Investigating Annual Variation in Fruit Quality Using Nutrient Assays and Multidimensional Fluorescence Spectroscopy

Harshita Sood

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Investigating Annual Variation in Fruit Quality Using Nutrient Assays and Multidimensional Fluorescence Spectroscopy

By Harshita Sood

A Thesis submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Environmental Science

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August, 2015

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Acknowledgements

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Thesis Abstract

Each year, billions of birds engage in migratory behavior in response to seasonal changes. During fall migration, many birds consume nutritionally rich fruits with high energy density to satisfy their energy requirements, and rich in antioxidant capacity to alleviate oxidative stress. The goal of this study was to investigate the variation in nutritional content and antioxidant content of fruits from two different years, and compare these with trends in temperature and precipitation during the years. The fruits of 12 shrubs were collected during autumn of 2012 and 2013 in Rochester, NY. Nutrient analyses were used to measure the energy density, fat content, acid detergent fiber content, total soluble solids content, ash content, and water content of these fruits. Extracts of fruits were analyzed using multidimensional fluorescence spectroscopy and parallel factor analysis (PARAFAC), a multiway decomposition method, was used to provide “scores” for fluorescent components in the dataset. Components were then correlated with phenol content and anthocyanin content of fruits, obtained using other analytical methods. No consistent trend in the concentration of any particular analyte or PARAFAC scores was observed. Red Osier Dogwood seemed to produce fruits with lower energy density, fat content, and sum of PARAFAC scores, and higher water and acid detergent fiber content in 2012, which experienced more extreme weather patterns than 2013, but Spicebush showed the opposite trend. Fruits that showed less variation in nutritional content between the two years include the high-quality, native Gray Dogwood, and lower-quality non-native Multiflora Rose and Autumn Olive. The variation in nutritional quality and PARAFAC scores of fruits is likely a result of the degree of resilience of individual plants to fluctuating weather patterns, and variation in growing and ripening time frames for fruits.
Chapter One: Annual Variation in Nutritional Quality of Fruits in Rochester, NY

Abstract

Migratory songbirds rely on high-quality sources of food during annual migrations. During fall migration in eastern North America, many birds switch to a diet of fruits at stopover sites. In order to sustain them during their journey, birds may select fruits rich in nutrients that provide high energy, such as fats. While studies have evaluated the nutritional value of a variety of fruits, a less studied phenomenon is the impact of changing weather conditions on the nutritional quality of fruits available for migratory birds to eat. In this study, fruits from 12 species of locally growing shrubs in Rochester, NY were collected in 2012 and 2013 and analyzed for their energy density, percent fat content, total soluble solids content, acid detergent fiber content, ash content, and water content. The goal of this study was to determine if there is a variation in the nutritional quality of fruits between the two years, and if the variation can be correlated with different weather and growing season conditions during the two years. Results indicate that Red Osier Dogwood, a drought intolerant native plant, produced fruit with lower energy density and fat content in 2012, which experienced higher temperatures than 2013 during every season. On the other hand, Gray Dogwood, a closely related native plant, showed less variation in nutritional quality between the two years. This was also the case for drought-resistant non-native plants, such as Autumn Olive and Multiflora Rose. Spicebush is a drought tolerant native plant that seemed to produce fruits with greater fat content and energy density during 2012. These results suggest that different fruits may respond differently to fluctuating weather conditions, which may be a result of variation in ripening time of the fruit, and the level of tolerance of the plant for different climatic conditions.
Introduction

Migration is a major life history event for many animals. Each year, species from all major animal groups, including birds, mammals, fish, reptiles, amphibians and insects, engage in some sort of migratory behavior. Of these, bird migration is an intriguing phenomenon that has been studied for the last 3000 years. Each year, approximately half of the 10,000 documented bird species engage in some sort of migratory behavior (Newton, 2010). These migrations are typically in response to shifting weather patterns, and subsequent changes in resource availability.

Bird migrations are completed in alternating periods of flights and stopovers. The cost of migration is significant during times when birds are in flight. Flying time may vary from a few hours to several days while crossing barriers such as lakes or deserts. During this time, birds rely on energetic stores (particularly fat stores) for fuel. In order to prepare for energy-intensive migratory flights, birds enter a state known as “hyperphagia”, during which food intake increases and birds increase their body mass in fat by up to 50% (Blem 1990). In addition to fueling in flight metabolic activities, energy expenditure at stopover sites is also of utmost importance. Birds have been estimated to spend up to 90% of their time, and up to 65% of their energy at stopover sites, where they rapidly refuel before continuing on (Lindstrom, 2005; McWilliams et al., 2004).

It is important for birds to minimize energetic costs while maximizing the use of food resources during migration (McWilliams et al., 2004). To achieve this goal, birds have several adaptations in terms of morphology, behavior, nutrition and metabolism that help minimize energetic expenditures, while maximizing metabolism of macronutrients for fuel, thus ensuring their survival during this strenuous activity. The energy and nutrients needed by birds for flights
are obtained exclusively from food sources and not from endogenous sources (McWilliams et al., 2004). It has been suggested that the change of birds’ foraging behavior from an insect-based diet to a fruit-based diet stems from their high energy demands during fall migration, as they spend less time foraging for fruits during stopovers (Parrish 1997), and some fruits can meet the daily energetic needs of birds during migration (Smith et al., 2007). However, the energy density and nutritional content of fruits may vary considerably. A study conducted by Smith et al. indicates that birds often select native fruits high in fat content and energy density over low-fat, low-energy invasive fruits (2013). On the other hand, other studies indicate that birds may select less energy-dense invasive fruits over native fruits (Lafleur et al., 2007; Drummond, 2005).

Studies have indicated that several factors may affect the fruit choice preferences of migratory birds, such as fruit accessibility, bicolored vs. unicolored displays of fruits (with bicolored being the more preferred) (Whelan and Willson, 1994), and the presence of certain phytochemicals such as anthocyanins and carotenoids in fruits (Bolser et al., 2013; Schaefer et al., 2008). Foraging behavior on fruits and diet composition may also vary for different species of frugivores (Malmborg and Willson, 1988).

**Important Dietary Nutrients for Migratory Birds**

Fruits differ in their nutritional composition (Smith et al., 2007; 2013), and careful diet selection is important during migration. Fruits containing high amounts of fatty acids are an important nutritional resource for birds, as they provide the most energy for birds. Fatty acids are more chemically reduced than other macronutrients, and therefore provide up to ten times more energy upon oxidation (Weber and Haman, 2004). Additionally, lipids can be stored in an anhydrous state as triacylglycerol, which increases the energy yield to wet mass ratio and
minimizes body mass (McWilliams et al., 2004). A secondary source of fuel is carbohydrates. Even though the energy yielded by carbohydrates (approximately 17.15 kJ/g) is lower than that of fatty acids (approximately 38.91 kJ/g), they are completely oxidized during respiration to form carbon dioxide and water. Carbohydrates are abundant in plants, in several forms including sucrose, starch and cellulose. Birds differ in their ability to digest and utilize various forms of carbohydrates. For example, starch and fibrous carbohydrates such as cellulose are not as easily digested by birds as they require symbiotic bacteria which (Klasing, 1998). Harder fibers such as lignin are not digested by most birds, and are excreted in the form of uric acid (See Stevens, 1997). In addition to energy depletion, a factor that can significantly impact long-distance avian migrants is dehydration. Fruits often have a high amount of water in their pulp (over 50%) (Smith et al., 2013; Izhaki, 1992). However, studies have shown that water metabolism is closely linked with energetic management (Engel, 2005; Klaassen 1995). Birds are able to compensate for water lost due to evaporation during flight in low humidity weather conditions through protein catabolism (Gerson and Guglielmo, 2011), and dietary sources of water may not be important for birds that rely on endogenous sources of water during flights. Minerals are another important nutrient for birds, for the purpose of maintaining osmotic balance and homeostasis (In: Izhaki, 1992).

The Impact of Climate Change on Migratory Bird Habitat Quality

An important factor that may affect the habitat quality of stopover sites is the impact of climate change on the nutritional composition of fruits. A study conducted on Cornus drummondii (Roughleaf Dogwood) indicates annual variation over a four year period in ripening time, as well as nutritional characteristics such as lipid content and nitrogen content (Willson and
Whelan, 1993). Another long-term (12-year) study indicates a positive correlation between the abundance of ripe fruits during autumn, and rainfall during the preceding spring (Hererra, 1998). Barnuud et al. suggest that increasing temperatures may lead to a decline in the anthocyanin content of berries, which could significantly affect the total antioxidant capacity of these fruits (2013). While changes in temperature during the course of the year is important, variation in temperature during specific seasons is imperative when determining factors that influence variation in nutritional quality of fruits. For example, a study conducted by Nicholas et al. indicates that phenolic concentrations in berries from Pinot noir vineyards is positively correlated with cooler temperatures post-harvest the previous year to maturity and from bloom to the onset of ripening, and warmer temperatures from budburst to bloom (2011). Another important consideration is the ripening time of the fruit. A study on seasonal variation in nutritional quality of fruits indicates variation between fruits with different ripening times, corresponding to the nutritional requirements of birds consuming the fruits during that time (Hererra, 1982).

