), and the high fat content of native fruits (White 1989; Smith et al. 2007a; Smith et al.

2013) could enhance antioxidant absorption. This effect could lead to a greater habitat difference in the actual acquisition than what is predictable through our calculations. We encourage future studies to take a more rigorous approach in linking dietary carotenoids and antioxidant capacity to these measures in circulation in birds.

Immune and Antioxidant Measures as Indicators of Habitat Quality

Applications of ecophysiology can provide information on individual performance related to fitness and hence have great potentials as indicators of habitat quality (Albano 2012). However, the utility of immune and antioxidant indices for assessing habitat quality depends on whether the observed habitat-related differences can be interpreted as consequences of differential habitat use (see Johnson 2007; Albano 2012). Based on our results, we do argue that habitat differences in health status may well represent consequences of differential use. Habitat-related differences in populations or pathogen exposure were unlikely due to the proximity of the two shrublands within the study area. However, alternative explanations should be considered. One such explanation is that individuals with superior health status were more likely to use native shrubland. For example, spatial segregation by health is conceivable if there was greater predation risk in native shrubland and healthier birds more willing to forage in that risker habitat. However, this explanation is unlikely because vegetation structure (i.e. cover) was similar between exotic and native shrublands (Y. Oguchi and J. Owen, unpublished data). The second explanation is that individuals sampled in exotic shrubland simply represented more recent arrivals, and that on average, birds captured in native shrubland had more time to recover or upregulate their health status. Consistent with this explanation, our capture-recapture record shows that birds initially captured in exotic shrubland were more likely to switch to native

shrubland than vice versa (Y. Oguchi and J. Owen, unpublished data). However, we believe that such variations in stay length was at least partially controlled for by inclusions of condition indices (fat and muscle scores) in ANCOVA models because stay length is generally linked to both condition and health indices (Owen and Moore 2008a; Buehler et al. 2010). In addition, several indices of immunity and antioxidant capacity were related to time after sunrise in at least some comparison groups (see Table 2.2), indicating these measures were sensitive to short-term change and reflected current health status (see also van de Crommenacker et al. 2010).

Results from this study provide the first evidence that differential use of stopover habitat may impact the health status of migrating landbirds, and conversely, that stopover habitat quality could be inferred from measures of health in birds. In light of the recent reviews that highlight the need for advancing knowledge on factors influencing migratory bird health (Albano 2012; Klaassen et al. 2012), we encourage future studies comparing habitat quality through measures of individual performance to include indices of immunity and antioxidant status. In addition, inclusions of immune and antioxidant measures may reveal differences in habitat quality that may not be reflected in plasma metabolites, such as habitat variation in nutrient acquisition.

We conclude that exotic shrubs in our study site may represent suboptimal stopover habitat compared to native counterparts for some, but not all, species of fall frugivorous migrants, and that the degree of habitat quality difference likely differ between years. We recommend future studies to test these same hypotheses through controlled laboratory experiments, which will help isolate and confirm the link among nutritional attributes of exotic and native fruits, refueling performance, immunity, and antioxidant capacity of individual birds.

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Table 2.1. Summary of health parameters (means \pm SE) in gray catbirds and Swainson's thrushes captured in exotic- or native-dominated shrubland near East Lansing, MI, USA in 2012 and 2013. Sample sizes are listed in parentheses. TRIG = triglycerides (mmol/l), BUTY = β -hydroxybutyrate (mmol/l), WBC = total leukocytes/10,000 erythrocytes, G:L = granulocyte:lymphocyte ratio, HL = hemolysis score, HA = hemagglutination score, Hp = haptoglobin (mg/ml), Car = total carotenoids (µg/ml), OXY = antioxidant capacity (mmol/l of HOCl neutralized).

