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Source: The Wilson Journal of Ornithology, 125(3):513-525. 2013.
Published By: The Wilson Ornithological Society
DOI: http://dx.doi.org/10.1676/13-014.1
URL: http://www.bioone.org/doi/full/10.1676/13-014.1

The Wilson Journal of Ornithology 125(3):513–525, 2013

THE IMPORTANCE OF ANTIOXIDANTS FOR AVIAN FRUIT SELECTION DURING AUTUMN MIGRATION

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ABSTRACT.—All vertebrates must contend with an increase in oxidative stress during intense exercise. Birds, in particular, may be exposed to increased oxidative stress during long-distance migration, and dietary antioxidants are likely important in alleviating the deleterious effects associated with such a stressor. We evaluated whether fruit selection by birds at a migratory stopover site in southern New England was related to the antioxidant and macronutrient content of fruits from seven commonly consumed fall-fruiting shrub species. Our objectives were to: (1) quantify, for the first time, total hydrophilic and lipophilic antioxidants, as well as two types of lipophilic antioxidants (i.e., carotenoids, and tocopherols) in wild fruits consumed by migrating birds, (2) test the hypothesis that antioxidant content of wild fruits is related to macronutrient composition, and (3) relate patterns of avian frugivory to antioxidant availability and macronutrient content of wild fruits during autumn migration. We found significant differences between fruits in total lipophilic antioxidants, carotenoids, and tocopherols, but not total hydrophilic antioxidants. *Viburnum* spp. and *Myrica pennsylvanica* had the most lipophilic antioxidants and tocopherols, whereas *Celastrus orbiculatus* and *Rosa multiflora* contained the most carotenoids. Carotenoid content was positively correlated with protein content but no significant relationships were evident between the other antioxidants and macronutrients. Fruit consumption was negatively correlated with carotenoid content and was not related to any other antioxidant measure. Interestingly, the most consumed fruit species, arrowwood, was among the highest in fat, total lipophilic antioxidants, and tocopherols. These data indicate that antioxidant content differs significantly between fruit species and suggest that (1) birds can acquire different types of antioxidants depending on the fruits they select and (2) lipophilic antioxidants, especially tocopherols, may be important antioxidants for birds during autumn migration. *Received 17 January 2013. Accepted 19 April 2013.*

Key words: antioxidant, carotenoid, fruit preference, migratory birds, tocopherol.

All aerobic organisms produce reactive oxygen species (ROS) as unavoidable byproducts of respiration and an over-production of these reactive species is associated with damage to key physiological systems (Barja 2000, Wiersma et al. 2004, Halliwell and Gutteridge 2007, Costantini 2008). Birds may be exposed to especially high levels of ROS production as a consequence of long-duration flight (Costantini et al. 2007, 2008). Birds and other vertebrates have evolved several mechanisms to cope with this oxidative burden, including an ability to increase endogenous antioxidant production (e.g., uric acid and enzymatic antioxidants) (Balaban et al. 2005, Tsahar et al. 2006, Costantini 2008, Monaghan et al. 2009) and to preferentially consume and metabolize dietary antioxidants (e.g., carotenoids, tocopherols, and polyphenolic compounds) (Catoni et al. 2008a, Costantini 2008, Monaghan et al. 2009). Currently, little is known about where birds might acquire specific antioxidants in their diet, because the antioxidant composition of wild foods is not yet well established (Schaefer et al. 2003, 2008; Hill and McGraw 2006).

During autumn migration, many birds that are primarily insectivorous during the summer months switch to eating seasonally-abundant fruits (Thompson and Willson 1979, Herrera 1984, Parrish 1997, Klasing 1998, McWilliams et al. 2004). Many of these fruits may be excellent sources of dietary antioxidants and could provide an important defense against oxidative stress for both fruiting plants and their consumers (Garcia-Alonso et al. 2004; Smith et al. 2007; Catoni et al. 2008a, b; Bolser et al. 2013). Most antioxidants in fruits can be classified either as lipophilic (e.g., carotenoids and tocopherols) or hydrophilic (e.g., ascorbic acid and polyphenolic compounds) (Crozier et al. 2006, Catoni et al. 2008b, Costantini 2008). Despite the potential importance of lipophilic antioxidants to health, very few studies have directly measured their concentration in fruits. Schaefer et al. (2008) measured the carotenoid content of 60 wild fruit species commonly consumed by birds and found that *Tamus communis*, *Streptopus amplexifolius*, *Polygonatum multiflorum*, and *Rosa glauca* had the

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highest amounts. Considerably more attention has been given to hydrophilic antioxidants and several studies have specifically measured these antioxidants in fruits. Benvenuti et al. (2004) observed high hydrophilic antioxidant activity in fruits from the genera *Rubus, Ribes,* and *Aronia*. Schaefer et al. (2008) found that fruits from American elderberry (*Sambucus canadensis*), black mulberry (*Morus nigra)*, burnet rose (*Rosa pimpinellifolia*), common dogwood (*Cornus sanguinea)*, and hawthorne (*Crataegus* spp.) contained the most anthocyanins of 60 plant species they studied. More recently, Bolser et al. (2013) quantified hydrophilic antioxidants in fruits from seven shrub species at a southern New England stopover site and found that arrowwood (*Viburnum* spp.) fruits contained high levels of anthocyanins and other phenolics, whereas fruits from multiflora rose (*Rosa multiflora*), Asiatic bittersweet (*Celastrus obiculatus*), and winterberry (*Ilex verticillata*) contained virtually none of these antioxidants. No previous studies have quantified both the lipophilic and hydrophilic antioxidant content of wild fruits nor have they related such measures to fruit selection by wild birds.