**Fruits Available in the Western New York Region**

This study focuses on the nutritional content of 12 shrubs found in the Rochester, NY area (Table 1-1). *Lindera benzoin*, commonly known as Spicebush, (Magnoliid subclass), is a native plant that produces fruits high in fat content (Smith et al., 2013) and is commonly consumed by birds (Moore & Willson, 1982). The plant grows best in moist soil and shady areas, but can tolerate sunny and drought conditions as well (USDA Plants Database, 2015; Herb Society of America, 2010).

Members of the Cornacea family (clade Asterid, sub class Non-Magnoliid) in this study include *Cornus amomum* (Silky Dogwood), *Cornus racemosa* (Gray Dogwood), and *Cornus*
sericea (Red Osier Dogwood). These fruits are typically high in fat and energy density (Smith et al., 2013). Silky Dogwood produces fleshy, blue colored fruit that matures in early fall makes this plant easy to distinguish. This plant is commonly found in the Great Lakes region, but it does not grow well outside its natural range of Eastern United States (from Michigan and Wisconsin to Maine, and South to Florida) (USDA Plants Database, 2015). This plant thrives in poorly drained soils, and is tolerant of shade but not drought conditions (USDA Plants Database, 2015). Gray Dogwood is found throughout northeastern United States. It produces fruits in the summer which turn white and fleshy during fall. It thrives in a variety of climatic conditions (USDA Plants Database, 2015). Red Osier Dogwood bears fleshy white fruits that ripen mid- to late-summer. Red Osier Dogwood grows best in nitrogen-rich and well-drained soils (Monsen et al., 2004), and is somewhat drought intolerant (Merigliano, 1996; Haeussler et al., 1990).

Members of the Caprifoliaceae family (clade Asterid, sub class Non-Magnoliid) in this study include Viburnum dentatum (Arrowwood Viburnum), Viburnum opulus (European Cranberrybush), and Lonicera spp. (Bush Honeysuckle). Arrowwood Viburnum produces dark blue-black colored berries (drupes) during early fall, that are known to be rich in fat and energy density (Smith et al., 2013), and are commonly consumed by frugivorous birds (Bolser et al., 2013; Meiners and Stiles, 1997; Smith et al. 2007). This plant prefers moist soil and partial shade, but is able to survive in drier and sunny conditions as well (USDA Plants Database). European Cranberrybush is a non-native plant species that produces bright red berries during late summer, which are low in nutritional quality for migratory birds due to their lower energy density and fat content (Smith et al., 2013). The plant is able to adapt to both high light and partial shade, and grows best in moist soils (University of Connecticut Plant Database, 2015). Bush Honeysuckle is a grouping of similar shrub species that are native to Asia and have been
introduced in the United States. Bush honeysuckles produce fleshy fruits during summer months, and its seeds are dispersed by frugivorous birds (Ingold and Craycraft, 1983). One study indicates that it positively impacts bird abundance in habitats with fleshy fruited plants (Gleditsch and Carlo, 2011), while others indicate that it can serve as an ecological trap for frugivores (Rodewald, 2012). While most Honeysuckle species thrive in sunlight, they are able to tolerate shade which helps them outcompete native shrubs (Smith and Smith, 2010).

*Ilex verticillata* (Common Winterberry) is a native shrub (Asterid clade) that produces reddish-orange colored fruits that mature during late summer months, and can remain on plants until mid-winter (USDA Plants Database, 2015). Common Winterberry seeds are dispersed by birds, and a study conducted in Trenton, New Jersey indicates that the seeds of Common Winterberry are preferentially consumed by seed predators (Meiners and Stiles, 1997). Published literature does not indicate ideal growing conditions for this plant, however horticultural guidelines for this shrub include planting in moist and shady areas (USDA Plants Database, 2015).

Members of the Rosales order (Rosid clade) include three non-native plants, *Elaeagnus umbellata* (Autumn Olive), *Rhamnus cathartica* (Common Buckthorn), and *Rosa multiflora* (Multiflora Rose). Autumn Olive produces fruits that appear silvery and brown when unripe, and turn red when they ripe during autumn. Autumn olive fruits are eaten by frugivorous birds (Suthers et al., 2000), which disperse their seeds widely (Michigan Department of Natural Resources, 2012). Autumn olive is drought tolerant (Virginia Department of Conservation and Recreation, retrieved 2015), and somewhat shade tolerant, and is able to adapt to a variety of soil types (Michigan Department of Natural Resources, 2012). Common Buckthorn is a non-native shrub that has invaded several parts of North America, including the Great Lakes region. This
shrub produces dark purple colored fruits which are low in fat content and energy density (Smith et al., 2013), and contains emodins that have negative physiological effects on animals that consume them (USDA Plant Database, 2015; Knight et al., 2007). Its success as an invader can be attributed to its ability to tolerate a wide range of climatic conditions, including sunshine, shade, moist and dry conditions (Kuryolo et al., 2007). Multiflora Rose produces small, firm, round-shaped fruits called ‘hips’ that are red in color during late summer, and persist during the winter (Szafoni, 2007). A single plant may produce up to 500,000 seeds, which may be distributed by birds and mammals that feed on the fruits (Maddox et al., 2007). However, the fruits have a low fat content and energy density (Smith et al., 2007; 2013) and are not considered as valuable of a food resource to wildlife (Maddox et al., 2007). Multiflora Rose is able to grow in a variety of habitats and climatic conditions, and is able to tolerate various kinds of soils, moisture levels and light availability (Missouri Department of Conservation, 2015).

Vitis riparia, commonly known as Riverbank Grape, is a native vine that thrives in wetland soils, but is occasionally found in non-wetland soils, and able to tolerate extremely low temperatures and can adapt to varying soil chemistry conditions (USDA Plants Database, 2015). The plant produces small, round, dark colored berries that can contain 2-4 seeds, and are readily consumed by frugivorous birds (Willson and Whelan, 1989).

Study Goals

While information is available on the general tolerance of plants to various climatic conditions, few studies focus on the change in the nutritional composition of fruits produced by these plants with changing weather conditions. The purpose of this study is to determine the how annual weather patterns relate to variation in the nutritional quality of fruits available for
migratory birds to eat in the Rochester, NY region during fall migration. Fruits from 12 species were collected in 2012 and 2013. The nutritional content of fruits was measured with the goal of correlating the nutritional value of fruits with the temperature and rainfall conditions for the two growing seasons. It is hypothesized that different fruits will respond differently to the weather patterns during the two years consistent with their different evolutionary histories and adaptations for growing conditions. It is predicted that fruits that are less resilient to variable weather conditions (such as Silky Dogwood and Red Osier Dogwood) may experience greater stress during growing seasons with harsher weather conditions, and may produce fruits with less energy density. On the other hand, plants that can tolerate a wide range of temperature and weather conditions (such as Gray Dogwood, Multiflora Rose, Autumn Olive, etc.) may be less likely to show nutritional variation between the two years.

**Methods**

**Study Sites**

Fruits were collected from two different sites – Braddock Bay Bird Observatory and Rochester Institute of Technology. Braddock Bay Bird Observatory (hereafter “BBBO”) is located on the southern shore of Lake Ontario (43°19’N, 77°43’W), northwest of Rochester, NY, and is an important stopover site for migratory birds during spring and fall migration (Bonter et al., 2007). The land cover of the site consists of abandoned fields and early-successional forests, dominated by native shrubs such *Viburnum dentatum* (Arrowwood Viburnum) and *Cornus racemosa* (Gray Dogwood), and invasive shrubs such as *Lonicera spp.* (Bush Honeysuckle).

Rochester Institute of Technology (hereafter “RIT”) is located in Henrietta, NY (43°54’N, 77°40’W), and is characterized by mid-successional forests and wetland, with adjacent suburban
areas. Much of the area was previously used for farming, and was converted into mitigation wetland more recently. The area includes several species of native shrubs, such as *Lindera benzoin* (Spicebush) and *Cornus racemosa* (Gray Dogwood), and invasive shrubs such as *Rosa multiflora* (Multiflora Rose) and *Rhamnus cathartica* (Common Buckthorn).

