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BIRDS SELECT FRUITS WITH MORE ANTHOCYANINS AND PHENOLIC COMPOUNDS DURING AUTUMN MIGRATION

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ABSTRACT.—We evaluated whether fruit selection by autumn-migrating birds at an important stopover site in southern New England was related to water-soluble antioxidant content of fruits. We measured total anthocyanins, total phenolics, and total antioxidant capacity in fruits from common native and non-native plant species and related this to estimates of fruit selection by free-living birds. Birds selected certain fruits over others, with arrowwood (*Viburnum recognitum*, *V. dentatum*) consumed at the highest rate, followed by Virginia creeper (*Parthenocissus quinquefolia*) with much lower consumption of other fruits (e.g., oriental bittersweet [*Celastrus orbiculatus*], multiflora rose [*Rosa multiflora*], winterberry [*Ilex verticillata*]). Antioxidant concentrations primarily differed by shrub species and less so between sites. Arrowwood spp. had the highest total antioxidants, followed by Virginia creeper, northern bayberry (*Myrica pennsylvanica*), chokeberry spp. (*Aronia prunifolia, A. melanocarpa*), multiflora rose, winterberry, and oriental bittersweet. These results are consistent with the hypothesis that free-living birds select fruits based, in part, on antioxidant content. We suggest birds may actively select polyphenol/anthocyanin-rich fruits during autumn migration to protect themselves against the potentially damaging effects of oxidative stress caused by long-distance fasting flight. *Received 4 April 2012. Accepted 29 June 2012.*

Key words: anthocyanin, Block Island, fall migration, fruit antioxidants, fruit selection, *Viburnum*.

All aerobic organisms produce reactive oxygen species (ROS) as unavoidable byproducts of respiration and animals have evolved several mechanisms for coping with this oxidative burden (Finkel and Holbrook 2000, Pamplona and Costantini 2011). Birds, in particular, are exposed to especially high levels of oxidative stress as a direct consequence of long-distance flight (Costantini et al. 2007). One way migratory birds might mitigate oxidative stress is through consumption of dietary antioxidants. Many birds that are primarily insectivorous during the breeding season switch to eating seasonally-abundant fruits during autumn migration. Many of these fruits are excellent sources of dietary antioxidants and may provide an important defense against oxidative stress for both fruiting plants and their consumers (Garcia-Alonso et al. 2004, Smith et al. 2007b, Catoni et al. 2008). Most antioxidants in fruits are secondary metabolites that include flavonoids and allied phenolic and polyphenolic compounds (Crozier et al. 2006). These compounds derived from diet may reduce oxidative damage and are referred to as 'dietary antioxidants,' although these compounds often have many other physiological functions and their antioxidant benefits to date are usually only measured *in vitro* (Halliwell and Gutteridge 2007). Wu and others (2004) analyzed phenolic content, specifically anthocyanins, of 100 plant-based foods, and found the three foods containing the highest levels were all fruits: chokeberry (*Aronia melanocarpa*), American elder (*Sambucus canadensis*), and wild blueberry (*Vaccinium corymbosum*). High antioxidant activity measured *in vitro* was also observed in other fruits from the genera *Rubus, Ribes*, and *Aronia* (Benvenuti et al. 2004). These studies provide insight into the antioxidant content of specific fruits but do not address how antioxidant composition might explain patterns of fruit selection by birds.

Many factors influence patterns of avian frugivory (Snow 1970, Stiles 1980, Levey and Martinez del Rio 2001) including macronutrient and mineral composition (Herrera 1987, Smith et al. 2007b), fruit and seed size and shape (Herrera 1984, Witmer and Van Soest 1998), fruit abundance and spatial arrangement (Denslow 1987, Sargent 1990, Carlo and Morales 2008), and anti-nutrient secondary metabolites (Cipollini and Levey 1997a, b; Levey and Cipollini 1998; Struempf et al. 1999; Tsahar et al. 2002; Schaefer et al. 2003). However, the influence of antioxidant levels on fruit preference has only recently

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received attention from ornithologists. Schaefer and others (2008) measured anthocyanin and carotenoid content in 100 fruit species and found that Eurasian Blackcaps (*Sylvia atricapilla*) preferred artificial diets supplemented with anthocyanins over foods without these supplements. Catoni and others (2008) showed, in behavioral choice trials, that Eurasian Blackcaps preferred food with flavonoids over that without flavonoids. Senar and others (2010), in a similar diet preference trial, artificially enriched mealworms with carotenoids and found Great Tits (*Parus major*) consistently chose carotenoid-enriched mealworms over non-enriched mealworms. This research suggests birds prefer food supplemented with antioxidants (Schaefer et al. 2008, Catoni et al. 2008), and birds are able to detect dietary antioxidants without visual signals (Senar et al. 2010); however, the positive effects of dietary antioxidants on oxidative stress have not yet been demonstrated.