To date, no studies have specifically investigated the relationship between the macronutrient content and antioxidant content of wild fruits consumed by free-living birds. In both natural and processed foods, the susceptibility of lipids to oxidation is a major cause of quality deterioration (Frankel 1996). One of the most effective means of retarding lipid oxidation in fatty foods is through antioxidant protection (Decker 1998a, b; Frankel 1996; Reisch et al. 1998). Specifically, lipophilic antioxidants extracted from plants (e.g., tocopherols) are especially potent in preventing oxidation in food emulsions (McClements and Decker 2000). A direct correlation has been established between the polarity of antioxidants and their effectiveness in inhibiting oxidation. Highly polar antioxidants (e.g., hydrophilic antioxidants) are much less efficient in inhibiting lipid oxidation than non-polar antioxidants (e.g., lipophilic antioxidants) (Schwarz et al. 1996), because hydrophilic antioxidants are located primarily in aqueous phases, away from where lipid oxidation occurs, whereas lipophilic antioxidants are located both in fatty tissues and in the lipid membranes of cells where the bulk of lipid oxidation occurs (McClements and Decker 2000). As such, it is possible that plants with high-fat fruits (e.g., arrowwood; Smith et al. 2007) may allocate more antioxidants, particularly lipophilic antioxidants, to these fruits to protect against oxidative damage, though this relationship has yet to be directly demonstrated.

The influence of antioxidant levels on avian fruit selection has only recently received attention from ornithologists. α-tocopherol, a form of vitamin E, is commonly used as a supplement to improve broiler chicken health and quality (Marusich et al. 1975, Boa-Amponsem et al. 2000, Leshchinsky and Klasing 2001, Surai 2002) and carotenoids are well-known contributors to plumage coloration and sexual signaling in several bird species (Olsen and Owens 1998, Hill 2000, Hill et al. 2002, Shawkey et al. 2006). Although lipophilic antioxidants provide these nutritional benefits, the influence of tocopherols on avian food preference has yet to be investigated. A few studies have directly assessed how carotenoids influence food selection by birds, although these have produced contradictory results. For example, Senar et al. (2010) found that Great Tits (*Parus major)* consistently chose carotenoid-enriched mealworms over non-enriched mealworms; however, male Society Finches (*Lonchura domestica)* offered a carotenoid-enriched diet did not consume more food than birds offered a non-enriched diet (McGraw et al. 2006) and Garden Warblers (*Sylvia borin)* did not preferentially consume carotenoids when offered a choice between cartenoid-supplemented and non-supplemented food (Catoni et al. 2011). Hydrophilic antioxidants, on the other hand, have been widely used in behavioral diet choice trials. Schaefer et al. (2008) found that Eurasian Blackcaps (*Sylvia atricapilla*) preferred artificial diets supplemented with anthocyanins over foods without these supplements, and Catoni et al. (2008a) showed that Eurasian Blackcaps preferred food with flavonoids over food without flavonoids. In addition, free-living birds selected arrowwood fruits containing more anthocyanins over lower-anthocyanin fruits from other fruiting-shrub species during autumn migration (Bolser et al. 2013).

No previous studies have determined the extent to which free-living birds select wild fruits based on their specific hydrophilic and lipophilic antioxidant content. In this study, we evaluated whether fruit selection by birds at an important stopover site in southern New England was related to the antioxidant and macronutrient content of wild fruits from seven fall-fruiting shrub species. Our specific objectives were to: (1) quantify total hydrophilic and lipophilic antioxidants, as well as two types of lipophilic antioxidants (i.e., carotenoids, and tocopherols) in wild fruits consumed by migrating birds, (2) test the hypothesis that antioxidant content of wild fruits is related to macronutrient composition, and (3) relate patterns of avian frugivory to antioxidant availability and macronutrient content of wild fruits during autumn migration.

METHODS

Study Area and Bird Species Composition.— Fieldwork was conducted on Block Island, Rhode Island (41° 13′ N, 71° 33′ W), an island located 15.5 km south of Rhode Island's coast and 22.5 km northeast of Long Island, New York, USA. The study site was located on the northeast portion of the island, within the Bay Rose and Clay Head preserves, where migratory birds are known to concentrate and fall-fruiting shrub species are abundant (Able 1977; Parrish 1997, 2000; Hammond 2002; Reinert et al. 2002; Comings 2005; Smith et al. 2007; Smith and McWilliams 2010). The site itself was 27 ha in size and was bounded by Corn Neck Road to the west, Mansion Road to the south, the coast to the east, and Kurz Road to the north. The majority of the site can be classified as maritime shrubland habitat with salt spray and wind disturbance as the primary determinants of plant species composition (Hammond 2002). Exposed areas were dominated by northern bayberry (*Myrica pensylvanica*), eastern poison ivy (*Toxico dendronradicans*), and downy goldenrod (*Solidago puberula*); whereas areas that were more sheltered supported arrowwood, chokeberry (*Aronia* sp.), and Virginia creeper (*Parthenocissus quinquefolia*). Two non-native species, Asiatic bittersweet and multiflora rose, were also abundant throughout the study site.

