

# Themed Issue Article: Conservation Physiology of Animal Migrations

## Local site variation in stopover physiology of migrating songbirds near the south shore of Lake Ontario is linked to fruit availability and quality

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Birds that migrate long distances between breeding and wintering grounds are challenged to find adequate stopover sites that can provide a high-quality source of nutrition in order to refuel quickly and continue on their migratory journeys. Wild fruits are a well-documented component in the diets of many passerines during autumn migration. Thus, fruit availability and the proliferation of shrubs that bear low-quality fruits at important stopover sites may dictate the quality of food resources available for refuelling birds and present a conservation concern. We profiled plasma metabolites of two migratory passerine species at two different stopover sites near the south shore of Lake Ontario during the peak of autumn migration. We also measured diversity, availability and nutritional quality of fruits present at these sites. Site explained most of the variation in plasma triglyceride for both bird species, but was less important than other confounding variables for explaining concentrations of plasma  $\beta$ -hydroxybutyrate and plasma uric acid concentrations. Site differences in fat deposition, as indicated by plasma triglyceride, may in part be explained by the large differences in diversity and availability of high-quality fruits between the two sites. Our results suggest that abundant, lipid-rich native fruits with high-energy density are associated with increased fat deposition during autumn stopovers for some species, although other factors, such as proximity to the Lake Ontario shoreline and the opportunities to refuel in the surrounding landscape, are likely to play a role in stopover site use by birds. It is possible that local site characteristics that influence growing conditions may impact the quality of fruits produced by a plant species, altering the availability of critical nutrients for avian consumers.

**Key words:** Bird migration, fruit quality, fruit resources, Lake Ontario, passerines, stopover physiology

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### Introduction

The extreme energetic demands of long-distance migration make it a challenging and vulnerable period for birds marked by high mortality rates and extreme physiological stress

(Silllett and Holmes, 2002; Klaassen *et al.*, 2014). Thus, conservation of migratory bird populations could be well informed by studying the habitat and physiological requirements of birds during these transitory phases. Migration in small passerines necessitates frequent refuelling periods at

stopover sites throughout migration, and these stopover periods may entail twice the energy expenditure as flight (McWilliams *et al.*, 2004). Thus, events and conditions experienced at the numerous stopover sites used by birds for fat deposition and resting points in between migratory flights are likely to influence migration success and, as a result, might contribute to the population dynamics of these species (Mehlman *et al.*, 2005; Newton, 2006).

Habitat selection by birds during migration can be driven by food abundance (Buler *et al.*, 2007), and birds may seek out early successional or edge habitats, or shrublands with ample availability of fruits (Rodewald *et al.*, 2004; Packett and Dunning, 2009; Carlisle *et al.*, 2012; Mudrzyński and Norment, 2013). The majority of passerine species stopping over in the northeastern USA in the autumn are omnivorous (Parrish, 1997) and rely heavily on the abundant fruit resources provided by these types of habitats, sometimes exhibiting a preference for habitats that provide certain fruits (Suthers *et al.*, 2000). Diet quality can affect refuelling rates of small songbirds (Pierce and McWilliams, 2004), and nutritional composition in particular is likely to influence the fattening rates of birds during migration stopovers (Bairlein, 1998) and the daily intake of foods, such as fruits, needed to balance energy and nutrient requirements (Smith *et al.*, 2007a). Autumn-ripening fruits can vary tremendously in the amount of energy and macronutrients they provide to birds (Smith *et al.*, 2007a, 2013), and birds often select higher quality fruits with important nutritional and biochemical characteristics during stopovers (Smith *et al.*, 2007a, 2013; Alan *et al.*, 2013; Bolser *et al.*, 2013). Thus, the availability and composition of fruit-bearing shrubs and plants at stopover sites is likely to impact foraging efforts and the rate at which birds can replenish the necessary energy to continue migration (Smith and McWilliams, 2010).

Anthropogenic landscape change that alters the availability and continuity of suitable stopover habitats can limit choices for birds during migration, although some studies have found that birds can use urbanized or developed areas during stopovers (Seewagen and Slayton, 2008; Seewagen *et al.*, 2011; Wagner *et al.*, 2014), and these more anthropogenic habitats can partly substitute for intact habitats for landbirds (Liu and Swanson, 2014). One example of the potential negative consequences of urbanized habitats that may be of particular relevance to migrating birds in autumn is the potential introduction and range expansion of non-native fruit-bearing plants in areas that may serve as stopover sites. Given the importance of fruits for autumn migrants (Parrish, 1997), it is possible that the ability of birds to refuel adequately during short stopovers may be compromised if non-native plant species bear fruits that are of lesser quality than those of native plants (Gosper and Vivian-Smith, 2009; Smith *et al.*, 2013). Similar results may also occur if critical habitat at stopover sites becomes dominated by lesser quality native species that require birds to spend more time foraging in order to continue migration (Smith and McWilliams, 2010).

The nutritional characteristics of fleshy fruits can also exhibit variation on broad geographical scales (Hampe, 2003; Traveset *et al.*, 2004; Voigt *et al.*, 2004), presumably in response to different climates and growing conditions along latitudinal gradients. In fact, chemical traits of fruits may be sensitive to local abiotic conditions (Lotan and Izhaki, 2013), and climate variables may have a dominating influence on variations in certain biochemical components of berries, such as anthocyanins (Barnuud *et al.*, 2013). On regional or local scales, fruit production can vary spatially and temporally among habitat types (Greenberg *et al.*, 2012), and the ability of certain fruit-bearing species to thrive in particular habitat types may relate to factors such as soil type/fertility, moisture levels or sunlight. But to our knowledge, less is understood about the variation in the nutrient content or quality of fruits on these smaller scales. Such information could be vital for understanding the dynamics of refuelling in migrants in urbanized landscapes where stopover sites may be limited.

Measuring the rate of mass gain, especially fat deposition, is a means to determine the quality of habitat for migrating birds, and there are multiple methods for assessing the refuelling performance of birds at stopover sites via measures of mass or mass change (Bonter *et al.*, 2007; Cherry, 1982; Dunn, 2000; Horton and Morris, 2012), each with their own benefits and limitations. Plasma metabolite profiling offers a reliable alternative method of determining instantaneous rates of energy utilization and nutritional state, and can therefore be extremely useful in assessing the physiological condition of birds captured at stopover sites in order to provide insight into habitat quality and use by birds during migration (Guglielmo *et al.*, 2005; Williams *et al.*, 2007; Smith and McWilliams, 2010; Smith, 2013). For example, plasma triglyceride concentrations rise with feeding and fat deposition, and  $\beta$ -hydroxybutyrate concentrations rise with fasting (Stevens, 2004), providing a useful index of fat utilization, whereas uric acid concentrations show an increase with dietary protein intake in feeding birds (Smith *et al.*, 2007b). Given that dietary macronutrient intake may affect fattening of songbirds and can be reflected in plasma metabolite profiles in captivity (Smith and McWilliams, 2009), studies that relate the physiology of free-living birds with the quality of available fruit resources may provide valuable insight into their potential refuelling performance during stopovers.