Para-	Gray catbird, 2012		Gray catbird, 2013		Swainson's thrush, 2012		Swainson's thrush, 2013	
meter	Exotic	Native	Exotic	Native	Exotic	Native	Exotic	Native
TRIG	$.67 \pm .04$	$.77 \pm .03$	$.62 \pm .07$	$.57 \pm .03$	$1.29 \pm .06$	$1.46 \pm .17$	$1.02 \pm .06$	$1.18\pm.09$
	(44)	(134)	(43)	(142)	(61)	(103)	(103)	(119)
BUTY	$9.36\pm.78$	$10.10 \pm .41$	$6.56\pm.66$	$8.48 \pm .37$	$6.87 \pm .63$	$7.98 \pm .49$	$6.43 \pm .47$	$7.02 \pm .41$
	(44)	(134)	(43)	(142)	(61)	(103)	(103)	(119)
WBC	61.00 ± 7.27	57.74 ± 3.48	78.93 ± 12.35	81.80 ± 5.84	42.21 ± 3.51	48.65 ± 4.03	43.43 ± 5.30	43.35 ± 3.21
	(25)	(116)	(14)	(61)	(33)	(54)	(44)	(51)
G:L	$.37 \pm .07$	$.26 \pm .02$	$.50 \pm .19$	$.24 \pm .04$	$.50 \pm .13$	$.33 \pm .07$	$.25 \pm .04$	$.26 \pm .03$
	(25)	(116)	(14)	(61)	(33)	(54)	(44)	(51)
HL	$5.53 \pm .23$	$5.29 \pm .13$	$4.33 \pm .27$	$4.78 \pm .11$	$4.12 \pm .15$	$3.84 \pm .13$	$3.89\pm.08$	$4.06\pm.10$
	(36)	(129)	(40)	(141)	(70)	(110)	(104)	(116)
HA	$7.07 \pm .36$	$7.17 \pm .21$	$6.45 \pm .38$	$7.17 \pm .18$	$5.21 \pm .24$	$4.70 \pm .14$	$4.92\pm.05$	$5.33\pm.17$
	(36)	(129)	(40)	(141)	(70)	(110)	(104)	(116)
Нр	$.78 \pm .04$	$.83 \pm .02$	$.73 \pm .06$	$.88 \pm .02$	$.39 \pm .02$	$.42 \pm .02$	$.50 \pm .02$	$.54 \pm .02$
	(25)	(113)	(24)	(99)	(61)	(94)	(86)	(95)
Car	20.24 ± 1.83	$22.89\pm.96$	16.93 ± 1.84	$19.87\pm.83$	34.28 ± 1.82	40.22 ± 1.86	36.99 ± 1.82	43.29 ± 1.98
	(37)	(126)	(36)	(133)	(72)	(109)	(100)	(116)
OXY	239.7 ± 5.0	255.3 ± 2.9	226.6 ± 7.1	240.4 ± 3.3	228.8 ± 4.1	226.9 ± 3.2	221.2 ± 2.6	225.3 ± 2.8
	(40)	(132)	(34)	(120)	(70)	(100)	(95)	(111)

Table 2.2. Summary of variables retained in multiple linear regressions with backward selection (P < 0.1). These variables were then included as covariates in ANCOVA testing for habitat effect on health parameters. TRIG = triglycerides, BUTY = β -hydroxybutyrate, WBC = total leukocytes, G:L = granulocyte:lymphocyte ratio, HL = hemolysis score, HA = hemagglutination score, Hp = haptoglobin, Car = total carotenoids, OXY = antioxidant capacity, D = ordinal date, T = time after sunrise, H = handling time, F = fat score, M = muscle score, W = wing chord, A = age, and N = no covariates. Plus or minus sign denote the direction of effects. Variables with parentheses indicate those dropped (P > 0.05) in the final, minimal ANCOVA model that included habitat.

Parameter	Gray catbird, 2012	Gray catbird, 2013	Swainson's thrush, 2012	Swainson's thrush, 2013
TRIG	+D, +T, -H, +F, (+A)	+D, +T, +F	+T, -H, +F	+T, (-H), +F
BUTY	-T, +H	-T, +H, -F	-T, +H, (-A)	-D, -T, +H, -F
WBC	-D, -H	Ν	(+T), (-H)	-H
G:L	(+A)	(+D), -M	+D, +H	-W
HL	+D, (+A)	(+D), (-A)	+D, (-H), -F, (-A)	(+H)
HA	(+D)	(+D), +T, (-H), -A	+D, (-H), (-A)	(+D), +W
Нр	-D, -T, (+M)	Ν	-D, +H	-H
Car	+F	+D, +F	+D, +F	+D, +F, (+M), (+W), -A
OXY	+H, +F, (+M), +W	+D, (-A)	+T, (+H)	(+D), +F

Table 2.3. Number of fecal samples from gray catbirds and Swainson's thrushes captured in exotic- and native-dominated shrublands with fruit species identified. U.S. Status indicates the origin of fruit species as either exotic or native, while Exotic or Native under bird species indicates shrubland habitat the samples were collected from. Counts of fecal samples were pooled from 2012 and 2013 captures and were interpreted to represent fruit consumption frequencies of individuals in their respective habitat.