No previous studies have examined the extent free-living migratory birds select wild fruits containing higher levels of antioxidants. Assessments of fruit 'selection' by free-living birds are complimentary to studies of diet or fruit 'preference.' Diet 'preference' is evident when birds consume more of certain diets when given equal access to alternative diets. Diet 'selection' refers to when free-living birds consume more of certain foods when availability of alternative diet choices may be quite different (Frazer and McWilliams 2002). Diet 'selection' is a function of the interaction between diet preference and availability of alternative diet choices. Our objectives were to: (1) examine relative patterns of avian fruit consumption of seven fall-fruiting shrub species during autumn migration, (2) qualitatively relate patterns of avian fruit selection to availability of antioxidants in fruits, and (3) examine island-scale spatial variation in antioxidant concentration of fruits for a select subset of fall-fruiting shrub species.

METHODS

Study Area.—All fieldwork was conducted on Block Island, Rhode Island (41° 13′ N, 71° 33′ W), a 2,900-ha, teardrop-shaped island 15.5 km south of the Rhode Island coast and 22.5 km northeast of Long Island, New York. Migrating passerines, especially hatch-year birds, fly south to the southern New England coast and are often pushed out to sea by northwest winds associated with passing cold fronts during autumn migration (Ralph 1981). Coastal locations, such as Block Island, are especially important stopover sites for many of these birds (Able 1977). The main study site was in the northeast portion of the island within the Bay Rose and Clay Head preserves. Previous research has shown this is an area where migratory birds concentrate on Block Island and it also supports numerous fall-fruiting shrub species (Able 1977; Parrish 1997, 2000; Hammond 2002; Reinert et al. 2002; Smith et al. 2007b; Smith and McWilliams 2010). The boundaries of the 27-ha site were arbitrarily chosen: Corn Neck Road to the west, Mansion Road to the south, the coast to the east, and Kurz Road to the north.

A variety of habitat types occur in southern New England, but most of the study site can be classified as a maritime shrubland habitat (Hammond 2002). Salt spray and wind disturbance are important determinants of the plant species composition. Shorter-statured northern bayberry (*Myrica pennsylvanica*), poison ivy (*Toxicodendron radicans*), and downy goldenrod (*Solidago puberula*) dominate the more exposed areas, whereas northern arrowwood (*Viburnum recognitum*), southern arrowwood (*V. dentatum*), purple chokeberry (*Aronia prunifolia*), black chokeberry (*A. melanocarpa*), winterberry (*Ilex verticillata*), and Virginia creeper (*Parthenocissus quinquefolia*) are common in areas with more protection. Two non-native species, oriental bittersweet (*Celastrus orbiculatus*) and multiflora rose (*Rosa multiflora*), are also common throughout this site.

Avian Fruit Consumption.—The fruit consumption study was conducted at seven randomly-chosen locations within the main study site on Block Island. The 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 seven species within 150 m of the center of a selected grid cell. The seven species included: northern bayberry, arrowwood spp., winterberry, chokeberry spp., Virginia creeper, oriental bittersweet, and multiflora rose. Identification of plant species was based upon criteria from multiple sources (Symonds 1963, Petrides 1988, USDA 2008). We selected the nearest plant from the central point that had a pair of branches with at least 25 fruits per branch if there was more than one fruiting plant of a given species at a location. One branch on the selected plant was covered with plastic mesh (0.5-cm grid), following Smith et al. (2007b), that prevented access to birds but not most invertebrates. 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.

We counted the number of fruits on each marked branch 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. We assessed the percent ripeness of fruits during each count by visually estimating the percentage of fruits on each branch that were unripe, ripe, and over-ripe so the sum was 100%. The mesh net was carefully removed from the netted branch prior to each count for northern bayberry and replaced afterwards because we were unable to accurately count these clustered fruits through the net.

Fruit Collection.—Fruit samples were collected from the same seven shrub species and locations. Fruit was harvested during peak ripeness for each species (early Sep for chokeberry spp., arrowwood spp., and northern bayberry; early Oct for winterberry and Virginia creeper; and early Nov for oriental bittersweet and multiflora rose). Ripe fruits from three plant species that were widely available on Block Island (chokeberry spp., southern arrowwood, and northern bayberry) were also collected within maritime shrubland habitat at two additional locations on the southern portion of the island to examine spatial variation in fruit composition on an island-scale. All collected fruit samples were kept frozen on Block Island until they were transported to the University of Rhode Island where they were stored at −80 °C until chemical analysis.