In this study, we used plasma metabolite profiles to correlate patterns of physiological refuelling with fruit resources in two passerine species, blackpoll warbler (*Setophaga striata*) and white-throated sparrow (*Zonotrichia albicollis*), captured at two sites with different landscape and vegetative characteristics near the south shore of Lake Ontario. This area of the Great Lakes basin region serves as an important stopover location for thousands of passerines each spring and autumn (Bonter *et al.*, 2009) and represents a region of important conservation interest in terms of maintaining suitable habitat that can support these large numbers of migrants each year. We hypothesized that plasma metabolite concentrations of birds captured at these two sites would reflect patterns of overall

fruit abundance and quality at the sites of capture. More specifically, we expected that birds captured where high-quality (e.g. high-fat and energy-dense) native fruit abundance is high would have higher plasma triglyceride, lower  $\beta$ -hydroxybutyrate and lower uric acid concentrations than birds captured at a site where fruit resources are less available and may be of lower nutritional quality during autumn stopovers.

## Materials and methods

### Study sites

We studied the stopover physiology of birds at two sites near Rochester, NY, USA. Braddock Bay Bird Observatory (hereafter 'BBBO') is located on the southern shoreline of Lake Ontario ~19 km northwest of the urban centre of Rochester, NY, USA. This site is the location of a long-term migration monitoring programme (since 1985) and serves as an important stopover site for many passerines in both the spring and autumn (Bonter *et al.*, 2007). The habitat is primarily early successional deciduous forest with patchy shrub habitat and abandoned agricultural fields in various stages of succession set within a landscape of state-managed land surrounded by low-density housing and agriculture.

Rochester Institute of Technology is located ~24 km south-southeast of Braddock Bay along the Genesee River corridor in the town of Henrietta, NY, USA and ~10 km to the southwest of the large metropolitan centre of Rochester. A small-scale banding effort was operated on campus at the RIT Bird Observatory (hereafter 'RIT') from 2003 to 2012 on an undeveloped wooded area located on the east side of campus. The plot is separated from campus by a wetland habitat along its west border and has a road with student housing and developed land along the east border, with patchy woodlands to the north and south. A habitat restoration project was initiated at this site in 2009 to replace woody shrub vegetation following a wetland mitigation project in 2007 on the adjacent RIT campus, after which an area along the northeast border of the banding habitat was permanently flooded. Plantings were sparse and had not yet produced fruit at the time of the study, and the banding area remains a mix of wet woodland with open patches dominated by woody shrubs and vines.

### Study species, capture and blood sampling

Blackpoll warblers and white-throated sparrows were selected as focal species for this study because of their reliable capture rates at both study sites and omnivorous dietary habits during autumn migration (Parrish, 1997). Banding and blood sampling efforts were carried out twice weekly from 13 September 2011 to 18 October 2011 at RIT and BBBO, with blackpoll warblers and white-throated sparrows being sampled on 3 and 5 days, respectively, at both sites. Birds were captured in 30 mm mesh mist nets that were opened at sunrise and subsequently checked every 30 min thereafter for up to 6 h at both sites as part of the established migration monitoring protocol. Birds sampled in this study were captured on corresponding

days between the first and sixth hour after sunrise at each site to reduce bias attributed to daily environmental variation (e.g. temperature and precipitation). Captured birds were taken to a central location at each site, where they were bled via brachial vein puncture using a 27.5 gauge needle, and up to 100  $\mu$ l of whole blood was collected in heparinized capillary tubes. Capture time was recorded as the hour after sunrise when the net was checked (hereafter 'time of day'), and exact time of blood collection was recorded. Bleed time was calculated as the number of minutes between net extraction and the start of blood sampling and was restricted to <30 min. Samples were sealed with clay (Fisher Scientific) and were kept cool until processing in the laboratory (within 6 h), which involved spinning capillary tubes in a haematocrit centrifuge for 10 min at 9500  $\times$  g to separate the plasma and red blood cells, and then transferring plasma to 1.2 ml cryogenic vials for storage at  $-80^{\circ}\text{C}$  until analysis.

After blood collection, birds were banded with serially numbered bands from the US Geological Survey bird banding laboratory. Biometric measurements were recorded, including unflattened wing chord (in millimetres), tarsal length (in millimetres), a fat class score on a six-point scale (Helms and Drury, 1960) and body mass (in grams). Capture data for each species at each site are summarized in Table 1. Age and sex were also recorded when possible, according to Pyle (1997). Of blackpoll warblers that could be aged reliably, 83 and 88% were aged as hatching year at the RIT site and the BBBO site, respectively. Of the white-throated sparrows that could be aged reliably, 80% at the RIT site and 92% at the BBBO were aged as hatching year. Overall, 72% of birds could not be sexed reliably, representing 50–100% of the birds at each site for each of the two study species.

### Analysis of plasma metabolites

Plasma samples were diluted 1:2 or 1:3 with 0.9% NaCl. Colorimetric end-point assays were performed to measure plasma concentrations of triglyceride and uric acid, and a kinetic assay was carried out to measure plasma concentrations of  $\beta$ -hydroxybutyrate, each using 5  $\mu$ l of sample per well, with two replicates for each sample, for which the coefficient of variation was  $\leq 10\%$ . The procedures and suppliers for these assays are the same as those followed by Smith and McWilliams (2010). All assays were run on a Thermo Scientific Varioskan Flash Multimode Reader. Plasma triglyceride was prioritized and then uric acid and  $\beta$ -hydroxybutyrate were measured as remaining sample volume allowed.

### Habitat and environmental assessment

A quantitative assessment of fruit availability and abundance was made at each site on the same date (24 September) during the peak of migration and general fruit ripening at both sites. Twenty 4 m<sup>2</sup> plots were established at evenly spaced intervals at 10 mist nets located along previously established and maintained net lanes at each site, with two plots located at each net ~0.5 m away from the net and on opposite ends. The presence

**Table 1:** Capture information and morphological data collected for blackpoll warblers and white-throated sparrows captured at Rochester Institute of Technology and Braddock Bay Bird Observatory during autumn 2011

Site	Capture day of year	Temperature at sunrise (°C)	Capture time (h past sunrise)	Bleed time (min)	Mass index (g) <sup>a</sup>	Fat class
Blackpoll warbler						
RIT (n = 17)	270 (263–287)	15.35 ± 0.97 (6.16–18.43)	2.4 ± 0.3 (1.0–5.0)	17 ± 1 (9–24)	14.4 ± 1.1 (9.7–24.3)	1 (0–5)
BBBO (n = 10)	273 (263–287)	13.71 ± 1.35 (6.57–18.71)	3.8 ± 0.4 (1.5–5.0)	17 ± 1 (10–26)	12.5 ± 0.9 (7.7–17.4)	1 (0–3)
White-throated sparrow						
RIT (n = 16)	277 (270–284)	12.32 ± 0.50 (10.55–18.43)	2.8 ± 0.8 (1.0–3.5)	18 ± 1 (10–25)	26.6 ± 0.5 (24.2–29.5)	2* (1–3)
BBBO (n = 15 <sup>b</sup> )	284 (273–287)	12.45 ± 0.49 (11.04–15.28)	3.4 ± 0.3 (1.5–5.5)	18 ± 1 (13–25)	26.1 ± 0.6 (22.3–29.7)	1 (0–3)

Values represent means ± SEM for all except capture day of year and fat class score, for which the median is presented. Samples sizes are provided below each site, and ranges are provided in parentheses below their corresponding variables. Abbreviations: BBBO, Braddock Bay Bird Observatory; and RIT, Rochester Institute of Technology.