**Collecting, Drying and Storing Fruits**

The fruits collected for analysis are summarized in Table 1-1. Fruits were collected during fall migration in 2012 and 2013 at the peak of ripeness (August-September), and immediately frozen at -20°C. Frozen fruits were thawed and then dissected to remove seeds, which provide no nutritional value to birds. The fruits were then freeze dried at -80°C and 0.051 Torr pressure in a Labconco TRIAD benchtop freeze dryer for two days, homogenized using a mortar and pestle, and stored in vials at -20°C until they were used for analyses.
Table 1-1: A summary of the fruits collected for analysis in this study. Information on the plants related to taxonomy, native to vs. non-native status in Rochester, NY, and level of drought tolerance (low, fair or high) is provided. Fruits were collected either from the Braddock Bay Bird Observatory, or from the Rochester Institute of Technology. All fruits were collected during 2012 and 2013, with the exception of Silky Dogwood, Bush Honeysuckle, and Arrowwood Viburnum which were only collected in 2013.

<table>
<thead>
<tr>
<th>Taxonomic Grouping</th>
<th>Scientific Name</th>
<th>Common Name</th>
<th>Native or Non-Native</th>
<th>Site</th>
<th>Drought Tolerance</th>
<th>Years Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnoliid</td>
<td><em>Lindera benzoin</em></td>
<td>Spicebush</td>
<td>Native</td>
<td>RIT</td>
<td>High</td>
<td>2012, 2013</td>
</tr>
<tr>
<td>Non-Magnoliid (Asterid)</td>
<td><em>Cornus amomum</em></td>
<td>Silky Dogwood</td>
<td>Native</td>
<td>BBBO</td>
<td>Low</td>
<td>2013</td>
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<tr>
<td></td>
<td><em>Cornus racemosa</em></td>
<td>Gray Dogwood</td>
<td>Native</td>
<td>BBBO</td>
<td>High</td>
<td>2012, 2013</td>
</tr>
<tr>
<td></td>
<td><em>Cornus sericea</em></td>
<td>Red Osier Dogwood</td>
<td>Native</td>
<td>BBBO</td>
<td>Low</td>
<td>2012, 2013</td>
</tr>
<tr>
<td></td>
<td><em>Lonicera spp.</em></td>
<td>Bush Honeysuckle</td>
<td>Non-native</td>
<td>BBBO</td>
<td>Fair</td>
<td>2013</td>
</tr>
<tr>
<td></td>
<td><em>Viburnum dentatum</em></td>
<td>Arrowwood Viburnum</td>
<td>Native</td>
<td>BBBO</td>
<td>Fair</td>
<td>2013</td>
</tr>
<tr>
<td></td>
<td><em>Viburnum opulus</em></td>
<td>European Cranberrybush</td>
<td>Non-native</td>
<td>BBBO</td>
<td>Fair</td>
<td>2012, 2013</td>
</tr>
<tr>
<td></td>
<td><em>Ilex verticillata</em></td>
<td>Common Winterberry</td>
<td>Native</td>
<td>BBBO</td>
<td>Fair</td>
<td>2012, 2013</td>
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<td></td>
<td><em>Rhamnus cathartica</em></td>
<td>Common Buckthorn</td>
<td>Non-native</td>
<td>BBBO</td>
<td>High</td>
<td>2012, 2013</td>
</tr>
<tr>
<td></td>
<td><em>Rosa multiflora</em></td>
<td>Multiflora Rose</td>
<td>Non-native</td>
<td>RIT</td>
<td>High</td>
<td>2012, 2013</td>
</tr>
<tr>
<td></td>
<td><em>Vitis riparia</em></td>
<td>Riverbank Grape</td>
<td>Native</td>
<td>BBBO</td>
<td>Fair</td>
<td>2012, 2013</td>
</tr>
</tbody>
</table>

**Measuring Water Content of Fruits**

Approximately 25 – 100 fruits of each species were dissected to remove seeds in aluminum weighing dishes. Once the seeds were removed, the weight of fruits before and after freeze-drying was recorded. The average mass from all trays for a fruit from a particular year was determined. The water content was determined as %mass lost following freeze-drying and is expressed as %wet mass per fruit.
Measuring the Energy Density of Fruits

The energy density of the fruits was measured using a Parr1341 bomb calorimeter. One gram pellets of dried fruits were combusted in an oxygen-filled chamber within the bomb, and the heat released upon combustion of the fruit was be recorded as temperature change in the water jacket. The energy density of the fruits was calculated and recorded in kJ/gram of sample using a benzoic acid standard (Smith et al. 2013).

Measuring the Percent Fat Content of Fruits

The total fat content of fruits was determined using an Ankom XT10 fat extractor (Ankom Technology). Approximately 0.5 – 1.0 grams of freeze-dried fruit samples were placed in XT-4 filter bags. The bags were sealed and placed in the fat extractor, and extracted for 60 minutes in petroleum ether solvent at 90°C. The weight of each bag was recorded before and after the extraction process to determine the percent fat content of the fruits.

Measuring the Percent Ash Content of Fruits

Ash content of fruits was measured by combusting Ankom XT-4 filter bags, following the fat extraction. The filter bags were placed in crucibles and combusted in a muffle furnace at 550°C for 3 hours. The crucibles were allowed to cool in a desiccator before the weight of the remaining sample was recorded. The weight of the crucibles was recorded prior to the procedure, and the dry weight of samples was obtained from the procedure to determine the fat content. The ash content was determined as % dry weight of the fruit.
Measuring the Acid Detergent Fiber content of fruits

Acid Detergent Fiber (ADF) content of the fruits, which predominantly includes indigestible cellulose and lignin, was determined using an A200 fiber analyzer (Ankom Technology). The reagent used in the process is Acid Detergent Solution, a mixture of cetyl trimethylammonium bromide (CTAB) and sulfuric acid. Approximately 0.4 grams of freeze-dried fruit is placed in filter bags and then digested for 60 minutes. Mass remaining in the bag following digestion and drying at 102°C was measured as %ADF.

Measuring the Total Soluble Solids (TSS) content of fruits

The total soluble solids content was determined by picking randomly selected ripe fruits of each species that were frozen at 20°C after harvesting, thawing them at room temperature, and squeezing the juice onto the prism of a digital refractometer (Reichert AR200). Temperature-compensated refractive index was measured after calibration with nanopure water and TSS was determined by comparing values to a % sucrose table (AOAC Official Methods of Analysis, 1990), which denotes TSS as °Brix. This method was not used to evaluate the TSS content of Common Winterberry, Multiflora Rose, Arrowwood Viburnum, and Autumn Olive due to their pulp consistency.

Collecting Temperature and Precipitation Data

Temperature and precipitation data for the months of January – October for 2012 and 2013 were obtained from the National Oceanic and Atmospheric Administration (NOAA) archives for station located in Webster, NY (Elev: 275 ft. Lat: 43.242° N Lon: 77.388° W). This station was selected due to its proximity to both study sites, and to Lake Ontario and because
complete dataset was archived for the study period. Differences in average temperature and total rainfall patterns between the months of January – October in 2012 and 2013 were examined using a paired t-test. The seasons were also divided between winter (January-February), spring (March-May), summer (June-July), and fall (August-October), and differences in average temperature and total rainfall between seasons were examined using a paired t-test. Patterns of extreme temperatures during growing seasons in 2012 and 2013 were also obtained (NOAA Database, 2015). Specifically, data on prolonged dry periods, and number of days with temperatures higher than 90°F or lower than 32°F were obtained. These results were used to compare nutritional differences between the two years with changes in weather conditions during growing season and fall migration season, when fruits are consumed by birds. Specifically, differences in precipitation and rainfall were compared with the nutritional variation for fruits with various levels of adaptability to climatic variation. The temperature and precipitation data were also compared with the ripening and harvesting time frame for the fruits.

Results

Temperature and Precipitation Data for 2012 and 2013

Average temperature and total precipitation data for Rochester, NY between the months of January – October in 2012 and 2013 were collected (NOAA Database, 2015). There was a significant difference in the average temperatures between the different seasons, as indicated by a paired t-test (p < 0.05, t-statistic = 4.09, df = 3). The average temperature between these months was 2.93°F higher in 2012 than in 2013. Every month in 2012 experienced higher temperatures than the corresponding month in 2013, with the exception of April (0.9°F higher in 2013). The greatest temperature differences were observed in March (12.6°F higher in 2012),
February (6°F higher in 2012) and June (3.1°F higher in 2012). The precipitation in 2012 was not significantly different from 2013 for the different seasons (p > 0.05, t-statistic = -0.03, df = 3, indicated by a paired t-test). However, the total precipitation was highest in June 2013 (6.43 inches), which is more than twice as high as the corresponding month in 2012 (2.96 inches).