Sample Preparation.—Preparation of the frozen fruit for analysis involved manually de-seeding 10–15 g of wet fruit from each sample with the exception of chokeberry spp. and multiflora rose which were analyzed whole. These fruits contain hundreds of small seeds that are not easily extracted from the fruit pulp. Fruit was then freeze-dried, weighed, and homogenized using a Wiley mill. Each dried/ground sample was divided into triplicate sub-samples that were analyzed separately to obtain an estimate of within-sample variation in chemical composition. Aqueous extracts from each fruit subsample were obtained using a double methanol wash method (Garcia-Alonso et al. 2004) in 10 ml of acidified (0.1% HCl) methanol collected into a glass vial. Samples of northern and southern arrowwood fruits were washed a third time because, unlike the other fruit species, the supernatant from the second wash was not visibly lighter than the first. We removed the solvent from the supernatant under a stream of nitrogen gas and under vacuum. Dry mass of the sample was recorded and the extract was frozen at −80 °C until chemical analysis.

Chemical Analysis.—A variety of measures has 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, Halliwell and Gutteridge 2007). The chemical analysis of the aqueous extracts in our study included a spectrophotometric assay (DPPH) that measures total antioxidant capacity of the sample (Ozgen et al. 2006, Seeram et al. 2008). The DPPH assay involves dissolving a sample in dimethyl sulfoxide and measuring its ability to reduce 50% of the DPPH radical (EC_{50}) based on standard calibration curves (Brand-Williams et al. 1995, Molyneux 2004). High DPPH values indicate only a small quantity of the radical has been reduced while lower DPPH values indicate a higher amount of radical reduction. The extract from a fruit with a higher total antioxidant capacity, and a higher ability to quench free radicals, will exhibit lower DPPH values. Total phenolics (TPH) in the fruit were measured spectrophotometrically following the widely-used Folin-Ciocalteau method (Singleton et al. 1999); total monomericanthocyanins (TA) in the samples were measured with the pH-differential method (Giusti and Wrolstad 2001). All assays were conducted in duplicate or triplicate and were repeated when the coefficient of variation between samples was >15%.

Statistical Analysis.—We constructed a log-linear (Poisson) model to compare numbers of fruits on a branch by species and treatment (branch enclosed or open) in SAS (GENMOD procedure; SAS Institute 2009). We inflated standard errors of parameter estimates with a dispersion parameter (i.e., Pearson's Chi-square statistic divided by the degrees of freedom) to account for over-dispersion in the data.

We calculated two metrics to evaluate the fruit preferences of birds: percentage of remaining fruit on each enclosed and open branch at each count period for each plant species, and a consumption index (CI) (Smith et al. 2007a) for each pair of branches on the same plant at each count period, where CI = 1 − (% remaining on open branch/% remaining on enclosed branch). These dependent variables did not conform to the assumptions of normality, even when transformed. Thus, we converted them to rank values to evaluate differences using repeated-measures ANOVA models with three main effects (species, sampling site, and count period). We also considered two interaction terms (species*count period, sampling site*count period). Sampling site was used as a fixed effect in the model to assess regional-spatial patterns in bird fruit preferences. We used the best-fitting covariance structure to account for multiple observations from the same individuals using second-order Akaike's Information Criterion (AIC_c) values (Burnham and Anderson 2002); a compound symmetric covariance structure was the best fit for all models. Non-significant interactions were removed from the full model. We report the least squares mean rank estimates of the response variables in the event of a significant count period*species interaction.

The three measures of antioxidant composition (TA, TPH, and DPPH) did not conform to the assumptions of normality even when transformed, and we converted TA, TPH, and DPPH to rank values (ties averaged) to examine correlations among the variables using Spearman's rho. All three variables were significantly correlated, and we used the Vegan package (Oksanen et al. 2011: R package Version 2.0-2) in R (R Core Development Team 2011: R Version 2.14.1) to examine multivariate differences in the three antioxidant variables among plant species, nested within sites. We performed a permutational multivariate analysis of variance (MANOVA; McArdle and Anderson 2001) of raw (untransformed) antioxidant values. Differences among species were evaluated in the permutational MANOVA with an F-test based on 5,000 permutations.

We evaluated regional differences (northern vs. southern Block Island) in antioxidant values for three species (arrowwood spp., chokeberry spp., and northern bayberry) using a permutational MANOVA. The initial model contained species, region (north or south), and the species*region effects. We also related the consumption index with the three antioxidant variables in another permutational MANOVA to investigate the relationship between fruit antioxidant composition and consumption by birds. We used the CI estimate from the count period when the fruit sample was collected; however, chokeberry spp. and arrowwood spp. were collected at the initial count period when it was not possible to calculate the CI, and we used the CI estimate for the second count period for these two species. We ran an additional permutational MANOVA model for CI that omitted DPPH to ascertain the specific association of total anthocyanins and total phenolics with fruit selection by avian consumers.