<sup>a</sup>Mass index is body mass (in grams) scaled for wing length (in millimetres) of captured birds according to the equation provided by Peig and Green (2009).

<sup>b</sup>n = 14 for mass index and fat class.

\*Significantly greater than BBBO; Mann–Whitney U-test, P < 0.01.

or absence of each fruit-bearing plant species in each plot was recorded. The species of each woody plant (shrubs and vines) was identified, and the number of mature individuals, qualified as being >1 m tall or capable of producing fruit, was recorded along with a ranking for the estimated number of ripe fruits produced by each individual (0 = 0 fruits, 1 = 1–50 fruits, 2 = 51–100 fruits, 3 = 101–250 fruits, 4 = 251–500 fruits and 5 = more than 500 fruits). To estimate the total ripe fruit crop for each species at a given site, the ripe fruit ranking was summed over all plots. Combined ripe fruit crop estimates for all species at a given site were used to generate the proportion accounted for by each species. In order to assess potential environmental differences between the sites further, air temperature (in degrees Celsius) was recorded every 10 min daily during the length of the study using HOBO temperature loggers (Onset Corporation) placed in shaded areas at both sites for the duration of the study. The temperature at sunrise on the day of capture for each sampled bird was extracted from the data and is summarized in Table 1.

### Fruit nutritional/biochemical analyses

The fruits of the following five plant species were the focus of nutritional comparisons: gray dogwood (*Cornus racemosa*), silky dogwood (*Cornus amomum*), common buckthorn (*Rhamnus cathartica*), arrowwood viburnum (*Viburnum dentatum*) and riverbank grape (*Vitis riparia*). These fruits were selected because they are present and in sufficient quantity at both sites to permit site comparisons. Other fruits present at one, but not both, sites include red osier dogwood (*Cornus sericea*), bush honeysuckle (*Lonicera* spp.), European cranberrybush (*Viburnum opulus*) and multiflora rose (*Rosa multiflora*). Fruits from both sites were collected in mid-September at the peak of ripeness and stored frozen at –20°C until analysis. To prepare for analysis, fruits were dissected to leave only skin and pulp, which was then placed in a –80°C freezer for at least 1 h before being freeze-dried for 48 h. Freeze-dried samples were then homogenized using a mortar and pestle, and

the ground samples were stored in air-tight scintillation vials at –20°C.

Fruits contain many biochemical compounds of biological significance, including polyphenols, which have been shown to contribute to the antioxidant properties of fruits (Vinson *et al.*, 2001; Moyer *et al.*, 2002). We measured total phenol content of the five focal fruits as an indicator for the potential antioxidant capacity of the fruits. Extracts of each fruit were prepared by vortexing 75 mg of sample in 1.5 ml of 0.1% acidified methanol (50% aqueous) for 30 s, which was then shaken on a wrist shaker for 30 min. The samples were then centrifuged at 6100 × g for 15 min, and the supernatant was collected. The pellet was resuspended with 1.5 ml of solvent, and the procedure was repeated three more times to ensure complete extraction of all phenolic compounds. Total phenol content was measured using a Folin–Ciocalteu microplate assay (Magalhães *et al.*, 2010), with modification to account for the effect of different sample dilutions on calculated concentration as described by Sipel *et al.* (2013), using three sample dilutions of four replicate wells for each fruit extract and a gallic acid standard curve. All blanks, samples and standards were run on a 96-well microplate using a Biotek Eon microplate spectrophotometer.

Energy density of each fruit was determined using 1 g of freeze-dried fruit formed into a pellet and placed in a Parr 1341 bomb calorimeter. Energy density (in kilojoules per gram) was calculated from the temperature change in the water jacket following ignition, using benzoic acid as a standard. Three trials were analysed for all fruits except arrowwood viburnum, gray dogwood, silky dogwood and common buckthorn at RIT, for which two trials were analysed. The percentage dry mass fat was determined using an Ankom XT-10 extractor. Approximately 0.7–1.0 g of freeze-dried fruit was sealed in an Ankom XT4 sample bag. Sample bags were extracted for 60 min at 90°C in petroleum ether, and the percentage of fat was calculated as mass lost from the bag

following extraction and drying at 102°C. Two trials were analysed for all fruits except BBBO riverbank grape, for which three trials were analysed. To determine inorganic content, the fat-extracted XT4 sample bags were placed in crucibles in a muffle furnace at 550°C for 3 h, and percentage ash content was calculated (indicating that sample sizes are the same as those for the percentage fat). The percentage dry mass protein was determined using the Association of Official Analytical Chemists procedure for micro-Kjeldahl analysis (Association of Official Analytical Chemists, 1990). Analysis was completed by digesting 0.5 g of freeze-dried fruit and a copper-titanium dioxide catalyst (Fisher Scientific) in a sulfuric acid solution followed by steam distillation using a Labconco micro-Kjeldahl still. Total nitrogen content of the product was measured using a Shimadzu TOC/TN analyser, with potassium nitrate calibration curve. Total nitrogen was multiplied by a conversion factor of 4.4 (Witmer, 1998) to obtain the percentage protein content of the fruit. The percentage of acid detergent fibre (%ADF) of freeze-dried samples was measured using an Ankom A200 fibre analyser, with 0.45–0.55 g of pre-ground fruit sealed in Ankom F57 filter bags, which were then digested in sulfuric acid and cetyl trimethylammonium bromide for 60 min at 90°C. The residue remaining in the bags after drying at 102°C was calculated as the %ADF (cellulose + lignin). Two trials were analysed for each fruit species except for riverbank grape ( $n = 3$ ) and common buckthorn ( $n = 4$ ) at BBBO. Low sample availability prohibited analysis of %ADF in arrowwood viburnum at RIT.

## Statistical analyses

We evaluated normality of the plasma metabolite data with Shapiro–Wilk tests. Plasma  $\beta$ -hydroxybutyrate concentrations of blackpoll warblers and white-throated sparrows and plasma uric acid concentrations of white-throated sparrows were  $\log_{10}(X + 1)$  transformed to normalize the data. Given that individual birds within a species may differ in structural size, and these structural differences may influence body mass, we adjusted body mass for individual variation in body size by calculating a ‘mass index’ (using wing chord as a scalar) as described by Peig and Green (2009). Wilcoxon Mann–Whitney  $U$ -tests were then used to examine site differences in mass index and fat class score for each focal bird species. We investigated the relative importance of potential covariates that may affect circulating plasma metabolite concentrations in the captured birds using Akaike information criterion (AIC) modelling procedures as applied by Smith and McWilliams (2010) and following Burnham and Anderson (2002). Candidate model sets contained all combinations of time of day, bleed time, mass index and site (coded as a dummy variable with ‘1’ assigned as RIT and ‘0’ assigned as BBBO) and were generated separately for each metabolite and each species. The AIC corrected for small sample sizes ( $AIC_c$ ) was calculated, and differences in  $AIC_c$  ( $\Delta AIC_c = AIC_{c_i} - AIC_{c_{\min}}$ , where  $AIC_{c_i}$  is the AIC value of the model of interest and  $AIC_{c_{\min}}$  is the minimum AIC value) were used to rank candidate models. Models with  $\Delta AIC_c < 2$  were considered to have substantial support (Burnham and