The winter preceding 2012 experienced fewer days with maximum temperatures below 32°F and 0°F (17 days, and 1 day respectively) than the winter preceding 2013 (30 days, and 3 days respectively). March 2012 had an average minimum temperature of 35°F and an average maximum temperature of 56.8°F, and experienced 10 days with minimum temperatures dropping lower than 32°F. March 2013 had an average minimum temperature of 25°F, an average maximum temperature of 41.5°F, and 28 days with minimum temperatures below 32°F.

The number of summer days that experienced maximum temperatures greater than 90°F was higher for 2012 (12 days) than for 2013 (7 days). 4 days in June 2012 and 3 days in August 2012 experienced temperatures higher than 90°F, while none of the corresponding months in 2013 experienced maximum temperatures as high as 90°F. On the other hand, September 2012 did not experience high temperatures of 90°F, whereas September 2013 did experience 2 days with 90°F temperatures.

Precipitation trends differed between late spring and early summer (May – June) months. Rainfall was distributed fairly evenly between days during May – July in 2013. May 2012, however, experienced 23 consecutive days with less than 0.10 inches of total rainfall (all of which was received in one day). During late June and early July in 2012, precipitation levels totaled 0.16 inches. Although August 2012 and August 2013 received a similar amount of rainfall (4.08 and 4.24 inches respectively), the rainfall was more scattered among several days in 2013. Over half of the rainfall received in August 2012 (2.71 inches) was received in 1 day.
Variation in Nutritional Quality of Fruits

No major trends in the change in concentration of fruit nutritional analytes were apparent with respect to the taxonomic grouping of plants (Table 1-3). In the case of individual fruits, the change in concentration of many analytes is likely not significant due to the overlap of standard deviation (denoted by error bars in Figures 1-1 to 1-6 and Table 1-2). The fruits that seemed to show greater variation in nutritional quality between the two years are Spicebush, Red Osier Dogwood, Common Winterberry, and Common Buckthorn. Spicebush showed a decreasing trend in average energy density, and fat content, and an increasing trend in ash content and water content (Tables 1-2 and 1-3). Red Osier Dogwood showed an increasing trend in energy density and fat content, and a decreasing trend in acid detergent fiber and water content (Tables 1-2 and 1-3). Common Winterberry showed a decreasing trend in energy density, fat content, and ash content (Tables 1-2 and 1-3). Common Buckthorn showed an increasing trend in energy density, fat content, and water content (Tables 1-2 and 1-3).
Table 1-2 – Summary of fruit nutritional analyses - energy density, % fat, % total soluble solids, % acid detergent fiber, water content and % ash for 2012 and 2013 fruit. Energy density is expressed in kJ/gram, and all other values are in % dry mass or wet mass (water, TSS). N = 3, unless otherwise indicated. Sample size for water content is summarized in the ‘methods’ section. a indicates sample size = 4, b indicates sample size = 5, c indicates sample size = 6, d indicates sample size = 7

<table>
<thead>
<tr>
<th>Fruit Species</th>
<th>Year</th>
<th>Energy Density (kJ/g)</th>
<th>% Fat Content</th>
<th>% Total Soluble Solids</th>
<th>% Acid Detergent Fiber</th>
<th>% Water Content</th>
<th>% Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spicebush</td>
<td>2012</td>
<td>26.98 ± 0.01</td>
<td>40.75 ± 0.76</td>
<td>N/A</td>
<td>5.22 ± 1.31^a</td>
<td>71.39 ± 1.17</td>
<td>3.49 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>26.00 ± 0.19</td>
<td>33.76 ± 0.63</td>
<td>N/A</td>
<td>5.02 ± 1.03^a</td>
<td>74.24 ± 1.02</td>
<td>6.28 ± 0.34</td>
</tr>
<tr>
<td>Silky Dogwood</td>
<td>2013</td>
<td>17.85 ± 0.27</td>
<td>5.54 ± 0.17</td>
<td>10.85 ± 0.63</td>
<td>10.6 ± 0.95</td>
<td>86.56 ± 5.82</td>
<td>4.96 ± 0.22</td>
</tr>
<tr>
<td>Gray Dogwood</td>
<td>2012</td>
<td>25.93 ± 0.29</td>
<td>39.18 ± 0.67</td>
<td>21.77 ± 1.09^b</td>
<td>4.07 ± 1.20^b</td>
<td>64.5 ± 2.02</td>
<td>2.82 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>25.51 ± 0.20</td>
<td>39.7 ± 0.73</td>
<td>19.55 ± 1.50</td>
<td>3.43 ± 0.68^a</td>
<td>75.74 ± 7.54</td>
<td>1.9 ± 0.07</td>
</tr>
<tr>
<td>Red Osier Dogwood</td>
<td>2012</td>
<td>19.57 ± 0.55</td>
<td>20.13 ± 1.37</td>
<td>10.97 ± 3.15</td>
<td>7.4 ± 0.43</td>
<td>84.56 ± 0.69</td>
<td>2.87 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>20.65 ± 0.38</td>
<td>26.67 ± 0.35</td>
<td>13.53 ± 1.18^b</td>
<td>4.19 ± 0.24</td>
<td>80.09 ± 0.26</td>
<td>2.99 ± 0.17</td>
</tr>
<tr>
<td>Bush Honeysuckle</td>
<td>2013</td>
<td>16.64 ± 0.31</td>
<td>1.09 ± 0.36</td>
<td>13.73 ± 1.9</td>
<td>3.53 ± 0.05</td>
<td>84.37 ± 1.41</td>
<td>3.36 ± 0.73</td>
</tr>
<tr>
<td>Arrowwood Viburnum</td>
<td>2012</td>
<td>26.76 ± 0.21</td>
<td>44.99 ± 0.53</td>
<td>N/A</td>
<td>14.34 ± 2.12^a</td>
<td>66.05 ± 3.7</td>
<td>2.75 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>26.25 ± 0.18</td>
<td>44.99 ± 0.53</td>
<td>N/A</td>
<td>14.34 ± 2.12^a</td>
<td>66.05 ± 3.7</td>
<td>2.75 ± 0.13</td>
</tr>
<tr>
<td>European Cranberrybush</td>
<td>2012</td>
<td>15.64 ± 0.17</td>
<td>0.02 ± 0.00</td>
<td>13.5 ± 0.76^a</td>
<td>9.52 ± 1.28^a</td>
<td>85.33 ± 0.34</td>
<td>2.28 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>15.96 ± 0.12</td>
<td>0.86 ± 0.10</td>
<td>14.3 ± 1.43^c</td>
<td>10.8 ± 0.93</td>
<td>83.19 ± 3.76</td>
<td>2.96 ± 0.42</td>
</tr>
<tr>
<td>Common Winterberry</td>
<td>2012</td>
<td>19.79 ± 0.27</td>
<td>2.13 ± 0.14</td>
<td>N/A</td>
<td>23.21 ± 2.08</td>
<td>72.97 ± 1.82</td>
<td>3.26 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>18.05 ± 0.29</td>
<td>1.41 ± 0.78</td>
<td>N/A</td>
<td>16.01 ± 0.52</td>
<td>N/A</td>
<td>2.6 ± 0.20</td>
</tr>
<tr>
<td>Autumn Olive</td>
<td>2012</td>
<td>16.00 ± 0.17</td>
<td>1.64 ± 0.11</td>
<td>N/A</td>
<td>13.64 ± 1.06</td>
<td>75.28 ± 1.50</td>
<td>2.14 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>15.69 ± 0.22</td>
<td>0.62 ± 0.02</td>
<td>N/A</td>
<td>11.22 ± 1.49^a</td>
<td>78.11 ± 0.12</td>
<td>1.57 ± 0.28</td>
</tr>
<tr>
<td>Common Buckthorn</td>
<td>2012</td>
<td>16.48 ± 0.38</td>
<td>0.44 ± 0.05</td>
<td>35.99 ± 3.39^b</td>
<td>6.32 ± 1.25^a</td>
<td>60.12 ± 3.47</td>
<td>4.29 ± 0.88</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>17.02 ± 0.16</td>
<td>1.27 ± 0.38</td>
<td>30.77 ± 4.15</td>
<td>6.29 ± 0.93^a</td>
<td>72.75 ± 1.19</td>
<td>3.26 ± 0.26</td>
</tr>
<tr>
<td>Multiflora Rose</td>
<td>2012</td>
<td>16.12 ± 0.15</td>
<td>2.29 ± 0.10</td>
<td>N/A</td>
<td>22.9 ± 2.19</td>
<td>52.24 ± 3.25</td>
<td>7.33 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>16.35 ± 0.04</td>
<td>2.29 ± 0.19</td>
<td>N/A</td>
<td>19.12 ± 1.64</td>
<td>32.77 ± 4.74</td>
<td>6.25 ± 1.64</td>
</tr>
<tr>
<td>Riverbank Grape</td>
<td>2012</td>
<td>15.57 ± 0.16</td>
<td>1.25 ± 0.22</td>
<td>25.24 ± 0.96^a</td>
<td>4.91 ± 0.25</td>
<td>71.81 ± 1.88</td>
<td>2.68 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>15.13 ± 0.28</td>
<td>1.23 ± 0.18</td>
<td>21.52 ± 0.82^a</td>
<td>5.35 ± 0.46</td>
<td>73.92 ± 1.88</td>
<td>3.66 ± 0.08</td>
</tr>
</tbody>
</table>
Table 1-3: The change in nutritional measures of fruits from 2012 to 2013. +/- indicates a less than 0.5 kJ/gram change in energy density, less than 5% change in water content, and less than 1% change in other nutrients. +/+- indicates a 0.5 – 1 kJ/gram change in energy density, a 5-10% change in water content, and a 1%-2% change in other nutrients. +++/- indicates greater than 1kJ/gram change in energy density, greater than 10% change in water content, and greater than 2% change in other nutrients.