We considered effects significant at $\alpha = 0.05$. We used R (R Core Development Team 2011) to test for normality, correlation, and for the permutational MANOVA models. All other statistical analyses were conducted using SAS 9.2 (SAS Institute 2009).

RESULTS

Avian Patterns of Fruit Consumption.—The initial number of fruits on open and enclosed branches was not significantly different within a species ($\chi^2 = 0.87$, df = 1, $P = 0.35$), although there were large differences among species in the initial number of fruits per branch ($\chi^2 = 4.70$, df = 6, $P < 0.001$). Plant species differed in fruit phenology as indicated by both the rate of natural abscission and percent of ripe fruit at each count period (Fig. 1). Percent of fruit remaining on the enclosed branches decreased during the six count periods indicating regular loss from natural abscission that was consistent among sampling sites (count: $F_{5,205} = 437.25$, $P < 0.001$; site: $F_{6,35} = 0.40$, $P = 0.87$; Fig. 1). Plant species differed in the rate of natural fruit loss (species: $F_{6,35} = 13.43$, $P < 0.001$; species*count: $F_{30,205} = 7.83$, $P < 0.001$). Early-fruiting species, such as chokeberry spp., had higher abscission rates during the first few count periods, whereas later-ripening species, such as winterberry, had higher abscission rates during later count periods.

The percent of remaining fruit on the open branches accessible to birds rapidly decreased during the six count periods and this pattern was consistent among sampling sites (count: $F_{5,205} = 358.42$, $P < 0.001$; site: $F_{6,35} = 0.90$, $P = 0.51$). Plant species differed in the rate of fruit decline (species: $F_{6,35} = 21.48$, $P < 0.001$; species*count: $F_{30,205} = 7.33$, $P < 0.001$) indicating that birds were selectively consuming specific species,