During fall migration, approximately 113 different bird species are known to stopover on Block Island (Reinert et al. 2002). The three most abundant bird taxa are warblers, mimic-thrushes, and New World sparrows. Within these taxa, the most common species include the Yellow-rumped Warbler (*Setophaga coronata*), Gray Catbird (*Dumetella carolinensis*), Golden-crowned Kinglet (*Regulus satrapa*), and Red-eyed Vireo (*Vireo olivaceus*) (Reinert et al. 2002). Average stopover length of songbirds on Block Island differs between species and has been estimated to range from a mean (± SD) of 2.57 ± 2.51 days in Golden-crowned Kinglets to 7.17 ± 6.42 days in Ovenbirds (*Seiurus aurocapilla*; Parrish 1997).

Fruit Collection and Consumption Data.—Avian fruit consumption was measured in a previous study (Bolser et al. 2013) from September–November 2009 at seven randomly chosen locations on Block Island. During this time, seven fall-fruiting shrub species were assessed for consumption rate and included: arrowwood, northern bayberry, Asiatic bittersweet, chokeberry, multiflora rose, Virginia creeper, and winterberry. Study locations were chosen by overlaying the study site with a numbered grid consisting of 300 × 300 m cells and randomly choosing cells within the grid. Locations were accepted if they contained at least one fruiting plant of each of the seven species within 150 m of the center of a selected grid cell. When a given location included more than one fruiting plant, we chose to sample fruits from the nearest plant to the central point that had a pair of branches with at least 25 fruits per branch. One branch on the selected plant was enclosed with thin-plastic mesh (0.5-cm grid) that prevented birds but not most invertebrates from accessing the fruits (more detailed description of these methods provided by Smith et al. 2007). A partner branch was marked and remained uncovered. Only nine terrestrial mammal species occur on Block Island, none of which are known to commonly consume fruits (Lang and Comings 2001). Thus, songbirds are the primary consumers of fruits on Block Island during fall, and the majority of these birds are stopping over during migration. The number of fruits on each marked branch was counted during the primary fall songbird migration beginning in early September and every 7–14 days thereafter for a total of five counts. The procedure involved repeated counting of the number of fruits on each branch until a consistent number was attained (Bolser et al. 2013).

We collected fruit samples at peak ripeness from the same seven plant species and locations during September–October 2011. Arrowwood, bayberry, and chokeberry were collected from early to mid September, winterberry and Virginia creeper were harvested from late September through early October, and bittersweet and multiflora rose were collected from mid through late October. All samples were stored at −80 °C until analysis.

Sample Preparation and Antioxidant Extraction.—Between 10–15 g of fruit were manually deseeded per sample and the resulting skin and pulp stored at −80 °C until analysis. Both total hydrophilic and total lipophilic antioxidants were extracted using procedures detailed by Thaipong et al. (2006). Briefly, total hydrophilic antioxidants were extracted by homogenizing 3 g fresh weight of deseeded skin and pulp in 25 mL of methanol using a Waring Blender (Waring Laboratory Science, Torring, CT) and allowing the homogenate to saponify for 12 hrs at 4 °C. Following saponification, the homogenate was spun at 6,825 RPM in a tabletop centrifuge (Allegra 21R, Beckman Coulter, Brea, CA) at 4 °C for 20 mins. The supernatant was then recovered and stored at −80 °C for later analysis. Total lipophilic antioxidants were extracted by homogenizing the leftover pellet with 20 mL of dichloromethane. The homogenate was then immediately centrifuged at 6,925 RPM at 4 °C for 20 mins, and the resulting supernatant was recovered and stored until analysis.

To extract tocopherols and carotenoids, fruit samples were weighed in 1.5 mL screw-cap microtubes to the nearest 0.0l mg and ground for 20 mins at 30 Hz using a ball mill (MM200, Retsch GmbH & Co. KG, Haan, Germany) in the presence of 1 mL hexane:methyltert butyl (1:1, v/v) and steel grinding balls (Toomey and McGraw 2010). The samples were then centrifuged for 3 mins at 10,000 RPM, after which time the supernatant was transferred to a fresh tube and evaporated to dryness under a stream of nitrogen.

Chemical Analysis of Extracts.—All of the fruit samples were assessed for concentrations of: (1) total hydrophilic antioxidants, (2) total lipophilic antioxidants, (3) composite antioxidants, (4) tocopherols, and (5) carotenoids. Various measures have been used to assess antioxidant status in biological samples, the advantages and disadvantages of which have been extensively reviewed and discussed (Prior and Cao 1999, Sanchez-Moreno 2002, Schlesier et al. 2002, Haung et al. 2005, Ozgen et al. 2006, Thaipong et al. 2006, Halliwell and Gutteridge 2007). We chose to use a modified DPPH (2,2-Diphenyl-1-picrylhydrazyl) spectrophotometric procedure, as detailed by Thaipong et al. (2006), to measure total hydrophilic and total lipophilic antioxidants and we further adjusted this protocol for analysis using 96-well microplates and a microplate spectrophotometer. Briefly, a DPPH stock solution was prepared by dissolving 0.024 g of DPPH in 50 mL of methanol and a working DPPH solution was subsequently prepared by adding 18 mL of the DPPH stock to 32 mL of methanol to yield an approximate absorbance of 1.5 at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a vitamin E analog, was used to construct a standard curve linear between 25 and 1,500 μM. 25 μL of either standard or sample were reacted with 475 μL of DPPH working solution under dark conditions for 24 hrs in 1.7 mL polypropylene microcentrifuge tubes. Following this 24-hr period, 300 μL of reacted sample or standard were pipetted into the wells of a 96-well microplate and the absorbance read at 515 nm in a microplate spectrophotometer (PowerWave 340, Bio-Tex, Winooski, VT). A standard curve was constructed by plotting the absorbance of the Trolox standards against their corresponding concentrations and the resulting regression equation was used to calculate the sample antioxidant concentrations. The DPPH protocol was performed twice for each fruit sample, once using the total hydrophilic extracts and once using the total lipophilic extracts, so that we could obtain separate measures for total hydrophilic and total lipophilic antioxidants. Composite antioxidant concentration was calculated by adding the total hydrophilic and total lipophilic antioxidant concentrations together for each sample. Total hydrophilic and lipophilic antioxidants were measured at the University of Rhode Island (Kingston, RI). All chemicals and reagents were purchased through Sigma-Aldrich Chemical Corporation (St. Louis, MO).