		U.S.	Gray catbird		Swainson's thrush	
Scientific name	Common name	Status	Exotic	Native	Exotic	Native
Lindera benzoin	Common spicebush	Native	3	63	22	86
Rhamnus cathartica	European buckthorn	Exotic	5	15	28	18
Solanum dulcamara	Bittersweet nightshade	Exotic	0	3	7	6
Ilex verticillata	Common winterberry	Native	1	3	4	8
Viburnum opulus	European cranberrybush	Exotic	1	3	7	2
Elaeagnus umbellata	Autumn olive	Exotic	1	3	5	2
Sambucus canadensis	Common elderberry	Native	1	1	3	5
Parthenocissus quinquefolia	Virginia creeper	Native	2	0	0	5
Rosa multiflora	Multiflora rose	Exotic	0	0	1	0
Lonicera spp.	Honeysuckle	Exotic	0	0	0	1
Total			14	91	77	133



Figure 2.1. Size-corrected fat mass estimates of gray catbirds captured near East Lansing, MI, USA during fall migration of 2012 and 2013 in relation to time after sunrise and exoticor native-dominated shrubland habitat. Regression lines through raw data uncorrected for ordinal date are shown. Controlling for ordinal date, significant time after sunrise × habitat interaction was found (P = 0.02), indicating no fat change over morning feeding hours in native shrubland (P = 0.23) and fat loss of 0.60 g/hr in exotic shrubland (P = 0.05).



Figure 2.2. Least square means (\pm SE) of plasma (A) triglyceride and (B) β hydroxybutyrate concentrations in gray catbirds and Swainson's thrushes refueling in exotic- or native-dominated shrubland near East Lansing, MI, USA during fall migration of 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 2.2). Significant differences or trends are denoted with *P*-value while NS describes non-significance.



Figure 2.3. Least square means $(\pm SE)$ of (A) total leukocyte count per 10,000 erythrocytes and (B) granulocyte:lymphocyte ratio in gray catbirds and Swainson's thrushes refueling in exotic- or native-dominated shrubland near East Lansing, MI, USA during fall migration of 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 2.2). Significant differences or trends are denoted with *P*-value while NS describes nonsignificance.



Figure 2.4. Least square means $(\pm SE)$ of (A) hemolysis score, (B) hemagglutination score, and (C) haptoglobin concentration in gray catbirds and Swainson's thrushes refueling in exotic- or native-dominated shrubland near East Lansing, MI, USA during fall migration of 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 2.2). Significant differences or trends are denoted with *P*-value while NS describes non-significance.



Figure 2.5. Least square means (\pm SE) of (A) plasma total carotenoids and (B) antioxidant capacity in gray catbirds and Swainson's thrushes refueling in exotic- or native-dominated shrubland near East Lansing, MI, USA during fall migration of 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 2.2). Significant differences or trends are denoted with *P*-value while NS describes non-significance.



Figure 2.6. Total carotenoids (A) and antioxidant capacity (B) of fruits found in shrublands used by migrating landbirds near East Lansing, MI, USA. Fruit species are ordered from highest to lowest means with centerline of boxes showing median values. Exotic fruits are shown as white boxes and native fruits are barred. Differences in letters above boxes represent significant differences (P < 0.05), and numbers below the letters denote sample size.



Figure 2.7. Expected dietary means (\pm SE) of (A) total carotenoids and (B) antioxidant capacity in gray catbirds and Swainson's thrushes refueling in exotic- or native-dominated shrubland in East Lansing, MI, USA. Means were calculated as values of assayed fruits (see Figure 2.6) weighted by consumption proportion (see Table 2.3). Significant differences are denoted with *P*-value while NS describes non-significance.

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