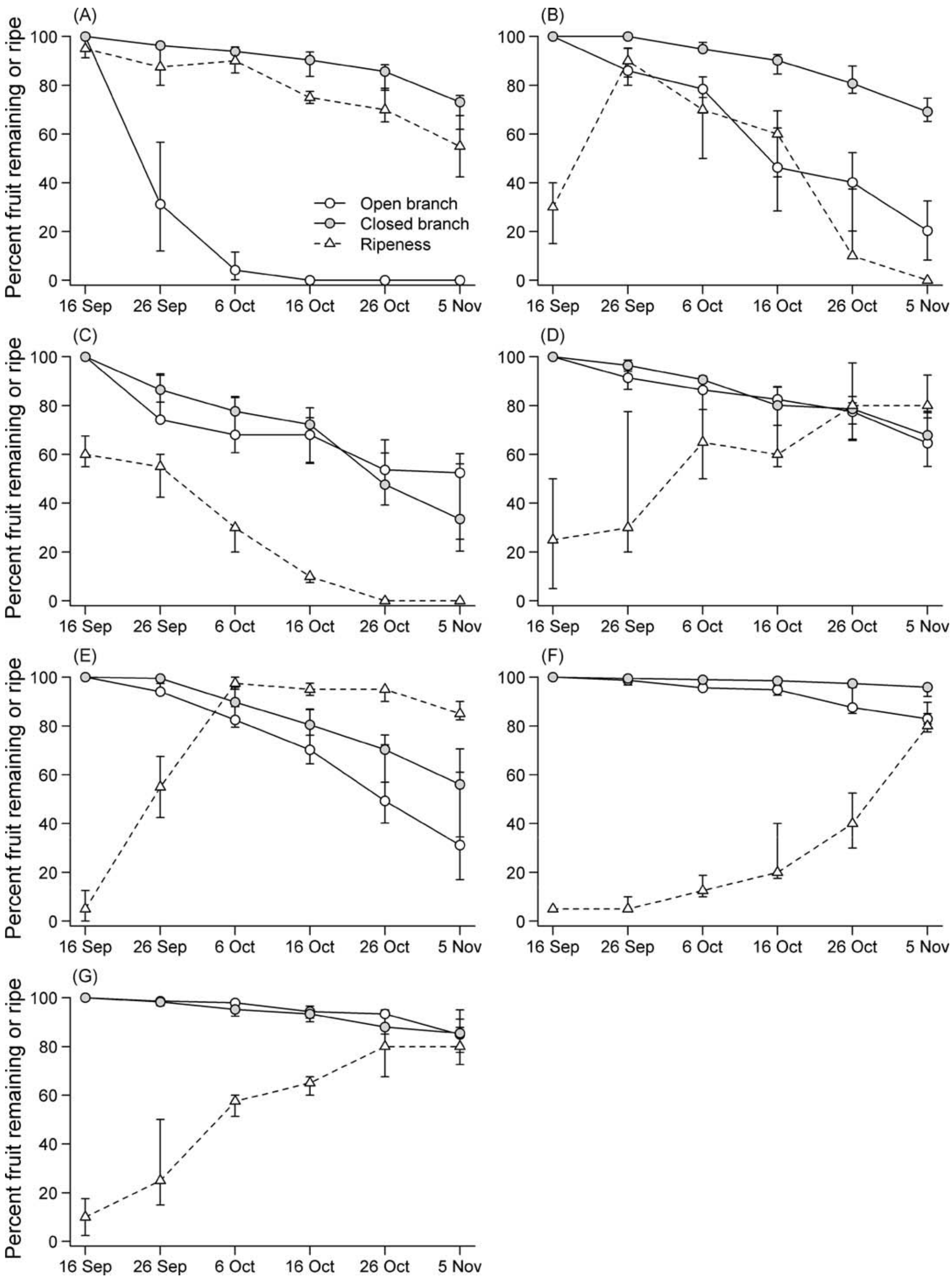

FIG. 1. Median percent of fruit remaining on open and closed branches and fruit ripeness over six count-periods during autumn 2008 on Block Island, Rhode Island; the interquartile range is indicated by whiskers. Species, sorted approximately from increasing to decreasing consumption, include (A) arrowwood spp., (B) Virginia creeper, (C) chokeberry spp., (D) northern bayberry, (E) winterberry, (F) oriental bittersweet, and (G) multiflora rose.

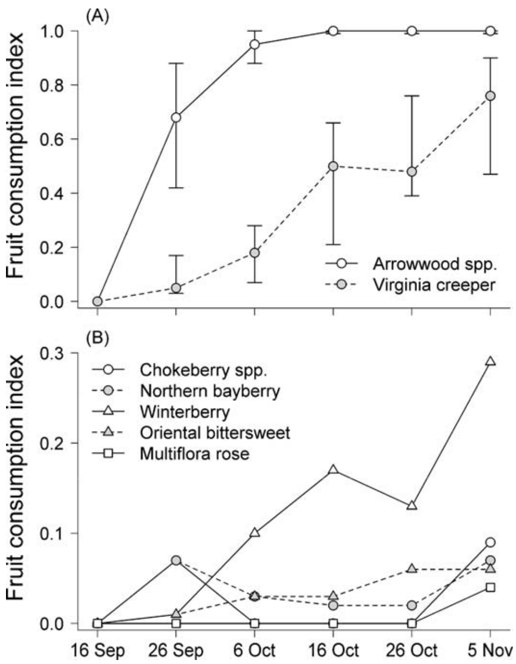

FIG. 2. Median fruit consumption index for seven species of fruiting shrubs over six count-periods during autumn 2008 on Block Island, Rhode Island; the interquartile range is indicated by whiskers. (A) Two species (arrowwood spp. and Virginia creeper) were consumed more regularly than (B) northern bayberry, oriental bittersweet, chokeberry spp., multiflora rose, and winterberry. A higher consumption index indicates a larger proportion of fruit consumed; consumption index (CI) = 1 − (% remaining on open branch/% remaining on enclosed branch). Interquartile range omitted from (B) to preserve clarity.

such as arrowwood spp. Arrowwood spp. and Virginia creeper had the highest consumption indices (Fig. 2A, B), which suggests these fruits were selected by birds relative to other available fruit species (species: $F_{6,35} = 11.05$, $P < 0.001$; count: $F_{5,205} = 75.78$, $P < 0.001$; species*count: $F_{30,205} = 4.27$, $P < 0.001$; site: $F_{6,35} = 1.02$, $P = 0.43$).

Antioxidant Composition of Fruits.—Total anthocyanins (TA), total antioxidant capacity (DPPH), and total phenolics (TPH) were significantly correlated with one another (TA*TPH: $r = 0.63$, $n = 55$, $P < 0.001$; TA*DPPH: $r = -0.78$, $n = 49$, $P < 0.001$; TPH*DPPH: $r = -0.81$, $n = 49$, $P < 0.001$). Fruit species varied in antioxidant content (Fig. 3A–C; permutational MANOVA: $F_{6,32} = 26.3$, $P < 0.001$). Arrowwood spp. contained the most anthocyanins, whereas multiflora rose, oriental bittersweet, and winterberry contained essentially no anthocyanins (Fig. 3A). Fruit from northern bayberry, chokeberry spp., and arrowwood spp. contained relatively more total phenolics, but there was overlap in values across species (Fig. 3B). DPPH, a reciprocal measure of total antioxidant capacity, was highest for fruit species with the lowest amount of antioxidants. For example, winterberry, oriental bittersweet, and multiflora rose had the lowest TA, but the highest DPPH values among species (Fig. 3C). This suggests these three fruits would be unlikely to offer much antioxidant protection to consumers.