Anderson, 2002). The relative importance [ $w + (j)$ ] of each variable was then calculated by summing Akaike weights ( $w_i$ ) across all candidate models in which a variable ( $j$ ) occurred (Burnham and Anderson, 2002). Analysis of covariance (ANCOVA) was then used to examine specific site differences in each metabolite for each species separately. Variables with AIC relative importance values (described above)  $\geq 0.5$  were included as covariates in ANCOVA models for each metabolite after testing for homogeneity of regression. All covariates exhibited homogeneity of regression except for time of day as a covariate for uric acid in white-throated sparrows. Thus, we corrected for time of day using a linear regression model of time of day and log-transformed uric acid concentrations, and residuals were then compared between the sites using Student’s unpaired  $t$ -test with Satterthwaite adjustment for unequal variance. Fruiting plant species diversity at the RIT site and the BBBO site was assessed descriptively by generating the Shannon–Wiener index ( $H'$ ) using data on the total number of mature individuals for each species at each site. Ripe fruit abundance and diversity were also compared between the two study sites using  $\chi^2$  goodness-of-fit tests on combined ripe fruit crop rankings for each species at a given site. Statistical analyses were calculated using SAS version 9.2 (SAS Institute, Carey, NC, USA). Significance values for all statistical tests were set at  $P < 0.05$ . Site variation in fruit nutritional traits was assessed by calculating the percentage difference in each analyte between the sites for each fruit individually. To analyse overall differences in nutritional characteristics between sites and among species, radar plots were created for each of the five fruits by normalizing the data for each analyte to a value between 0 and 1 using the minimal and maximal values for a given analyte. For these plots, the percentage of soluble carbohydrates/other were calculated as follows:  $100\% - (\% \text{fat} + \% \text{protein} + \% \text{ADF} + \% \text{ash})$ . For arrowwood viburnum, ADF was not available for the RIT site, the percentage of total carbohydrate/other for that species was calculated as follows:  $100\% - (\% \text{fat} + \% \text{protein} + \% \text{ash})$ .

## Results

### Bird captures at two sites

Blackpoll warblers were captured on 5 days at both RIT and BBBO, and white-throated sparrows were captured on 3 days at both sites (Table 1). Mass index calculations indicated that blackpoll warblers displayed a trend toward better condition at RIT compared with BBBO, although this was not significant, and a broader range of fat scores (Table 1). White-throated sparrows had nearly identical mass indices at both sites, and results of a Mann–Whitney  $U$ -test revealed that birds of this species captured at RIT had significantly higher fat class scores compared with those captured at BBBO (see Table 1), although overall the fat class scores were relatively low. The temperature at sunrise on capture dates was not significantly different between the sites for either blackpoll warblers ( $U = 141$ ,  $P = 0.97$ ) or white-throated sparrows ( $U = 246$ ,  $P = 0.83$ ).

### Modelling of plasma metabolites

The AIC model selection results for each metabolite and for each species are summarized in Table 2. The top-ranked models for plasma triglyceride included site and mass index for blackpoll warblers (RIT,  $n = 17$ ; BBBO,  $n = 10$ ) and site and bleed time for white-throated sparrows (RIT,  $n = 16$ ; BBBO,  $n = 15$ ). Relative importance values confirmed that site explained the majority of the variation in triglyceride for both species (Table 3). Model selection results for  $\beta$ -hydroxybutyrate displayed substantially more uncertainty because there were several plausible models identified for both species (Table 2). Relative importance values clarified that time of day explained the most variation in  $\beta$ -hydroxybutyrate for blackpoll warblers (RIT,  $n = 12$ ; BBBO,  $n = 4$ ), and both time of day and bleed time were dominant factors in explaining this metabolite for white-throated sparrows (RIT,  $n = 11$ ; BBBO,  $n = 12$ ). Uric acid was found to have considerable model selection uncertainty for blackpoll warblers, with four plausible models identified (Table 2; RIT,  $n = 15$ ; BBBO,  $n = 6$ ). Relative

importance values were also unclear and did not identify any single variable as being important in explaining variation in uric acid concentrations (Table 3). Model selection results for uric acid concentrations in white-throated sparrows were considerably more straightforward and suggested that time of day is important in explaining the variation in this metabolite for this species (Tables 2 and 3; RIT,  $n = 15$ ; BBBO,  $n = 14$ ).

After correcting for the covariates identified in the model selection analyses, ANCOVA showed explicit site differences in plasma triglyceride (Fig. 1A). Blackpoll warblers had significantly higher concentrations of plasma triglyceride at BBBO compared with RIT (site,  $F_{1,24} = 18.09$ ,  $P > 0.001$ ; mass index,  $F_{1,24} = 5.42$ ,  $P = 0.03$ ), as did white-throated sparrows (site,  $F_{1,28} = 6.67$ ,  $P = 0.02$ ; bleed time,  $F_{1,28} = 3.87$ ,  $P = 0.05$ ).  $\beta$ -Hydroxybutyrate did not differ between sites for blackpoll warblers (site,  $F_{1,13} = 0.17$ ,  $P = 0.69$ ; time of day,  $F_{1,23} = 3.41$ ,  $P = 0.09$ ; Fig. 1B) or white-throated sparrows (site,  $F_{1,19} = 0.87$ ,  $P = 0.36$ ; time of day,  $F_{1,19} = 1.89$ ,  $P < 0.001$ ; bleed time,  $F_{1,19} = 39.93$ ,  $P < 0.0001$ ; Fig. 1B). Likewise, uric acid was similar at both

**Table 2:** Model selection results for the top-ranked models explaining plasma triglyceride,  $\beta$ -hydroxybutyrate and uric acid concentrations based on Akaike information criterion model weights for blackpoll warblers (A) and white-throated sparrows (B)

	Model <sup>a</sup>	$k$	$AIC_c$	$\Delta AIC_c$	$w_i$
<b>(A) Blackpoll warbler</b>					
Triglyceride					
	TRIG = site + mass index	3	-36.80	0.00	0.51
$\beta$ -Hydroxybutyrate					
	LOG(BUTY + 1) = time of day	2	-53.95	0.00	0.25
	LOG(BUTY + 1) = time of day + bleed time	3	-53.83	0.13	0.23
	LOG(BUTY + 1) = bleed time	2	-52.57	1.38	0.12
	LOG(BUTY + 1) = site	2	-52.47	1.48	0.12
Uric acid					
	UA = bleed time	2	-43.18	0.00	0.25
	UA = mass index	2	-42.46	0.72	0.17
	UA = site	2	-41.92	1.27	0.13
	UA = time of day	2	-41.91	1.27	0.13
<b>(B) White-throated sparrow</b>					
Triglyceride					
	TRIG = site + bleed time	3	-46.31	0.00	0.28
	TRIG = site	2	-45.26	1.05	0.16
$\beta$ -Hydroxybutyrate					
	LOG(BUTY + 1) = time of day + bleed time	3	-98.34	0.00	0.68
Uric acid					
	LOG(UA + 1) = time of day	2	-135.55	0.00	0.39

Models with  $\Delta AIC_c < 2.0$  are provided. Abbreviations:  $AIC_c$ , small-sample Akaike information criterion;  $\Delta AIC_c$ , difference between  $AIC_c$  value of the model of interest and the minimum  $AIC_c$  value;  $w_i$ , Akaike weight; TRIG, plasma triglyceride concentration; BUTY, plasma  $\beta$ -hydroxybutyrate concentration; and UA, plasma uric acid concentration.