Carotenoids and tocopherols were analyzed at Arizona State University (Tempe, AZ) using spectrophotometric protocols as described by Toomey and McGraw (2010). Briefly, fruit extracts were resuspended in 1 mL hexane:MTBE (1:1, v/v) and transferred to a clean quartz cuvette for measurement with an absorbance spectrophotometer. Absorbance data were gathered at 450 nm for carotenoids and 301 nm for tocopherols. The extinction coefficients for calculating concentrations of carotenoids (Clotfelter et al. 2007) and tocopherols (Budvari 1989) were 2,550 and 3,270, respectively.

Statistical Analysis.—We assessed the Pearson's Product Moment Correlations between each of the five dependent variables (total hydrophilic antioxidants, total lipophilic antioxidants, composite antioxidants, tocopherols, and carotenoids) to investigate the relationships between the different classes of antioxidants. Total lipophilic

TABLE 1. Pearson's Product Moment Correlations for amounts of five different measures of antioxidants in fruits from seven fall-fruiting shrub species on Block Island, Rhode Island, in southern New England. Significant correlations are denoted by bolded text.

	Total hydrophilic	Total lipophilic	Composite antioxidants	Carotenoids
Total Lipophilic				
r =	−0.087	—	—	—
P =	0.58			
Composite Antioxidants				
r =	0.208	0.956	—	—
P =	0.18	**<0.0001**		
Carotenoids				
r =	0.177	−0.024	0.029	—
P =	0.26	0.88	0.86	
Tocopherols				
r =	−0.147	0.336	0.287	0.307
P =	0.34	**0.03**	**0.06**	**0.05**

and composite antioxidants exhibited an almost perfect correlation with one another (Table 1) suggesting that lipophilic antioxidants were responsible for driving composite antioxidant values. As such, we dropped composite antioxidants from the remaining statistical analyses. We constructed separate One-Way ANOVA models for each of the four remaining dependent variables to determine whether there were significant differences in antioxidants between fruit species. One-Way ANOVA models were also constructed to assess differences in three macronutrient measures (i.e., fat, carbohydrate, protein; Smith et al. 2007) between fruit species. Post-hoc Tukey's HSD Multiple Comparisons Analyses were performed to compare nutrient differences between each pair of fruit species for three antioxidant metrics (total lipophilic antioxidants, carotenoids, and tocopherols) and for the three macronutrient metrics. Total hydrophilic antioxidants were excluded from these comparisons as we did not find any overall significant differences between fruit species.

We performed an additional Pearson's Product Moment Correlations analysis to assess the relationship between macronutrient and antioxidant content of the seven plant species. We compared the average fat, protein, and carbohydrate content (from Smith et al. 2007) for each plant species to the average values of the four antioxidant metrics that we measured. To assess the relationship between avian fruit consumption, antioxidant content, and macronutrient content, we performed a Spearman's Rho Correlation analysis using ranked consumption data (from Bolser et al. 2013). The plant species with the lowest consumption index was ranked 1 and the plant species with the highest was ranked 7. For each antioxidant and macronutrient metric, we used the average rank for each plant species whenever there were non-significant differences between specific plant species as indicated by our multiple comparisons analyses. We did not include total hydrophilic antioxidants in this analysis because we found no overall differences in these antioxidants between plant species.

All statistical analyses were performed using SAS 9.2 statistical software (SAS Institute 2009) and results were considered significant at the $\alpha = 0.05$ level.

RESULTS

There was an exceptionally strong positive correlation between total lipophilic and composite antioxidants and moderate, but significant, positive correlations between total lipophilic antioxidants and tocopherols and between tocopherols and carotenoids (Table 1). The results of our One-Way ANOVA models indicated that there were significant differences in lipophilic antioxidants ($F_{6,36} = 32.65$, $P < 0.001$; Fig. 1A), carotenoids ($F_{6,36} = 33.15$, $P < 0.001$; Fig. 1B), and tocopherols ($F_{3,36} = 6.86$, $P < 0.001$; Fig. 1B) in fruits from the seven shrub species and no significant differences in total hydrophilic antioxidant content ($F_{6,36} = 1.71$, $P = 0.15$; Fig. 1B).