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Energy Density</th>
<th>% Fat</th>
<th>% Total Soluble Solids</th>
<th>% Acid Detergent Fiber</th>
<th>% Ash</th>
<th>% Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spicebush</td>
<td>--</td>
<td>---</td>
<td>N/A</td>
<td>-</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Gray Dogwood</td>
<td>-</td>
<td>+</td>
<td>---</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>Red Osier Dogwood</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>---</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>European Cranberrybush</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Autumn Olive</td>
<td>-</td>
<td>--</td>
<td>N/A</td>
<td>--</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Common Winterberry</td>
<td>---</td>
<td>-</td>
<td>N/A</td>
<td>---</td>
<td>-</td>
<td>N/A</td>
</tr>
<tr>
<td>Common Buckthorn</td>
<td>+</td>
<td>++</td>
<td>---</td>
<td>-</td>
<td>--</td>
<td>+++</td>
</tr>
<tr>
<td>Multiflora Rose</td>
<td>+</td>
<td>0</td>
<td>N/A</td>
<td>---</td>
<td>--</td>
<td>---</td>
</tr>
<tr>
<td>Riverbank Grape</td>
<td>-</td>
<td>-</td>
<td>---</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Energy Density of Fruits in 2012 vs. 2013*

The average energy density for Common Buckthorn, European Cranberrybush, Multiflora Rose and Red Osier Dogwood indicate an increasing trend from 2012 to 2013 (Figure 1-1, Table 1-3). Of these fruits, Red Osier Dogwood showed the greatest increase in average energy density (approximately 1.07 kJ/gram). The energy density for Spicebush, Gray Dogwood, Autumn Olive, Common Winterberry and Riverbank Grape showed a decreasing trend from 2012 to 2013 (Figure 1-1; Table 1-3). The largest decrease in energy density was observed in the case of Common Winterberry (approximately 1.7 kJ/gram) followed by Spicebush (approximately 0.98 kJ/gram). However, the overlap in error bars for Gray Dogwood and Autumn Olive suggests that the difference in energy density between the two years is not likely to be significant (Table 1-2; Figure 1-1).
Figure 1-1: Average Energy density of fruits in 2012 vs. 2013. Error bars indicate standard deviation, n = 3 for all fruits.

**Fat Content of Fruits in 2012 vs. 2013**

Six out of nine fruits shown in Figure 1-2 (European Cranberrybush, Autumn Olive, Common Winterberry, Common Buckthorn, Multiflora Rose, and Riverbank Grape) contained less than 5% fat. In the case of these low-fat fruits, increase in the average fat content of Common Buckthorn and European Cranberrybush (which contained a negligible amount of fat in 2012 – Table 1-2) was observed, by 0.83% and 0.84% respectively. The average fat content for Autumn Olive was less than half the 2012 value in 2013 (by 1.02%) (Figure 1-2). As indicated by the error bars, there was considerable overlap in the standard deviation of the average fat content values of Common Winterberry, Multiflora Rose, and Riverbank Grape fruits in 2012.
and 2013 (Table 1-2; Figure 1-2). Therefore, the change in fat content of these fruits between the two years is not likely to be significant.

In the case of fruits with more than 5% fat, the fat content of Red Osier Dogwood increased from 2012 to 2013 by 6.54%, whereas the fat content of Spicebush decreased between those years by 6.99% (Figure 1-3, Table 1-3). The fat content of Gray Dogwood increased by 0.52%, however, there was considerable overlap in the standard deviation of the fat content values of the samples between the two years, as indicated by the error bars (Table 1-2; Figure 1-2).

Figure 1-2: Fat content of fruits in 2012 vs. 2013. Error bars indicate standard deviation, n = 3.
Total Soluble Solids of Fruits in 2012 vs. 2013

The TSS content of European Cranberrybush and Red Osier Dogwood showed an increasing trend from 2013 to 2012 (Table 1-3), however, there was considerable overlap between the standard deviation of the values of these samples from 2012 and 2013 (Table 1-2; Figure 1-3). This was also the case for Common Buckthorn and Gray Dogwood, which showed a decreasing trend in TSS content (Table 1-3). The TSS content of Riverbank Grape decreased from 25.24 ± 0.96% in 2012 to 21.52 ± 0.82% in 2013 (Table 1-2).

Acid Detergent Fiber of Fruits in 2012 vs. 2013

The percent ADF for many fruits showed a decreasing trend from 2012 to 2013. However, there was considerable overlap between the standard deviation values of the samples for the two years for all fruits, with the exception of Red Osier Dogwood and Common Winterberry (Figure 1-4). Common Winterberry showed the greatest decrease in ADF value, from 23.21 ± 2.08% to 16.01 ± 0.52% (approximately 7.2%) (Table 1-2).

![Graph showing ADF content of fruits in 2012 vs. 2013]


Ash Content of Fruits in 2012 vs. 2013

The ash content of Spicebush, European Cranberrybush, and Riverbank Grape showed an increasing trend from 2012 to 2013 (Table 1-3). Of these, Spicebush showed the greatest
increase, from $3.49 \pm 0.16\%$ to $6.28 \pm 0.34\%$ (approximately 2.79\% increase; Table 1-2; Figure 1-6). The ash content of Autumn Olive, Gray Dogwood and Common Winterberry decreased from 2012 to 2013, with Gray Dogwood showing the greatest decrease, from $2.82 \pm 0.22\%$ to $1.9 \pm 0.07\%$ (approximately 0.92\% decrease) (Table 1-2; Figure 1-2). There was overlap in the standard deviation for fruit samples of Red Osier Dogwood, Common Buckthorn, and Multiflora Rose (Table 1-2; Figure 1-5).

**Figure 1-5:** Average \% ash content of fruits in 2012 vs. 2013. Error bars indicate standard deviation, $n = 3$

**Water Content of Fruits in 2012 vs. 2013**

The water content for Common Buckthorn, Gray Dogwood and Spicebush increased from 2012 to 2013 (Table 1-3). Of these, Common Buckthorn showed the highest increase, from
60.12 ± 3.47% to 72.75 ± 1.19% (approximately 12.63% increase; Table 1-2; Figure 1-6). The water content for Multiflora Rose and Red Osier Dogwood decreased from 2012 to 2013 (Figure 1-6). Multiflora Rose showed the greatest change in water content, which decreased from 52.24 ± 3.25% in 2012 to 32.77 ± 4.74% in 2013 (approximately 19.47% decrease; Table 1-2; Figure 1-6). The change in water content for European Cranberrybush and Riverbank Grape is not likely to be significant due to the overlap of error bars (Table 1-2; Figure 1-6).

Figure 1-6: Percent water content of fruits in 2012 vs. 2013. Error bars indicate standard deviation. N = 1-3 for all fruits, where each sample contained between 25-100 fruits that were freeze-dried.
Discussion

Data Trends with respect to Ecological and Taxonomic Considerations

No consistent trends were apparent in the overall variation in nutritional quality of all fruits between the two years, though individual fruits showed changes in some but not all nutritional analytes from 2021 to 2013. Additionally, this study has limited sample sizes, which makes it difficult to conclusively determine statistically significant differences in nutrient concentrations between 2012 and 2013 fruits. Extensive overlap in standard deviation for some analytes suggests a lack of a statistically significant difference; though there was not overlap in error bars for some analytes in some fruits. Trends in the change in nutritional composition of fruits may be further clarified by grouping fruits taxonomically to identify trends in closely related fruits. These trends may also be related to the degree of adaptability of the fruits to changing weather patterns.