<sup>a</sup>Time of day is the hour after sunrise of capture. Bleed time is minutes after capture when blood was drawn. Mass index is body mass (in grams) scaled for wing length (in millimetres) of captured birds according to the equation provided by Peig and Green (2009).

**Table 3:** Relative importance ( $\sum w_i$ ) of predictor variables<sup>a</sup> in Akaike information criterion models for blackpoll warblers (A) and white-throated sparrows (B)

	Site	Time of day	Bleed time	Mass index
<b>(A) Blackpoll warbler</b>				
Triglyceride	1.00*	0.20	0.21	0.78*
$\beta$ -Hydroxybutyrate	0.26	0.62*	0.36	0.16
Uric acid	0.27	0.27	0.46	0.35
<b>(B) White-throated sparrow</b>				
Triglyceride	0.78*	0.38	0.56*	0.23
$\beta$ -Hydroxybutyrate	0.19	1.00*	1.00*	0.15
Uric acid	0.25	0.80*	0.24	0.26

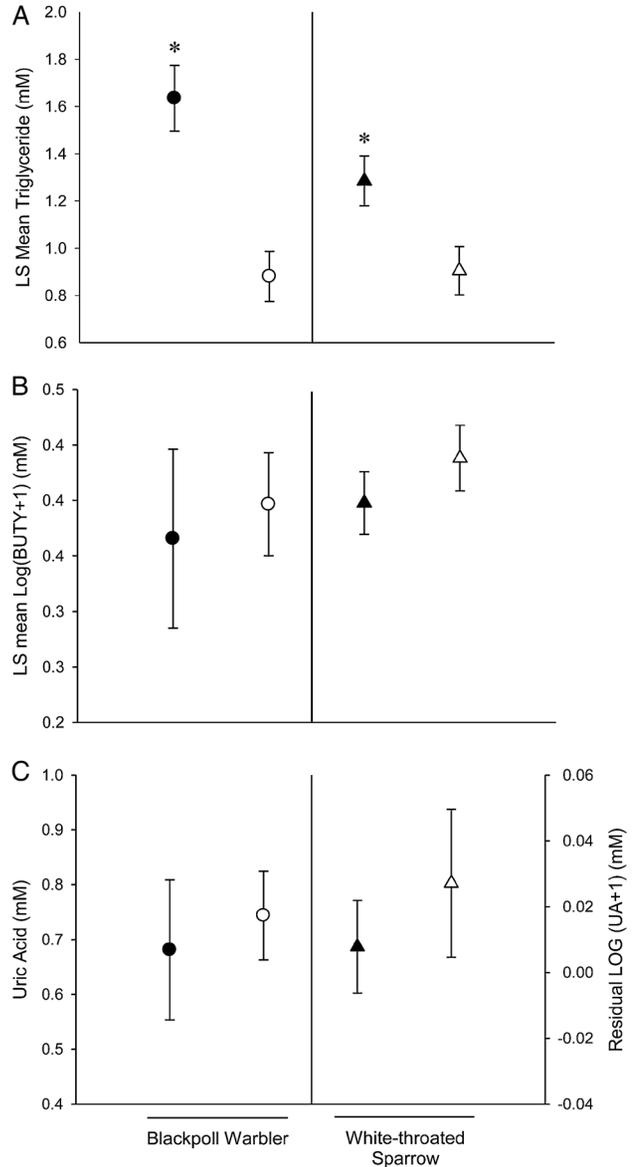
<sup>a</sup>Time of day is the hour after sunrise of capture. Bleed time is minutes after capture when blood was drawn. Mass index is body mass (in grams) corrected for wing length (in millimetres) of captured birds according to the equation provided by Peig and Green (2009).

\*Indicates covariates with relative importance values  $\geq 0.5$  that were included in ANCOVA models to compare metabolite concentrations among sites.

sites for blackpoll warblers (ANOVA:  $F_{1,19} = 0.17$ ,  $P = 0.68$ ; Fig. 1C) and also for white-throated sparrows ( $t = 0.49$ , d.f. = 20.95,  $P = 0.6273$ ; Fig. 1C). Note that site comparisons for  $\beta$ -hydroxybutyrate and uric acid for blackpoll warblers should be interpreted with caution given the disproportionately small sample size for these analytes at the BBBO site.

### Habitat assessment and fruit quality

The overall diversity of fruiting shrubs and vines was greater at BBBO ( $H' = 1.69$ ) compared with RIT ( $H' = 1.30$ ). When plant species were divided by native/non-native status, BBBO had greater native diversity ( $H' = 1.33$ ) than RIT ( $H' = 1.11$ ) and lower non-native diversity ( $H' = 0.40$ ) than RIT ( $H' = 0.50$ ). A  $\chi^2$  analysis revealed that the total combined ripe fruit crop by site was significantly higher at BBBO compared with RIT ( $\chi^2 = 1716.6$ , d.f. = 1,  $P < 0.0001$ ; Table 4). In addition, the distribution of ripe fruits among the plants species observed at each site was significantly different ( $\chi^2 = 5880.2$ , d.f. = 8,  $P < 0.0001$ ), with the ripe fruit crop at BBBO predominantly composed of arrowwood and dogwood fruits, and the ripe fruit crop at RIT being dominated by grape (Table 4). The five focal fruits analysed for nutritional and biochemical characteristics exhibited site variation in the analytes that ranged from 1.8 to 59.54% difference (Table 5), although the biological significance of these differences for each analyte has not been investigated. The magnitude of the differences varied among the analytes and fruits, with %ADF generally exhibiting the largest percentage difference between sites for three of the fruit species, but neither site was consistently higher than the other for a given analyte across all fruit species (Table 5). Differences in analytes for each species between the two sites can be visualized with radar plots of all normalized nutritional components. Examination of these plots revealed that each



**Figure 1:** Plasma concentrations of triglyceride (A),  $\beta$ -hydroxybutyrate (BUTY; B) and uric acid (UA; C) of blackpoll warblers (left panels, circles) and white-throated sparrows (right panels, triangles) captured at two sites during autumn of 2011 [Braddock Bay Bird Observatory (BBBO) site, filled symbols; Rochester Institute of Technology (RIT) site, open symbols].  $\beta$ -Hydroxybutyrate concentrations were  $\text{Log}(X + 1)$  transformed prior to analysis, and all metabolite values, except uric acid, are least-squares means (LS means) generated from ANCOVA models, correcting for variables with relative importance values  $> 0.5$  as shown in Table 3. Transformed uric acid concentrations for white-throated sparrows are represented as the residuals after correcting for time of day and were compared with Student's  $t$ -test. Uric acid concentrations for blackpoll warblers were compared using ANOVA because there were no covariates of importance identified by Akaike information criterion model selection. Asterisks indicate that plasma concentrations of birds captured at BBBO are significantly higher than those of birds captured at RIT.