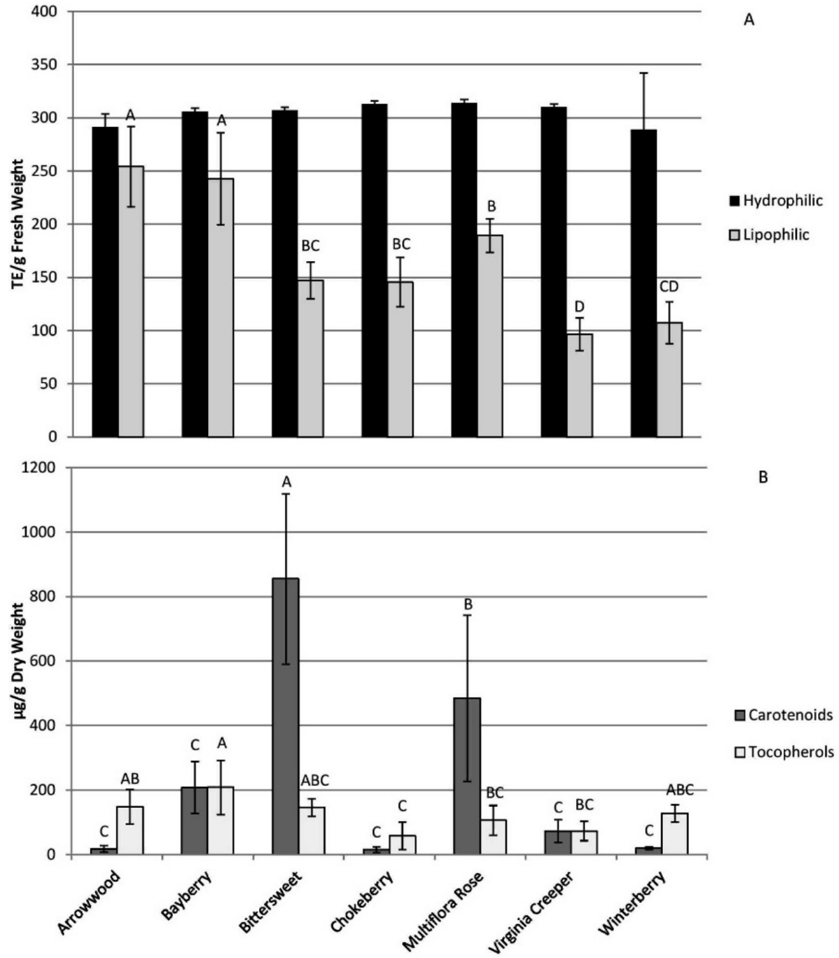

FIG. 1. (A) Mean total hydrophilic and total lipophilic antioxidant content (± SD) in fruits from seven fall-fruiting shrub species on Block Island, Rhode Island, an important fall migration stopover site: arrowwood, northern bayberry, Asiatic bittersweet, chokeberry, multiflora rose, Virginia creeper, and winterberry. Differences in letters above bars indicate significant differences ($P < 0.05$) between species for total lipophilic antioxidants. Total hydrophilic antioxidants were not significantly different among species. (B) Mean carotenoid and tocopherol content (± SD) in fruits from the same seven plant species. Differences in letters above bars indicate significant differences ($P < 0.05$) between species for a given antioxidant.

Total lipophilic antioxidants were highest in fruits from arrowwood and bayberry, moderately high in multiflora rose, bittersweet, chokeberry, and winterberry, and lowest in Virginia creeper (Fig. 1A). Carotenoids were much higher in fruits from bittersweet and multiflora rose than in any of the other shrub species, whereas tocopherols were highest in fruits from bayberry and arrowwood, moderately high in bittersweet, winterberry, multiflora rose, and Virginia creeper, and lowest in chokeberry (Fig. 1B).

We found no significant correlations between any of our fruit antioxidant metrics and macronutrient content except for a fairly strong positive relationship between protein and carotenoids in fruits (Table 2). Consumption rank was negatively correlated with carotenoid rank (carotenoids: $r_s = -0.76$, $P = 0.05$) but was not correlated with either of the other ranked antioxidant metrics (total lipophilic antioxidants: $r_s = 0.036$, $P = 0.94$; tocopherols: $r_s = 0.36$, $P = 0.42$) or with ranked macronutrient content (fat: $r_s = 0.611$, P

TABLE 2. Pearson's Product Moment Correlations for antioxidant and macronutrient content of fruits from seven fall-fruiting shrub species on Block Island, Rhode Island, in southern New England. Significant correlations are denoted by bolded text.

Type of antioxidant	Type of macronutrient		
	Fat	Protein	Carbohydrate
Total Hydrophilic			
r =	−0.260	0.505	0.250
P =	0.57	0.25	0.59
Total Lipophilic			
r =	0.677	−0.393	−0.688
P =	0.09	0.38	0.09
Carotenoids			
r =	−0.337	0.840	0.327
P =	0.46	**0.02**	0.47
Tocopherols			
r =	0.632	−0.171	−0.632
P =	0.13	0.71	0.13

= 0.15; protein: $r_s = -0.655$, $P = 0.11$; carbohydrate: $r_s = -0.611$, $P = 0.15$) (Fig. 2).