Antioxidants and Avian Consumption of Fruits.—Avian consumers exhibited marginal selection of fruits associated with antioxidant content when considering all three measures simultaneously (permutational MANOVA: $F_{1,37} = 3.1$, $P = 0.060$). However, selection of fruits was particularly strong when considering only total anthocyanins and total phenolics but not total DPPH (permutational MANOVA: $F_{1,37} = 8.9$, $P < 0.001$). For example, arrowwood spp. had the highest consumption index and relatively high anthocyanin and phenolic values, whereas oriental bittersweet had the opposite pattern. Fruits with the lowest DPPH values were also selected most commonly by birds (Fig. 3C). However, DPPH was the least effective of the three antioxidant measures in explaining patterns of avian fruit consumption; DPPH was also the least specific measure examined in this study. We did not find any consistent regional differences in antioxidant values for those species tested (region: $F_{1,35} = 2.32$, $P = 0.11$; species: $F_{2,35} = 6.35$, $P = 0.002$; Fig. 4).

DISCUSSION

Birds consumed certain fruit species (arrowwood spp.) more rapidly than others (oriental bittersweet, multiflora rose, winterberry). These patterns of fruit selection suggest birds consume fruits with high antioxidant content more rapidly than those with low levels of antioxidants. Antioxidants in fruits varied somewhat among sites on Block Island, although these were relatively subtle compared to differences in antioxidants between plant species.

Patterns of Avian Fruit Consumption.—Birds rapidly consumed northern and southern arrowwood fruits in the maritime shrubland habitat of northern Block Island. Previous work on Block Island repeatedly documented arrowwood as the most readily consumed fruit species (Parrish

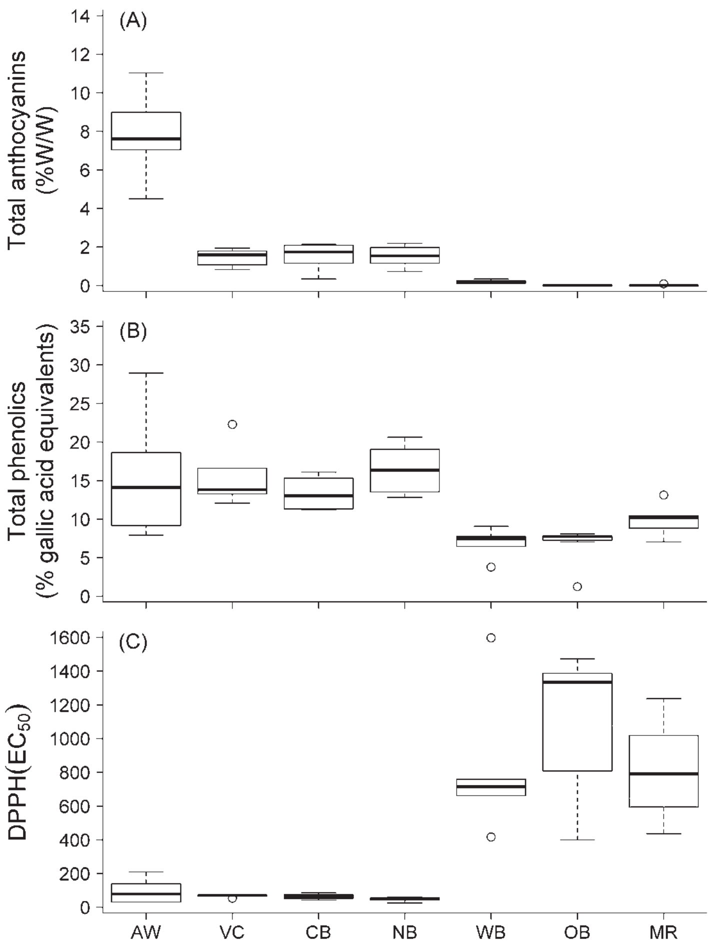

FIG. 3. (A) Total anthocyanins, (B) total phenolics, and (C) total antioxidant capacity (DPPH) for seven species of fruiting shrubs during autumn 2008 on Block Island, Rhode Island. High DPPH values indicate only a small quantity of the radical has been reduced while lower DPPH values indicate a higher amount of radical reduction. Fruit extracts with higher antioxidant capacity will have lower DPPH values. Arrowwood spp. (AW) fruits consistently contained the highest antioxidant measures. Antioxidant measures from Virginia creeper (VC), chokeberry spp. (CB), and northern bayberry (NB) were lower than those in arrowwood but relatively higher than those of winterberry (WB), oriental bittersweet (OB), and multiflora rose (MR). Box plots show the median, interquartile range, and 10th and 90th percentiles; outliers are indicated by unshaded circles.