fruit had fairly similar-shaped polygons to their nutritional and biochemical composition at both sites, although some nutritional analytes for each fruit varied by site as indicated by

**Table 4:** Estimated total ripe fruit crop of native and non-native plants at RIT and BBBO during autumn 2011

Species	Total fruit crop		Proportion of total fruit crop	
	RIT	BBBO	RIT	BBBO
<b>Native</b>				
Arrowwood	0	1700	0.00	0.26
Gray dogwood	150	2700	0.07	0.42
Silky dogwood	75	925	0.03	0.14
Red osier dogwood	0	25	0.00	0.00
Riverbank grape	1825	300	0.84	0.05
<b>Non-native</b>				
Common buckthorn	75	0	0.03	0.00
Multiflora rose	50	0	0.02	0.00
Bush honeysuckle	0	425	0.00	0.07
European cranberrybush	0	375	0.00	0.06
<b>Total</b>	<b>2175</b>	<b>6450</b>		

Total ripe fruit crops represent the sum of fruit rankings across all plants for each species in all sampled plots at each site (see Materials and methods section).

shifts on their respective axes (Fig. 2). Arrowwood viburnum was generally high in energy density, percentage of fat and total phenols compared with other fruits. The relative proportions of each analyte for arrowwood viburnum were fairly similar at the two sites, with BBBO being greater in energy density and percentage of fat, but lower with respect to %ADF, percentage of carbohydrate/other and percentage of ash than RIT (Fig. 2). Grape was relatively high in the percentage of soluble carbohydrates/other and percentage of ash, but relatively low in the percentage of protein, percentage of fat and energy density compared with the other fruits. Site differences for the measured analytes were relatively small for grape, with only the percentage of ash being greater at RIT (Fig. 2). Common buckthorn at the RIT site had the highest phenol content of all fruits and overall was highest in the percentage of soluble carbohydrate/other, but relatively low in energy density and the percentage of fat compared with other fruits. Furthermore, the percentage of ash was higher at BBBO and %ADF was higher at the RIT site for common buckthorn (Fig. 2). Gray dogwood and silky dogwood were generally more variable in analyte content between sites and had the relative highest percentage of protein compared with the other fruits, with the percentage of protein being even higher at RIT and the percentage of ash being higher at BBBO (Fig. 2).

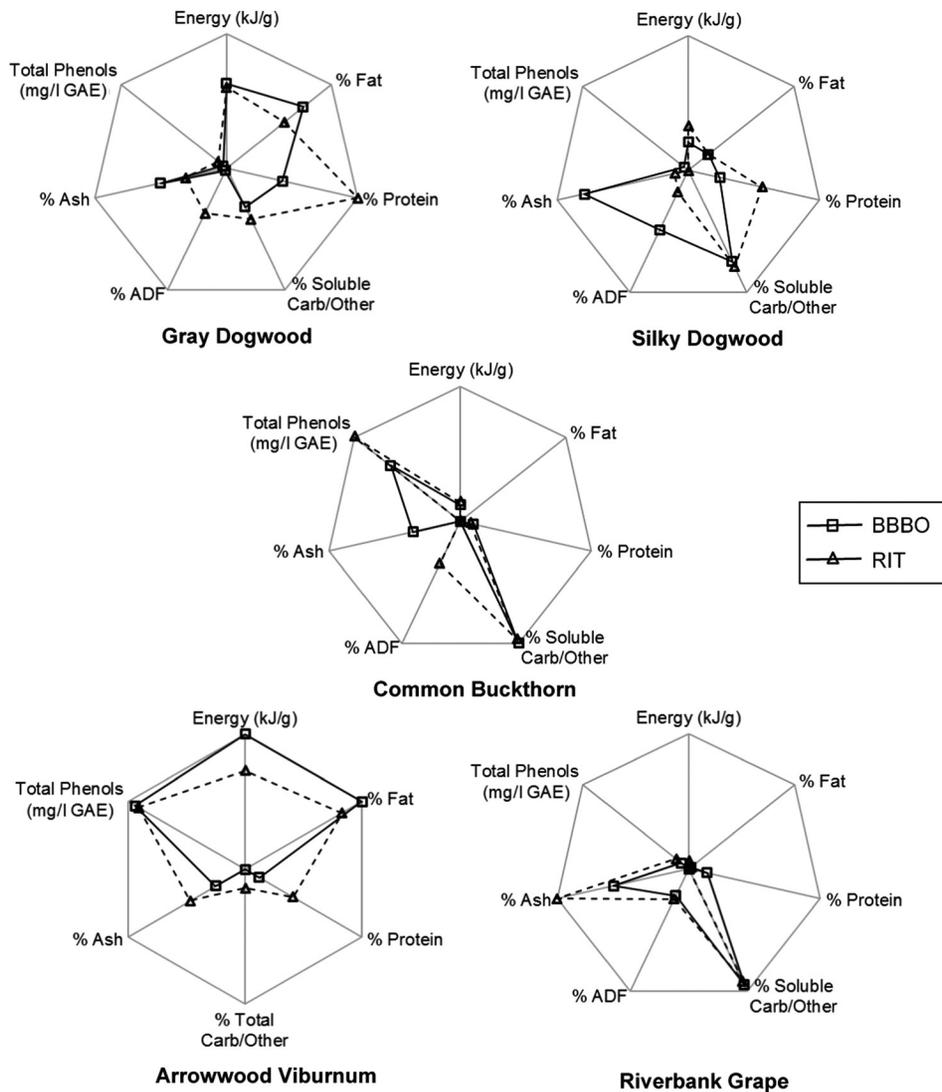
## Discussion

Our study provides an integrative evaluation of local fruit resources for autumn-migrating passerines at an important

**Table 5:** Nutritional and biochemical analytes measured in five fruit species collected at RIT and BBBO during autumn 2011

Species	Site	Energy density (kJ/g)	Fat (%)	Protein (%)	ADF (%)	Ash (%)	Total phenols (mg/l GAE)
Gray dogwood	RIT	24.00 ± 0.57	27.88 ± 0.10	5.39 ± 0.12	5.24 ± 0.30	2.93 ± 0.31	302.26 ± 12.76
	BBBO	24.48 ± 0.40	37.08 ± 2.04	3.47 ± 0.01	2.89 ± 0.00	3.34 ± 0.21	230.62 ± 11.12
	% Diff.	1.98%	28.33%	43.34%	57.81%	13.08%	26.91%
Silky dogwood	RIT	19.81 ± 0.20	9.86 ± 0.05	3.91 ± 0.08	3.94 ± 0.24	2.48 ± 0.05	189.90 ± 5.32
	BBBO	17.86 ± 0.83	10.08 ± 0.02	2.81 ± 0.21	6.05 ± 0.01	3.97 ± 0.31	242.22 ± 16.08
	% Diff.	10.35%	2.21%	32.74%	42.24%	46.20%	24.21%
Common buckthorn	RIT	16.78 ± 0.21	0.36 ± 0.04	2.28 ± 0.20	5.10 ± 0.43	2.27 ± 0.15	1647.52 ± 60.34
	BBBO	16.43 ± 0.14	0.34 ± 0.03	2.34 ± 0.10	2.76 ± 0.09	3.04 ± 0.03	1147.70 ± 40.85
	% Diff.	2.11%	5.72%	2.60%	59.54%	29.00%	35.80%
Arrowwood viburnum	RIT	25.99 ± 0.27	41.79 ± 0.11	3.39		3.28 ± 0.33	1516.85 ± 40.24
	BBBO	30.22 ± 0.42	50.48 ± 0.61	2.42 ± 0.15	9.44 ± 0.52	2.80 ± 0.16	1543.99 ± 89.03
	% Diff.	15.06%	18.84%	33.39%		15.79%	1.80%
Riverbank grape	RIT	15.48 ± 0.84	1.89 ± 0.14	2.01 ± 0.31	4.42 ± 0.22	4.42 ± 0.40	363.85 ± 14.62
	BBBO	14.61 ± 0.11	1.30 ± 0.10	2.48 ± 0.34	4.25 ± 0.18	3.50 ± 0.16	291.02 ± 12.12
	% Diff.	5.78%	36.99%	20.94%	3.92%	23.23%	22.20%