Spicebush is the only plant belonging to the Magnoliid group of plants. The fat content and energy density of Spicebush was higher during the warmer and dryer 2012, while the ash content and water content of the fruit was lower in 2012. This is not unexpected as Spicebush is known to be able to tolerate drought conditions (USDA Plants Database, 2015), and may not have been impacted by the lower precipitation levels during summer 2013.

There is no common trend in the increase or decrease of any analyte for all fruits belonging to the Asterid clade. Silky Dogwood and Red Osier Dogwood are not known to grow well in drought conditions (USDA Plants Database, 2015, Merigliano, 1996; Haeussler et al., 1990), and were the least drought-tolerant plants in this study. Of these, Silky Dogwood was not collected in 2012, as the fruits dried up during ripening (personal communication – S. Smith). This may be a result of the lower levels of precipitation experienced in summer 2012 (6.32
inches in 2012, compared to 10.13 inches in 2013), prolonged periods with little or no rain during summer 2012, and greater number of days with average temperatures higher than 90°F. Red Osier Dogwood showed an increase in fat content and energy density, a negligible increase in ash content, and a decrease in ADF and water content from 2012 to 2013 (Table 1-2). There was also a slight increase in the total soluble solids content, though likely not significant. However, the data suggest that Red Osier Dogwood may produce higher quality fruits during growing seasons with less extreme weather conditions, like those experienced in 2013 (rainfall scattered over multiple days, and fewer summer days with temperatures greater than 90°F). Additionally, the fruits for these plants ripen during late summer or early fall, and the high temperatures and low rainfall in summer 2012 may have adversely impacted the quality of the fruits produced. Gray Dogwood, on the other hand, is a high-quality native fruit with high fat content (Smith et al., 2013) and high level of drought tolerance (USDA Plants Database, 2015). The data indicates little difference in the nutrient content of Gray Dogwood from 2012 to 2013 (Table 1-2), which suggests that this fruit may be affected less by annual variations in weather conditions.

The three plants in this study that belong to the Caprifoliaceae family are Bush Honeysuckle, Arrowwood Viburnum, and European Cranberrybush. Of these, Bush Honeysuckle and Arrowwood Viburnum were not available in 2012 as the fruits dried up during summer ripening season. Although Red Honeysuckle is somewhat drought tolerant, it ripens during early summer and may have been negatively impacted by the high temperatures (four days in June 2012 with temperatures higher than 90°F) and several consecutive weeks with less than 0.16 inches of rainfall experienced in summer 2012. Arrowwood Viburnum, which prefers moist soils (USDA Plants Database, 2015), may have been negatively impacted if the soil
moisture content was lower due to the dryer conditions experienced during summer 2012. European Cranberrybush, on the other hand, is also fairly drought-tolerant, and it showed an increasing trend in energy density, fat content, and ash content from 2012 to 2013. However, European Cranberrybush has an extremely low fat content (Table 1-2) and these differences are not likely to be significant.

Three of the four fruits in this study that belong to the Order Rosales (Autumn Olive, Common Buckthorn, and Multiflora Rose) are invasive to the Rochester, NY area, and are known to be highly adaptable to various weather conditions, sunlight and shade levels, and moisture levels (USDA Plants Database, 2015). These fruits are lower in energy density and fat content than many native fruits (Figure 1-1, and 1-3). Each of these fruits showed a decreasing trend in ADF content and ash content from 2012 to 2013, although these differences are may not be significant due to overlap in error bars (Table 1-3). Autumn Olive showed a decreasing trend in energy density, fat content, and ash content, and an increasing trend in water content in 2013. Common Buckthorn experiences an increase in water content, and likely insignificant changes in other nutrients. As fall-harvested fruits, these trends may be explained by the lower precipitation levels and higher temperatures in summer 2012, and is consistent with trends in nutritional analyses in other studies. A study conducted on kiwifruit vines (Actinidia deliciosa) indicates that water stress during late summer (approximately 10 weeks prior to harvesting) increases TSS accumulation in fruits (Miller et al., 1998). Similar trends in soluble carbohydrate concentrations have been observed in leaves of other plants, including cherry (Ranney et al., 1991), and strawberry (Zhang and Archbold, 1993). Another study conducted on kiwifruit indicates a higher concentration of ash in fruits growing in warmer regions with less rainfall (Walton and De Jong, 1990). However, Miller et al. also indicated a hastening in the ripening time of water-stressed
fruits (1998). A similar trend in fruits at migratory bird stopover habitats may be detrimental to birds that are showing a delay in autumn migration timing. Multiflora Rose, which has lower levels of water content than Autumn Olive and Common Buckthorn (Table 1-2), experienced a decrease in the water content in 2013. This may be because Multiflora Rose fruits ripen later than the others, and may persist during winter (Szafoni, 2007), and the fall precipitation levels were lower in 2013 (9.91 inches) than in 2012 (13.17 inches). Riverbank Grape, on the other hand, is a native fruit that had a lower fat content than all other native fruits (Table 1-2), and is somewhat drought tolerant (USDA Plants Database 2015). Riverbank Grape showed an increasing trend in TSS content, and an increasing trend in ash content, which may indicate that this fruit is more resilient to changing climate patterns.

*Management and Conservation Implications*

Studies have indicated that populations of migratory birds are declining at substantial rates (Holmes and Sherry, 2001; Howe et al., 1989). Likely contributing factors for this declining trend include habitat loss (Robbins et al., 1989), changes in habitat vegetation structure and local disturbances (Holmes and Sherry, 2011), food availability (Both et al., 2006; Holmes and Sherry, 2011), and climate change (Both et al., 2006; See Crick, 2004). While many studies have focused on the impact of climate change on avian phenology (See Crick, 2004), few have focused on the impact of climate change on the nutritional quality of fruit resources available for birds during autumn migration. Understanding the impact of climate change on fruits is important, as it could be used to anticipate potential changes in the nutritional content of fruits that may impact the physiology of birds, and can therefore be made useful for conservation purposes. Invasive fruits in New York state have been show to produce fruits with lower fat content and energy density.
than native fruits (Smith et al., 2013), which is consistent with our results. However, this work did not focus on the resilience of invasive species vs. native species of plants to changing weather patterns. If invasive species such as Multiflora Rose, Autumn Olive, and Common Buckthorn are more adaptable to annual variation in temperature and precipitation, they may further outcompete high-quality native plants, such as Silky Dogwood and Red Osier Dogwood, with climate change in the years to come. On the other hand, Spicebush produced higher quality fruit during 2012. Gray Dogwood, showed little variation in nutritional quality between the years. This indicates that Spicebush and Gray Dogwood may be more resilient to changing climatic conditions, and may be a beneficial fruit resource for migratory birds in areas that are more prone to climate change. Therefore, habitat conservation efforts at stopover sites should focus not only on planting high-quality fruits, but also on planting those that are more resilient to climate change, to ensure the long-term availability of nutritionally adequate food sources for migratory birds.

**Scope for Further Research**

An important macronutrient that is not a part of this study is protein. Protein is an essential macronutrient for migratory birds at every stage of their life. In addition to being the building blocks of animal tissues, proteins may be especially important for migratory birds during long-distance flights - approximately 5-10% of protein may be catabolized during flights to compensate for depleting energy reserves (McWilliams et al., 2004). Studies have shown that the protein content of fruits is typically low (Smith et al., 2007; Izhaki 1998). A study conducted on seasonal variation in nutritional quality of fruits indicates that protein content does not significantly vary among fruits ripening during different seasons (Herrera, 1982). Additionally,
nitrogen content of fruit pulp is known to differ for fruits collected during different years (Willson and Whelan, 1993), which may be correlated with protein content of fruits. Therefore, it is important for future studies to focus on the annual variation in protein content of fruits due to changing climate.

While this study suggests that weather patterns may be associated with nutritional variations of some fruits, it is limited by small sample sizes. A more complete understanding of the impact of changing weather conditions on fruit quality could be obtained by expanding the study to include samples from more years. Trends may also be better understood if additional measurements of factors such as soil moisture content, and nutrient availability of the soil were taken into consideration and correlated with the nutritional composition of fruits.