DISCUSSION

Antioxidant and Macronutrient Content of Wild Fruits.—Antioxidants in fruits are secondary metabolites that plants may synthesize to protect themselves against the oxidative stress brought about by lipid metabolism (Catoni et al. 2008a). Specifically, lipophilic antioxidants extracted from plants (e.g., tocopherols) are especially potent in preventing oxidation in natural and processed foods (Decker 1998a, b; Frankel 1996; Reisch et al. 1998; McClements and Decker 2000). As such, plants that produce high-fat fruits may allocate more lipophilic antioxidants to these fruits to protect against oxidative damage. Consistent with this hypothesis, arrowwood and bayberry fruits had the highest fat content of the seven plant species (Smith et al. 2007) and they contained the highest concentrations of total lipophilic antioxidants (Fig. 1A). In contrast, Virginia creeper fruits were also high in fat, yet they contained some of the lowest concentrations of total lipophilic antioxidants (Fig. 1A). This is especially surprising considering that Virginia creeper fruits are comprised primarily of 18:2 polyunsaturated fatty acids (PUFA; Boyles 2011) (fatty acid nomenclature = C:D, where C = the number of carbons and D = the number of double bonds), which are much more susceptible to oxidative attack than either monounsaturated fatty acids (MUFA) or saturated fatty acids (Klasing 1998, Hulbert 2007). Arrowwood fruits are comprised mostly of 16:1 and 18:1 MUFA and bayberry fruits contain mostly 16:0 and 18:0 saturated fatty acids (Boyles 2011). As such, the benefit of fruit antioxidants for these plants may be related more to the quantity rather than the composition of the fats in these fruits. In general, antioxidant protection may not always be the main function of lipophilic antioxidants in fruits. For example, secondary metabolites (e.g., antioxidants) may function as attractants for seed dispersers by signaling a nutritional reward (Cipollini and Levey 1997a). In this case, arrowwood and bayberry shrubs might produce high-antioxidant fruits with more anthocyanins (Bolser et al. 2013) as a strategy by which to attract migratory songbirds that require dietary antioxidants to meet their nutritional needs during stopover. In addition, all of the fruits we studied contained high levels of total hydrophilic antioxidants, which may be sufficiently effective at mitigating oxidative damage without the additional protection of lipophilic antioxidants. In fact, Bolser et al. (2013) measured hydrophilic antioxidants in fruits from the same seven shrub-species and found that Virginia creeper fruits had among the highest phenolic content.

We also detected significant differences in carotenoids between the seven fall-fruiting shrubs we studied. Bittersweet and multiflora rose fruits had exceptionally high levels of carotenoids in comparison to all of the other shrub species (Fig. 1B) and also contained the highest protein concentrations (Table 2) (Smith et al. 2007). Virginia creeper fruits contained very few carotenoids (Fig. 1B), but they were also high in protein (Smith et al. 2007). Both bittersweet and multiflora rose fruits are bright red in color when ripe and this hue is well known to be associated with the presence of carotenoids, such as canthaxanthin (Bartley and Scolnik 1995, Hill 2000). Interestingly, winterberry also produces bright red fruits but had relatively low amounts of carotenoids. In general, little evidence has been found in support of a direct association between fruit color and nutrient content (Willson and Whelan 1990, Schaefer et al. 2008), though birds have been shown to exhibit a foraging preference for both red-hued food sources (Stockton-Shields 1997) and purple-hued food sources (Zhang et al.