All values are means ± SEM, and the percentage difference (% Diff.) between the means for a given fruit at each site is provided. Abbreviation: GAE, gallic acid equivalents.



**Figure 2:** Radar plots of nutritional and biochemical analytes measured in the five fruit species collected in autumn 2011. Values on each axis are normalized to a range of 0–1.

stopover location on the south shore of Lake Ontario that is coupled with information about the physiological condition of birds via plasma metabolite profiling. We have shown that patterns of refuelling in these birds differed between sites in a way that is consistent with the availability and quality of wild fruits in habitats used by birds during stopovers. Our results suggest that birds deposit more fat where high-quality (e.g. energy-dense, high-fat, high-phenol-content) native fruits are abundant, although plasma uric acid concentrations did not indicate site differences in dietary protein intake, suggesting that other high-protein foods, such as insects, did not comprise a greater proportion of the diet at the site dominated by lower quality fruits. Although we did not find clear site differences in the overall nutritional biochemistry across all fruit species, we found evidence that fruit-bearing plant species can

respond individually in different ways to local growing conditions, resulting in small-scale geographical variation in fruit quality. These local site differences in fruit quality may become important when they involve the critical energy-providing nutrients in fruits that are highly preferred by birds during migration as demonstrated for arrowwood viburnum (Smith *et al.*, 2013).

Our results for plasma triglyceride concentrations indicated that birds were depositing more fat at BBBO, where the total ripe fruit crop was approximately three times greater than that of the RIT site. This result is consistent with previous work that demonstrated higher plasma concentrations associated with high fruit availability (Smith and McWilliams, 2010) and that higher refuelling rates in frugivores are correlated with

habitats that support abundant fruit resources (Evans Ogden *et al.*, 2013). More generally, energetic condition and favourable energy budgets of birds during autumn stopover have also been associated with frugivory (Parrish, 1997, 2000). In fact, frugivorous thrushes migrating through our study area have higher plasma triglyceride and lower uric acid in autumn compared with spring, presumably because of the abundant fruit resources available as a primary food resource in autumn (Smith, 2013). Our results also suggest that the diversity and quality of available fruits are important factors that should be considered when evaluating the importance of fruit resources at stopover sites. Both warblers and sparrows exhibited higher levels of fat deposition at BBBO, and ~83% of the ripe fruit crop measured at this site are native fruits with high fat and high energy density (defined generally here as >5% fat and >19 kJ/g from the range of species reported at these sites by Smith *et al.*, 2013), whereas only 10% of the measured ripe fruit crop at RIT falls into this category. Furthermore, birds are known to prefer these high-quality native fruits during stopovers (Smith *et al.*, 2007a, 2013), providing additional support for their importance as food resources during autumn stopovers. At RIT, the dominant fruit was instead riverbank grape, which is among the lowest in terms of energy-providing nutrients and phenol content of the fruits sampled. Furthermore, grape fruits are often avoided by birds during migration at BBBO when other, more nutritious fruits are available, often persisting into the winter (S. Smith, personal observation). According to Smith *et al.* (2013), the most energy-dense and preferred of the available fruits in this area is arrowwood viburnum. This fruit was absent from the plots sampled at RIT and is sparsely present as patchy single shrubs in the vicinity of the RIT banding area. The noticeable lack of this important fruit and the relatively lower proportions of other fatty fruits, such as dogwoods, at RIT may impact the ability of birds to refuel efficiently at this site if they are limited to primarily lower energy, watery fruits, such as grape (Smith *et al.*, 2007a).

One possible explanation for the disparate triglyceride concentrations of birds at the two sites is that they might have consumed less fruit at RIT, perhaps in response to the lower availability of preferred fruit, and instead incorporated other food items, such as insects or seeds, into their diet that may be of lower quality than the dominant fruits at BBBO. Our results for uric acid suggest that dietary protein intake was similar at the two sites and so birds were likely not to be consuming more protein-rich insects at RIT, although the possibility that sparrows incorporated more seeds in their diet may not be reflected in the metabolite data. It is also possible that different demographic populations were captured at the two sites. Age is not likely to be a factor in explaining refuelling differences between the sites because the majority of the birds analysed for this study were hatching year, and adults and juveniles do not typically differ in their refuelling performance as measured by plasma metabolites in autumn (Seewagen *et al.*, 2013). Although an analysis of potential sex-based differences in refuelling was not possible in our study, generally, sex differences in stopover ecology are less commonly

reported in autumn compared with spring because of expected similar pressures for arrival at wintering grounds; hence, similar stopover behaviour (Morris and Glasgow, 2001). Competition for food resources may also impact foraging efficiency and subsequent fat deposition during stopovers (Newton, 2006), particularly when many birds with similar dietary habits may potentially deplete the available food resources (Moore and Yong, 1991). Fruits are a major food resource for many species of passerines during autumn, which could represent a potential source of resource competition at our sites. Although we lack data on resource competition during stopovers at our sites, decreased foraging due to competition is not likely to explain the lower triglyceride concentrations of birds at the RIT site because birds tend to be more abundant and may concentrate at stopover sites near the shoreline during autumn migration in this area (France *et al.*, 2012). Fruits are typically present throughout the autumn season at our sites, but certain fruits that are highly preferred by birds, such as arrowwood viburnum, may become depleted faster than other, lower quality fruits (Smith *et al.*, 2013). It is possible that competition for these higher quality fruits, which generally were more abundant at the BBBO site near the shoreline, led to a lower foraging efficiency and fat deposition of birds at the inland RIT site.

While our results for plasma triglycerides are significant and suggest clear site differences in refuelling between the sites, our data for  $\beta$ -hydroxybutyrate were inconclusive. We recognize that our data set for  $\beta$ -hydroxybutyrate was small for warblers at the RIT site, and this may have contributed to the considerable model selection uncertainty in this metabolite. Nevertheless, our results suggest that  $\beta$ -hydroxybutyrate is subject to more variation and can be sensitive to covariates such as bleed time, thus concurring with previous studies suggesting that plasma  $\beta$ -hydroxybutyrate is less reliable as an indicator of refuelling than plasma triglyceride in migrating birds at stopover sites (Guglielmo *et al.*, 2005; Smith and McWilliams, 2010; Evans Ogden *et al.*, 2013).