Conclusion

This study focused on fruits collected in 2012 and 2013, of which 2012 was a generally warmer year. No consistent trends in the change in nutritional quality of fruits with respect to changing weather conditions between 2012 and 2013 were observed. Some fruits, such as Red Osier Dogwood, seemed to be of “higher quality” in 2013, as indicated by their increasing trend in fat and energy density, and a decreasing trend in acid detergent fiber and water content. On the other hand, Spicebush produced “lower quality” fruits in 2013, as indicated by a decreasing trend in energy density and fat, and an increasing trend in ash and water content. Gray Dogwood, Autumn Olive, Multiflora Rose, and Common Buckthorn showed less nutritional variation between the two years. This is a likely due to the difference in adaptability and resilience of each plant to changing climate, which are important considerations to take into account for management purposes, to ensure adequate conservation of bird stopover habitats.
Chapter Two: Using of Multidimensional Fluorescence Spectroscopy and Parallel Factor Analysis in Investigating Annual Variation in Nutritional Quality of Fruits

Abstract

Migratory birds experience oxidative stress due to the production of free radicals during long-distance migration, which may cause tissue and cellular damage. Consuming fruits high in antioxidant content may help combat free radicals, and alleviate oxidative stress. Polyphenols, including anthocyanins, are known for their antioxidant capacity and also fluoresce due to the presence of aromatic rings in their chemical structure. This property of phenolic compounds is utilized in this study to identify the presence of polyphenols in fruits available for migratory birds to eat at stopover sites during fall migration. The goal of this study was to investigate differences in fluorophore composition of fruits of 11 different species of shrubs available in the Rochester, NY area between two years. Extracts of fruits collected in 2012 and 2013 were analyzed using multidimensional fluorescence spectroscopy. Parallel factor analysis (PARAFAC), a multiway decomposition method that provides spectral characterizations and “scores” for fluorescent components in a sample set, was performed on the fluorescence excitation-emission matrices (EEMs). Component scores were then correlated with nutrients measured by traditional analytical methods in the fruits. The excitation-emission spectrum for component 2 resembled that of catechin, a phenolic compound, and correlated with the total phenol content of the fruits. The excitation-emission spectrum of component 3 resembled that of monomeric anthocyanin, but did not correlate with the monomeric anthocyanin data collected using a pH differential method. Each fruit showed a unique pattern of variation in its fluorophore concentration between the two years. The sum of PARAFAC scores decreased from 2012 to
2013 for Red Osier Dogwood, Multiflora Rose and Common Winterberry, and increased for Spicebush, Gray Dogwood, European Cranberrybush, Autumn Olive and Riverbank Grape. No trends in changes in fluorophore concentration were observed based on the relatedness or drought and temperature tolerance of species, and are likely a result of environmental factors including, but not limited to, differences in weather patterns during growing and harvesting seasons of each fruit during the two years.

Introduction

Migration is physiologically and behaviorally challenging for birds. In addition to an increase in energetic costs, annual migrations cause oxidative stress in birds, due to the formation of Reactive Oxygen Species (ROS), which are by-products of aerobic respiration. ROS include ‘free radicals’ or ‘oxidants’ which contain one or more unpaired electrons. Because electrons tend to exist in paired states, these free radicals actively scavenge for electrons from other molecules, including DNA, proteins and lipids. This can cause chain reactions which lead to the disintegration of cellular molecules. In the case that the level of oxidants exceeds the equivalent level of antioxidants needed to prevent damage, it leads to a condition known as ‘oxidative stress’ (Sies H 1996). Studies have shown that birds experience higher rates of oxidative stress during long-distance flights (Constantini et al, 2007). Therefore, obtaining dietary antioxidants during stopover periods during long-distance migration may be critical for birds to prevent oxidative stress.

Antioxidants are molecules which stabilize free radicals by donating electrons to these radicals, thus preventing cellular damage. One common group of antioxidants in fruits is anthocyanins, a colored sub-category of flavanoids (Jovanovic et al, 1994; El-Agamey et al.,
Anthocyanins are pigments that are responsible for producing blue, purple, and red colors in various parts of plants, including flowers, fruits, shoots and leaves (Simon, 1997). Anthocyanins are considered powerful antioxidants because of their ability to scavenge free radicals (Gould 2004). Due to their antioxidant capacity, anthocyanins have been linked with anti-carcinogenic, anti-inflammatory and anti-angiogenic properties (Bagchi et al., 2004).

Behavioral studies indicate that polyphenols are of dietary importance to birds. A study conducted on wild-caught blackcaps (Sylvia atricapilla), a frugivorous European songbird, suggests that the birds preferentially select foods rich in flavonoids (Catoni et al., 2008). Studies indicate that birds are able to detect, and often seek, fruits rich in anthocyanin, which they use as an honest signal of antioxidant rewards in the fruits (Schaefer et al., 2008). A study conducted by Schaefer et al. studied the color, anthocyanin content and carotenoid content of 60 fruits dispersed by birds (2008). An avian eye model was also used, to understand how birds are able to discriminate between fruits with different nutritional qualities. They found that birds are capable of visually detecting differences in anthocyanin content, and select anthocyanin-rich fruits over anthocyanin-deficient fruits. Bolser et al. (2013) evaluated the total antioxidant, total phenolic content and total anthocyanin content in several fruits in Rhode Island, coupled with measurements of fruit consumption by birds during fall migration. Their results suggest that birds prefer fruits with more anthocyanins and other phenolic compounds.

Studying the nutritional quality of fruits available for migratory birds is challenging and time consuming because of the wide variety of nutrients that must be quantified. Different assays and protocols exist for each nutrient. It is therefore important to explore novel research techniques that can be used for comprehensive analysis of the nutritional quality of fruits. One technique that may be useful in measuring nutritional quality is multidimensional fluorescence.
spectroscopy, coupled with chemometric analysis. The spectrofluorometry technique involves recording 3-dimensional “fingerprints” for each fruit, which provides the excitation and emission spectra of fluorescent molecules in the fruits at various wavelengths. The Excitation-Emission Matrices (EEMs) can be correlated with the nutritional content of the fruits using parallel factor analysis (PARAFAC), a multiway decomposition method that provides a “score” for each fluorophore contained in the sample. The scores are then compared to the concentrations of the fluorophores obtained through chemometric analyses of the fruit samples. PARAFAC was successfully used by a study done by Pagano et al. to study dissolved organic carbon in natural water samples (2012). This technique has also been used on red wines (Airado-Rodriguez et al., 2009), yogurt (Christensen et al. 2005) and vinegars (Callejón et al. 2012).

The purpose of this study is to assess the phenolic content of fruits available for migratory birds during fall migration using multidimensional fluorescence spectroscopy and PARAFAC. Fruits from 2012 and 2013 were used, in order to evaluate if this technique can be useful in detecting variation in fruit quality between two different years. Data obtained from the National Oceanic and Atmospheric Administration archives indicates that the average temperature was 2.93°F higher in 2012 than 2013, between January - October. As indicated by Barnuud et al. (2013), anthocyanin content declines with increasing temperatures. Therefore, we hypothesize that the EEMs of fruits will indicate that the fluorescence of fruits will be higher in 2013 than in 2012.
Methods

Collecting, Drying and Storing Fruits

The fruits collected for analysis are summarized in Table 1-1. Each fruit was collected from one of two study sites, the Rochester Institute of Technology campus, or Braddock Bay Bird Observatory. Fruits were collected during autumn migration in 2012 and 2013 at the peak of ripeness (August-September), and immediately frozen at -20°C. The fruits were then dissected to remove seeds, and freeze dried at -80°C and 0.051 Torr pressure for two days. Frozen fruits were homogenized and stored in glass vials at -20°C until they were used extracted for analyses.

Fruit Extraction

An acidified methanol extract was prepared for each fruit to obtain the fluorescence EEMs, to analyze the phenolic content, and to determine the total monomeric anthocyanin content of fruits. 0.5 g of freeze-dried fruit was dissolved in a solvent containing 0.1% HCl, 49.9% nanopure water and 50% methanol. The solution was vortexed for 30 seconds, shaken for 30 minutes, and centrifuged for 15 minutes. The supernatant was collected, and the process was repeated two additional times using the same fruit sample before the fruit ‘pellet’ was discarded. The methanol was then evaporated from the solution using a vacufuge, and stored at -20°C until it was used for analyses.

Obtaining Excitation Emission Matrices (EEMs) of Fruits

Fluorescence EEMs of fruit extracts were generated using the protocol described by Pagano et al. (2012). The extracts were measured at room temperature in a 10 mm pathlength quartz cuvette using a Varian Cary Eclipse fluorometer. Scans were collected at excitation
wavelengths ranging from 240 nm to 540 nm, at 5 nm intervals. Emission was scanned at wavelengths ranging from 295 nm to 550 nm, at 1 nm intervals. The raw EEMs were corrected for inner filter effects and Rayleigh and Raman scatter were removed per Pagano et al. (2012), using a MATLAB Program developed by Hall et al. (2005). Rayleigh and Raman scatter (reflection) form due to the presence of small particles in the sample that do not contribute to the fluorescence profile of the fruits need to be removed prior to PARAFAC modelling (Andersen and Bro, 2003). Nanopure water was used as a blank to remove scatter effects from raw fruit EEMs.