1997, 2000; Smith et al. 2007b). The latter authors measured avian consumption of three fruit species (arrowwood spp., black chokeberry, and American pokeweed [*Phytolacca americana*]) during autumn migration on Block Island in 2004. They found arrowwood spp. had the highest consumption index of the three species they measured. However, there were differences in the timing of fruit removal during fall and the overall abundance of fruit between 2004 (Smith et al. 2007b) and our study, conducted in 2008. Fruits were abundant in 2004, all arrowwood was ripe by the first census on 9 October, and >70% of fruits on open branches was consumed by 16 October (Smith et al. 2007b). In contrast, fruits were much less abundant in 2008, all arrowwood was ripe by mid-September, and at least 70% of fruits on open branches were consumed by the first days of October. It is evident that, even in a year with low fruit yield, as 2008 was for northern arrowwood, birds still sought these fruits and consumed them at a higher rate than those species whose fruits were more abundant, despite differences in the timing of fruit consumption between years.

Consumption rates for the remaining five fruit species were relatively low (<0.2; Fig. 2A), indicating minimal avian consumption of these

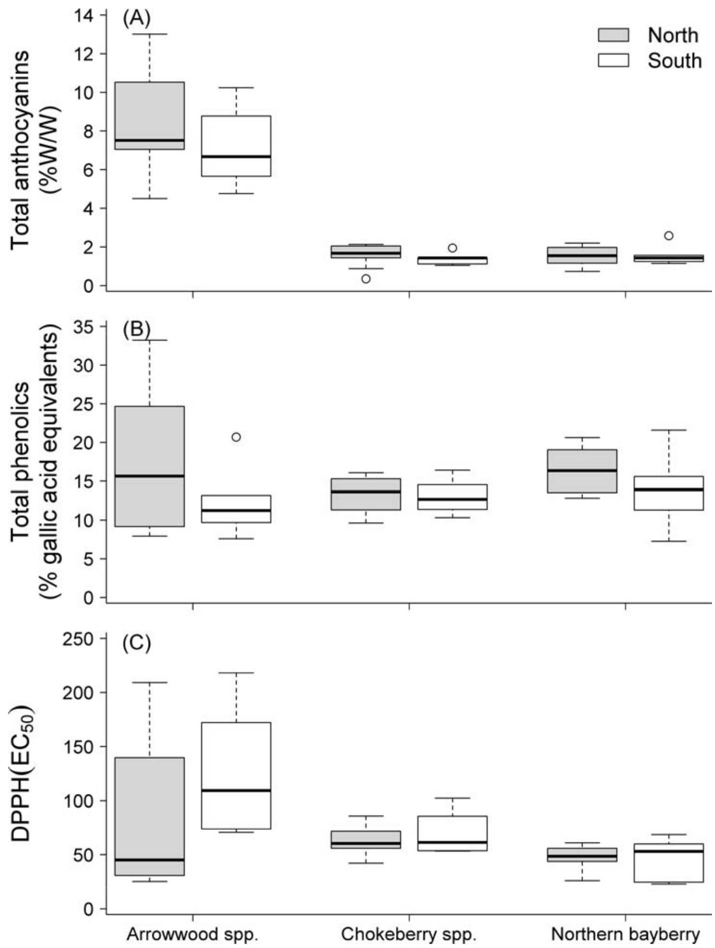

FIG. 4. Regional variation in (A) total anthocyanins, (B) total phenolics, and (C) total antioxidant capacity (DPPH) in fruits from three plant species during autumn 2008 on Block Island, Rhode Island. We detected no consistent regional differences in any antioxidant measures within these three plant species. Samples were collected from the main study site on the northern end of the island (gray) and from three sites in the southern half of the island (white). Box plots show the median, interquartile range, and 10th and 90th percentiles; outliers are indicated by unshaded circles.

species. Smith and others (2007b) found birds ate high-fat fruits, such as northern arrowwood, more frequently than fruits with more carbohydrates, such as black chokeberry, which agrees with our results. A similar study found that, of all the fruiting plant species available to birds on Block Island, fruits of northern arrowwood, northern bayberry, and American pokeweed predominated in fecal samples of a variety of migratory bird species (Parrish 1997). The fruiting phenology of later-ripening species, such as oriental bittersweet, might partially explain why birds did not selectively consume these fruits. Additional studies are needed that document fruit selection of specific bird species in relation to timing of their migration, the distribution and abundance of fruit over this same migration period, and the chemical composition of these fruits.