We recognize that our study sites may differ in other ways that may influence stopover site use by birds in the autumn. Braddock Bay Bird Observatory is located immediately along the lake shoreline and may represent an area where some birds are fat depleted after lake crossing. In fact, many birds readily cross Lake Ontario in autumn (Diehl *et al.*, 2003) and use the habitats surrounding BBBO on the south shore of Lake Ontario for foraging and replenishing energy (Bonter *et al.*, 2007), perhaps taking advantage of the abundant fruit resources before continuing on their southward migration. Birds that continue along the Genesee River corridor will encounter heavily urbanized areas, with unpredictable availability of habitat for adequate stopover refuelling. Urban habitats may represent suitable areas for birds to gain mass during stopovers in some locations (Seewagen and Slayton, 2008; Seewagen *et al.*, 2011), and these areas may also concentrate migrants. The vicinity of the RIT campus may represent one such area that is used by birds during the autumn, although bird densities and capture rates have historically

been lower at this site compared with more pristine areas, such as BBBO and birds banded at BBBO were never recaptured at the RIT site. There was a trend toward higher mass in both bird species captured at RIT compared with BBBO, and sparrows captured at RIT had significantly higher fat loads, though overall median fat scores were low at both sites. Thus, birds arriving at the RIT site in relatively good condition and with adequate fat stores may not need to stay for multiple days, which is a scenario that may also be preferable owing to the relatively lower fruit resources and proximity to developed areas. It is also unclear which routes the birds take after departing from the RIT site and whether they continue along the Genesee River, where they may encounter more suburban/agricultural and forested habitat patches.

To our knowledge, site differences in wild fruit quality have not commonly been examined on small spatial scales approaching the local scale in our study. We found that fruits can vary in nutritional analytes within a species on this small scale; however, this depended on the fruit species and analyte, although the biological significance of these differences has not been investigated. Generalizable differences between the sites were confounded by the fact that some fruits exhibited higher values for certain energy-related analytes (e.g. energy density, fat) but not others (protein, carbohydrate). Furthermore, for some fruits, such as arrowwood viburnum and gray dogwood, shifts toward higher fat and/or carbohydrates appear to co-occur with a shift in the opposite direction for other analytes, such as protein or ADF, at a given site. In these cases, the production of more energetically costly nutrients, such as fat, could offset the production of other biomolecules in the fruit. Figure 2 shows that fruits collected at the RIT site generally tended towards higher levels on the percentage of protein and %ADF and/or percentage of carbohydrate axes. A shift in the fruit pulp toward higher ADF (e.g. dogwoods, common buckthorn in our study) may represent a lowering in quality for birds because this fraction of fibre is typically indigestible by passerines (Klasing, 1998). Site variation in protein content of the fruits was observed for multiple species; regardless, all fruits at both sites are likely to meet the protein requirements of migrating passerines (Langlois and McWilliams, 2010). When considering site differences in fat content, Fig. 2 shows that arrowwood viburnum and gray dogwood were shifted higher on the energy-providing fat axis at BBBO, with arrowwood viburnum also displaying the largest site inequality in energy density; however, as mentioned above, the biological reasons for these site differences in the percentage of fat and energy density of arrowwood viburnum fruits are unknown. It is possible that local growing conditions contributed to these observed differences in the fat and energy of arrowwood viburnum fruit, particularly the amount of sunlight available at the two sites. At RIT, arrowwood viburnum is restricted to the understory, where light may be limiting, possibly resulting in the observed smaller and less abundant fruit on panicles of this shrub at the RIT site. Except for ADF and total phenols, nutritional analytes for common buckthorn had very similar values at the two sites. This species is known to be fast growing

and able to withstand a variety of conditions and may therefore be more resilient to local variation in habitat/environmental conditions (Knight *et al.*, 2007).

Arrowwood viburnum and common buckthorn had the highest measured phenols of all fruits, and the total phenol content in the former species was similar at both sites (1.8% difference), suggesting exceptional antioxidant potential of the fruit, regardless of location, and agreeing with the strong antioxidant capacity reported by others for this species (Alan *et al.*, 2013). These phenol concentrations may benefit birds during stopover given the powerful oxidative stress imposed by migratory flight (Jenni-Eiermann *et al.*, 2014), but it is important to note that the employed total phenol assay is a non-specific test that may include both biologically relevant compounds of antioxidant capacity importance, such as certain flavonols and anthocyanins, and other phenolic plant secondary compounds that may have potentially deleterious effects on the physiology of consumers. For example, common buckthorn displayed markedly higher total phenol content at the RIT site, but the high measured phenolic content of this fruit may not necessarily represent beneficial nutriment and could instead be indicative of plant secondary compounds, such as emodins, that are present in the plant vegetative parts, including fruit pulp, of *Rhamnus* species (Tsahar *et al.*, 2002). These secondary compounds serve as a feeding deterrent to herbivores, small mammals and birds through a variety of biological and digestive effects (Izhaki, 2002), thus potentially making this fruit that is more abundant at the RIT site less desirable. Common buckthorn is among the least preferred of the fruits at the RIT site (Smith *et al.*, 2013), which suggests that birds are not actively selecting it for potential nutritive properties as they typically would for other darkly pigmented fruits with high phenolic and anthocyanin content, such as arrowwood viburnum (Bolser *et al.*, 2013). Arrowwood viburnum has been shown to be highly preferred by birds at BBBO, where it is also more abundant (Smith *et al.*, 2013), which is consistent with other studies that have shown a preference by birds during autumn migration for this species (Smith *et al.*, 2007a; Bolser *et al.*, 2013). Given that both fruits display relatively high total phenol measurements and given their pigmentation, both might also contain relatively high anthocyanin content. The presence of detrimental secondary compounds that are phenolic in nature in common buckthorn probably indicates that other beneficial phenols represent the larger portion of the phenol profile in arrowwood viburnum. In fact, we have found (our unpublished data) that arrowwood viburnum contains a much higher concentration of antioxidant-rich catechin-like compounds compared with common buckthorn.

Our results suggest that fruit resources are an important component of autumn stopovers and complement a growing recent body of data to support their important physiological role for migratory passerines in the northeastern USA (Parrish, 1997; Smith *et al.*, 2007a, 2013; Smith and McWilliams, 2010; Bolser *et al.*, 2013; Mudrzyński and Norment, 2013). We suggest that quality, in addition to availability of fruits, is a key

consideration for management of important stopover sites. Establishing a link between the quality and quantity of fruit resources and physiological condition of birds during refuelling may help to inform conservation initiatives about critical habitats and resources for migratory bird populations. Despite the fact that efforts to examine the biological significance of the differences are ongoing, we believe that fruits can vary in their nutritional value to birds on small spatial scales in ways that may potentially affect the refuelling physiology of birds during short stopovers, although some high-quality native fruits (e.g. arrowwood viburnum) are likely to be superior resources for birds consistently, regardless of site variation. Future work that incorporates foraging observations or diet sampling with physiological assessments of refuelling may shed more light on the role of specific fruits in the diets of birds in relation to their energetic requirements. Furthermore, examination of the comprehensive nutritional and biochemical composition of fruits across landscape or urban gradients, but also annually at a given site, may provide insight into how this potential variation in fruit nutritional composition will affect migrating birds in light of predicted environmental change.

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