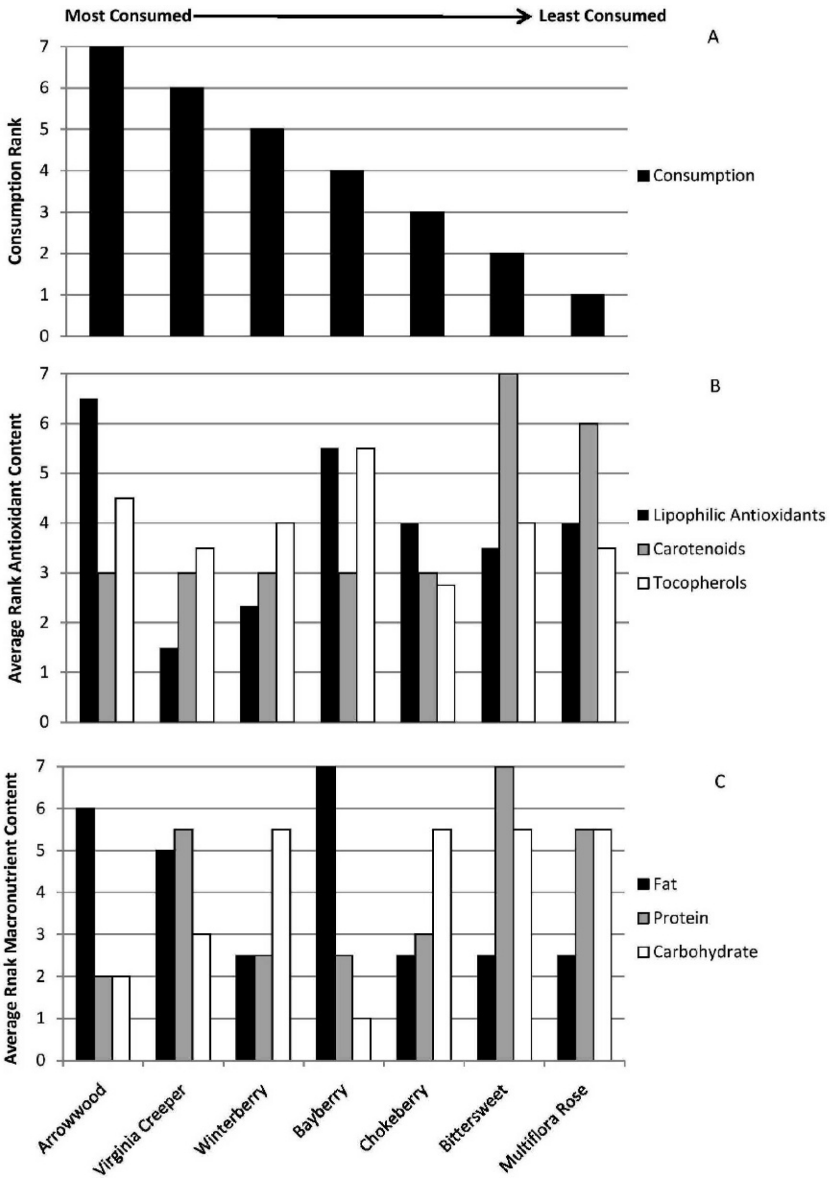

FIG. 2. (A) Rank order in which songbirds during fall migration on Block Island, Rhode Island, consumed fruits from seven fall-fruiting shrub species including: arrowwood, Virginia creeper, winterberry, northern bayberry, chokeberry, Asiatic bittersweet, and multiflora rose. Note that a higher rank indicates higher consumption (e.g., arrowwood was consumed most commonly of the seven species). (B) Mean rank antioxidant content including total lipophilic antioxidants, carotenoids, and tocopherols in fruits from the seven shrub species. Total hydrophilic antioxidants were excluded because they were not significantly different between fruit species. (C) Mean rank macronutrient content including fat, protein, and carbohydrate in fruits from the seven shrub species.

2012). In addition, low-quality fruits may mimic the color of high-quality fruits as a way to attract seed dispersers without investing energy in producing high-cost fruits (Cipollini and Levey 1997a).

The pattern of tocopherol abundance in fruits was similar to that of total lipophilic antioxidants but different than that of carotenoids, the other specific class of lipophilic antioxidants that we measured. Specifically, tocopherols were highest in fruits from both bayberry and arrowwood, moderate in bittersweet, winterberry, multiflora rose, and Virginia creeper and lowest in chokeberry (Fig. 2–1B). The high fat content of bayberry and arrowwood fruits may contribute to high concentrations of lipophilic antioxidants, such as tocopherols, in their pulp. α-tocopherol is well-documented as a potent antioxidant for improving immune function and mitigating oxidative damage in broiler chickens (Marusich et al. 1975, Boa-Amponsem et al. 2000, Leshchinsky and Klasing 2001, Surai 2002) and may provide similar benefits to free-living birds. If wild songbirds require such antioxidants to mitigate oxidative damage during migration, it is possible that the tocopherols in wild fruits may act as attractants for seed dispersers.

Total hydrophilic antioxidants were not different between fruit species and this finding is in disagreement with the findings of a similar study that found significantly higher levels of two specific hydrophilic antioxidants, phenolics and anthocyanins, in arrowwood fruits than in fruits from other shrub species on Block Island (Bolser et al. 2013). This apparent contradiction likely reflects the fact that our measure of hydrophilic antioxidants was more general than those used by Bolser et al. (2013). Songbirds differentiate between diets that differ in anthocyanins in both controlled studies (Schaefer et al. 2008) and in field studies (Bolser et al. 2013), suggesting that the amounts of specific types of hydrophilic antioxidants (e.g., anthocyanins, phenolics, flavonoids) in fruits and other foods may be more relevant than total hydrophilic antioxidant content alone.

In assessing the relationships between different classes of antioxidants, we expected to find significant correlations between total lipophilic antioxidants, carotenoids, and tocopherols given that the latter two are a subset of the former. We found significant positive relationships between total lipophilic antioxidants and tocopherols and between tocopherols and carotenoids (Table 1); however, no such relationship was evident between total lipophilic antioxidants and carotenoids. The two fruits highest in carotenoid content, bittersweet and multiflora rose, only contained moderate amounts of total lipophilic antioxidants and the two fruits with the highest total lipophilic antioxidants, arrowwood and bittersweet, contained some of the lowest carotenoid concentrations. Of the fruits that had the highest total lipophilic antioxidants, tocopherols made up the majority of those lipophilic antioxidants, whereas carotenoids were fairly minor contributors (Fig. 1). This may indicate that tocopherols are the more important lipophilic antioxidants during autumn migration, both for plants and their consumers, at least in these fall-fruiting shrub species in southern New England.

The relative amounts of antioxidants that we found in wild fruits in southern New England were similar to those reported in the few other studies that have quantified the antioxidant content of commercial and wild fruits. Wu et al. (2004) quantified the total hydrophilic and total lipophilic antioxidant capacities of common United States foods and found that, of the fruits they measured, Haas avocado (*Persea americana*), a high fat fruit, had exceptionally high amounts of lipophilic antioxidants. Other fruits with high concentrations of lipophilic antioxidants included cranberry (*Vaccinium macrocarpon*), raspberry (*Rubus* spp.), and shrubby blackberry (*Rubus fruticosus*), though these levels were considerably lower than those of avocado. Cranberry and blackberry fruits also had very high concentrations of hydrophilic antioxidants. Schaefer et al. (2008) measured carotenoids in fruits commonly consumed by birds and found that fruits from *Streptopus amplexifolius, Polygonatum multiflorum, Tamus communis,* and *Rosa glauca* contained the highest amounts. They also found low levels of carotenoids in fruits from Virginia creeper, which is in agreement with our results. Lastly, Chun et al. (2006) measured total tocopherol content in raw and processed fruits and vegetables and found that green olives (*Olea europaea*), which are high in fat, contained the most tocopherols followed closely by blackberry and raspberry. The findings of these studies, along with our own, support the hypotheses that: (1) high fat fruits often also contain high levels of lipophilic antioxidants, though there are many exceptions, and (2) many fruits are good sources of both hydrophilic and lipophilic antioxidants. Our results demonstrate for the first time that the relationships between the amounts of different types of antioxidants and macronutrients are complex and that consumers cannot eat only one or a few fruit types to satisfy their nutritional requirements.