Antioxidant Composition of Fruits and Spatial Variation.—Antioxidant concentration has frequently been measured in fruit species regularly consumed by humans, but only rarely for fruits more commonly eaten by wildlife. We found that arrowwood spp. had relatively high levels of antioxidants compared to other species. This is the first study to our knowledge to report the antioxidant content of arrowwood fruits. In contrast, the antioxidant composition and health benefits of black chokeberry have been extensively reported (Jurgonski et al. 2008); however, direct comparison of our results to those of other studies is challenging because analytical methods and standards vary. Jurgonski and others (2008) found anthocyanins were the primary phenolic constituent in black chokeberry, and that supplementing rat (*Rattus* spp.) diets with these fruit extracts lowered levels of oxidative stress. Anthocyanins and phenolic compounds are also known to have antioxidant properties both *in vitro* and *in vivo* (Halliwell and Gutteridge 2007). We found black chokeberry had relatively moderate amounts of antioxidants compared to the other species. The three different antioxidant metrics we assessed are measured on different scales and use assays with differing chemical properties. Thus, we cannot make an exact comparison between the three antioxidant metrics and it is difficult to directly assess the contributions of TA and TPH to DPPH. Our statistical analyses suggest that each of these three metrics is significantly correlated with the others. The antioxidants measured in our study represent only the hydrophilic antioxidants present in fruits. Future studies should investigate the lipophilic antioxidants in fruits, such as vitamin E and carotenoids, in relation to both hydrophilic antioxidants and fruit selection of birds.

Numerous environmental and genetic variables have been studied in relation to within-plant species variation in antioxidant levels (Connor et al. 2002) with some evidence this creates large-scale differences in antioxidant levels in the same plant species (Latti et al. 2008). We suspect the relatively small-scale spatial variation in antioxidant levels within plant species we documented were produced by differences in microclimate, soil type, or soil moisture, although this requires further study. The lack of significant within-species variation in fruit antioxidant levels that we documented suggests birds can effectively increase their antioxidant intake by selecting fruits from certain plant species without the need to extensively sample fruits from the same plant species.

Antioxidants and Avian Consumption of Fruits.—Antioxidants in fruits may benefit both fruiting plants as well as migrating birds that consume these fruits. Migrating birds use fat as a primary fuel, and fat metabolism results in production of free radicals (Bairlein and Gwinner 1994, Surai 2002, McWilliams et al. 2004, Pierce and McWilliams 2005); thus, long-distance flight likely causes oxidative damage, although this remains to be directly demonstrated. Plants may produce antioxidants, in part, to defend against oxidative stress while also providing a nutritional reward for consumers. The two fruit species in our study with the highest antioxidant concentrations were also the highest in fat content (northern arrowwood: 41.3 ± 5.8%; Virginia creeper: 23.6 ± 4.8% dry weight ± SE) (Smith et al. 2007b). These high levels of antioxidants might protect the plant, as well as the consumer, from the oxidative stress associated with fat metabolism (Klasing 1998). Plants with higher concentrations of antioxidants in their fruits, in addition to other nutritional benefits of fruit for consumers, may attract birds and achieve higher rates and distances of seed dispersal.

Numerous studies have explored patterns in avian fruit consumption (Parrish 1997, 2000; Smith et al. 2007b) with varying results. It is likely birds select certain fruits based upon several factors, including antioxidants, that work at different spatial scales and which may vary seasonally (Parrish 2000, Levey and Martinez del Rio 2001). Flörchinger and others (2010) found fruit size and color were more important than phenolic compounds in affecting which fruits were consumed by birds. Garden Warblers (*Sylivia borin*) presented with a paired choice between carotenoid enriched and non-enriched foods did not prefer a diet high in carotenoids overall, but individual birds consumed consistent levels of carotenoids throughout the experiment (Catoni et al. 2011). These results suggest birds can detect carotenoids in food and consequently regulate intake of antioxidants. The antioxidants that we measured included anthocyanins, which are pigmented (dark purple), and provide a visual signal of a nutritional reward. Our results are consistent with recent research showing birds in captivity prefer foods with anthocyanins over foods without (Schaefer et al. 2008) and represent one of the first field experiments to support the hypothesis that wild birds may select fruits, in part, based on antioxidant content.

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