Antioxidants and Fruit Consumption.—We expected birds to consume fruits with higher antioxidant content at a higher rate than fruits with lower amounts of antioxidants since these dietary antioxidants may act to mitigate the negative effects of oxidative stress (Garcia-Alonso et al. 2004; Catoni et al. 2008a, b; Costantini 2008, Monaghan et al. 2009). We found that the most highly consumed fruit species, arrowwood, also had among the highest fat content, highest total lipophilic antioxidants, and highest tocopherols of the seven shrub species (Fig. 2). The second most consumed species, Virginia creeper, also had high fat content (Smith et al. 2007). Interestingly, the least consumed species (chokeberry, bittersweet, and multiflora rose) were lowest in fat, highest in carbohydrate, and among the lowest in both total lipophilic antioxidants and tocopherols (Fig. 2). There was also a significant negative relationship between fruit consumption and carotenoid content (Table 2), because two of the least consumed fruits, bittersweet and multiflora rose, were exceptionally high in carotenoids. Other studies of birds have suggested that the antioxidant benefits derived from carotenoid consumption are meager (Costantini and Møller 2008, Simons et al. 2012) and thus support the hypothesis that other types of antioxidants, such as tocopherols, are more important for protecting against oxidative damage. Bolser et al. (2013) measured two specific types of hydrophilic antioxidants, phenolics and anthocyanins, in fruits from these same seven fall-fruiting species on Block Island and found that arrowwood fruits had the highest amounts. Interestingly, recent studies have suggested that the absorption of dietary antioxidants may be improved by concomitant consumption of dietary fat (Pietta 2000, Prior 2003). This may explain why high-fat fruits with high antioxidant content may be preferentially consumed over fruits with less fat and low antioxidant content. The trends we observed suggest that birds on Block Island during fall migration select high-fat fruits and might also select fruits based on high antioxidant content in order to meet their nutritional needs during stopover.

Multiple factors influence patterns of avian fruit selection and this likely explains why we found no significant correlation between consumption rank and the relative amounts of antioxidants that we measured in our study. Studies of both wild and captive songbirds have shown that fruit preference can be influenced by fat content (Stiles 1993, Fuentes 1994), sugar content (Levey 1987, Lepczyk et al. 2000), specific amino acids (Parrish and Martin 1977), fatty acids (McWilliams et al. 2002, Pierce et al. 2004), fruit color (Willson et al. 1990, Puckey et al. 1996), and pulp-to-seed ratio (Sorenson 1984, Izhaki 1992, Murray et al. 1993, Stanley and Lill 2002). In addition, birds may diversify their diet to avoid consuming toxic levels of particular secondary chemical compounds (Barnea et al. 1993; Cipollini and Levey 1997a, b; Levey and Cipollini 1998; Schaefer et al. 2003). Abundance and distribution of plant species in the environment may also contribute to the fruit selection patterns of wild birds (Baird 1980, Moermond and Denslow 1983, Sargent 1990, Whelan and Willson 1994). For example, arrowwood is one of the most abundant fruiting plant species on Block Island while other fruits, such as Virginia creeper, are much less abundant, not nearly as widely distributed, and are thus less available to birds during stopover (Smith et al. 2007). Unlike controlled laboratory or captive bird studies where single factors can be manipulated, field studies such as ours can, at best, detect general trends in bird biology and behavior. Though multiple factors may influence patterns of avian frugivory, the results of our study demonstrate that there are significant differences in antioxidant content between fruit species and, therefore, birds may be capable of altering the amounts and types of dietary antioxidants they consume based on the fruits they select. The trends we observed suggest that birds on Block Island during fall migration tend to select high-fat fruits, such as arrowwood, and that these fruits also contain high levels of total lipophilic antioxidants and tocopherols, in particular. Given these trends, it is possible that lipophilic antioxidants, particularly tocopherols, are especially important for birds and may influence patterns of frugivory at stopover during autumn migration.

CONCLUSIONS

The results of our study provide novel insights into the specific antioxidant content of wild fruits that are commonly consumed by free-living migratory songbirds during autumn migration. Our study is the first to measure total lipophilic antioxidants and total hydrophilic antioxidants in wild fruits on Block Island and to relate antioxidant content to both macronutrient composition and fruit consumption by free-living birds. Future research should investigate the food preferences of captive birds for specific macronutrients and antioxidants (e.g., tocopherols and carotenoids) using controlled diet choice trials. Such studies should supplement semi-synthetic diets with high levels of these antioxidants and vary macronutrient content to determine how different antioxidant/macronutrient combinations interact to influence the dietary preferences of captive birds. Understanding the nutritional requirements of songbirds during autumn migration is complex but essential for the effective management of bird habitats at stopover. Better knowledge about wild songbird nutrition and feeding behavior will allow conservationists to manage important stopover sites for preferred fruit species and will ultimately increase the value of such sites to the migrants that use them.

ACKNOWLEDGMENTS

We would like to thank the Block Island Division of the Nature Conservancy, and especially Scott Comings, for facilitating our field research. We would also like to thank all those who assisted both in the field and in the laboratory for their invaluable help collecting and analyzing fruit samples including: Marie Alan, Megan Skrip, Jacqueline Hall, and the students of the McGraw Lab at Arizona State University. Funding for this research was provided by the National Science Foundation (IBN-9984920, IOS-0748349) and the Rhode Island Agricultural Experiment Station, U.S. Department of Agriculture (538748). This is contribution #5330 from the University of Rhode Island Agricultural Experiment Station.

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