Obtaining a PARAFAC Model

Corrected EEMs from 19 fruits samples (8 from 2012 and 11 from 2013) were organized into a three-way data array and modelled using PARAFAC routines, per the PLS_Toolbox 7.0 software (Eigenvector Research, Inc., 2012), according to the tutorial by Stedmon and Bro (2008). A four component PARAFAC model was selected based on the percent variance explained by the model, residual analysis, core consistency analysis, and visual inspection of components per Andersen and Bro (2003). Each component in the PARAFAC model has distinct excitation and emission spectra, and a loading “score” for each fruit sample in the dataset. Initially, extracts of Common Buckthorn (*Rhamnus carthartica*) from 2012 and 2013 were included in the PARAFAC analysis. However, this fruit were removed from the final PARAFAC analysis because the intensity of the signal of one of the fluorescent molecules in Buckthorn was confounding the PARAFAC model, making the model convergence poor. As a result, Common Buckthorn will be the subject of future studies to determine its unique fluorescence fingerprint, but was not included in the PARAFAC model presented here.
Measuring the Total Phenol Content of Fruits

A modified microplate version of the Folin-Ciocalteu assay described by Magalhães et al. (2010) was used to determine the total phenolic content of the fruits. This method involves the reduction of the Folin reagent by the phenolic compounds in the fruit, which leads to the formation of a colored complex, the absorbance of which was measured at 760 nm. Gallic acid was used as the standard. To minimize effects of sample dilution and obtain accurate results, three dilutions were prepared for each fruit samples, such that the absorbance of each was within the absorbance range of the standard curve, per Sipel et al. (2013). The total phenolic content is reported in mg/L Gallic Acid Equivalents (GAE).

Measuring the Total Monomeric Anthocyanin Content of Fruits

The total monomeric anthocyanin of fruits was measured using a pH differential method described by Lee et al. (2005). This method is based on a property of anthocyanin that allows it to change color at different pH levels (colored oxonium at pH 1.0, and colorless hemiketal at pH 4.5). Two buffers were used as reagents – a 0.025M potassium chloride buffer adjusted to pH 1.0, and a 0.4M sodium acetate buffer adjusted to pH 4.5. The method described by Lee et al. (2005) was modified from a cuvette to a 96-well microplate, by adjusting the volume of solutions used. The difference in absorbance of the fruits in each buffer was calculated at 520nm to determine the monomeric anthocyanin concentration of the fruits, using the following equation (per Lee et al., 2005).

\[
\text{Anthocyanin Pigment Concentration} = \frac{A \times MW \times DF \times 1000}{\exp}
\]

Where \( A = (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH 1.0}} - (A_{520\text{nm}} - A_{700\text{nm}})_{\text{pH 4.5}} \)

\( \text{MW} = \text{molecular weight of cyanidin-3-glucoside} = 449.2 \text{ g/mol} \)
DF = dilution factor

\[ \varepsilon = \text{molar extinction coefficient} = 26900 \, \text{L/mol/cm for cyanidin-3-glucoside (Jurd and Asen, 1966; see also Lee et al., 2005)} \]

P = pathlength, in cm

1000 = conversion factor from g to mg

The anthocyanin concentration is reported in mg/L cyanidin-3-glucoside equivalents.

**Data Analysis**

The PARAFAC model was selected based on visual inspection of components, and modeling statistics including the percent of variance explained by the model, and residual analysis. The excitation and emission loadings of relevant components were identified by visual inspection, and by comparing them with spectra reported in the literature. The EEMs, PARAFAC scores, total phenol content and total anthocyanin content were examined to identify variation in nutritional composition of fruits between 2012 and 2013. Differences in PARAFAC scores of fruits between the two years were tested using a paired t-test. The data were tested for normality using the Anderson-Darling test. The PARAFAC scores of fruits were correlated with the total phenol content and the total monomeric anthocyanin content of fruits using Spearman rho correlation.
Results

Unique 3-dimensional EEMs were obtained for each individual fruit, which were used to obtain a PARAFAC model for the complete dataset. Examples of some of the fruit EEMs are shown in Figure 2-3. Unique EEMs with strong signals were observed for all samples in the dataset.

The Four-Component Model

The four component model was selected as the optimum model for our comparison (Table 2-1, Figure 2-1). This model explained 97.34% of variance in the dataset. There was little visible difference between the corrected EEMs and modelled EEMs for fruits, and a low amount of residual fluorescence (see Figure A-1), which supports the validity of the four component PARAFAC model used.

Table 2-1: PARAFAC models obtained for extracts of 11 fruits collected in 2012 and 2013.

<table>
<thead>
<tr>
<th>Components</th>
<th>Iterations</th>
<th>% Variance Explained</th>
<th>Sum of Squares Residuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>73.572</td>
<td>1.05E+09</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>92.176</td>
<td>3.12E+08</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>96.146</td>
<td>1.54E+08</td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>97.348</td>
<td>1.06E+08</td>
</tr>
<tr>
<td>5</td>
<td>255</td>
<td>98.013</td>
<td>7.92E+07</td>
</tr>
<tr>
<td>6</td>
<td>219</td>
<td>99.261</td>
<td>2.95E+07</td>
</tr>
<tr>
<td>7</td>
<td>347</td>
<td>99.517</td>
<td>1.92E+07</td>
</tr>
</tbody>
</table>
The excitation and emission loadings for each component were visually inspected, and compared to similarly fluorescing fluorophores in the literature. Component 1 has an excitation maxima at < 240nm, and an emission maxima at 324nm (Figure 2-1). Component 2 has a peak excitation at 275nm, and a peak emission at 316nm (Figure 2-1). Component 3 has peak excitation wavelengths of 265nm, and peak emission wavelengths at 350nm (Figure 2-1). Component 4 has peak excitations at <240 and 310nm, and emission maxima at 420nm.
PARAFAC Scores and Annual Variation in Fluorescence of Fruits

No consistent trends were observed in the variation in component scores and sum of PARAFAC scores from 2012 to 2013 (p<0.05 for all components, as indicated by a paired t-test). Each of the 19 fruit samples has a unique PARAFAC score for each component, as well as a unique sum of PARAFAC scores. Some samples may be missing certain components entirely. For example, the PARAFAC profile for 2013 Arrowwood Viburnum indicates the absence of component 3, and the PARAFAC profile for 2012 and 2013 Riverbank Grape indicates the absence of component 2 (Table 2-2). The change in the amount of each component between the two years varies between each species (Table 2-2 and Table 2-3).

The sum of PARAFAC scores varies for each fruit between the two years. Figure 2-2 depict radar plots of fruits, and shows the increase or decrease in the sum of PARAFAC scores from 2012 to 2013. Spicebush, Gray Dogwood, European Cranberrybush, Autumn Olive, and Riverbank Grape fruits show an increase in the sum of their component scores in 2013, whereas Red Osier Dogwood, Common Winterberry, and Multiflora Rose show a decrease in the sum of their components in 2013 (Figure 2-2). Autumn Olive stands out as the fruit that had the largest change in its sum of PARAFAC scores, which increased over 11 times from 2012 to 2013 (Figure 2-2). Spicebush and European Cranberrybush also showed an increase in their PARAFAC score (2.9 and 2.5 time increase, respectively; Table 2-2; Figure 2-2). Common Winterberry showed the greatest decrease in sum of PARAFAC score, which decreased by 2.3 times from 2012 to 2013 (Table 2-2; Figure 2-2).

The relative percent of each PARAFAC component remained somewhat unchanged between the two years for Red Osier Dogwood, European Cranberrybush, Common Winterberry, and Riverbank Grape (Table 2-3, Figure A-2), and changed between years in the case of
Spicebush, Gray Dogwood, Autumn Olive, and Multiflora Rose (Table 2-3, Figure A-2). Of these, Autumn Olive stands out as the fruit that experienced the most variation between the two years. The 2012 sample of Autumn Olive contained 15.58% component 1, whereas the 2013 sample contained 76.19% component 1 (Figure A-2). Gray Dogwood also showed variation in chemical composition between years with component 1 absent from the 2012 sample of Gray Dogwood but comprising 14.4% of the sample in 2013 (Figure A-2).

Table 2-2: Summary of the concentration of monomeric anthocyanin, phenol, and each component present in each sample. The concentration of each component, monomeric anthocyanin and phenol content is in Raman units, mg/L cyanidin-3-glucoside equivalents, and mg/L gallic acid equivalents respectively.

<table>
<thead>
<tr>
<th>Fruit Species</th>
<th>Year</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>Monomeric Anthocyanin Content</th>
<th>Phenol Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spicebush</td>
<td>2012</td>
<td>0.00</td>
<td>983.26</td>
<td>2549.20</td>
<td>2257.47</td>
<td>0.00</td>
<td>534.26</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>746.61</td>
<td>5669.14</td>
<td>8272.85</td>
<td>1983.36</td>
<td>17.45</td>
<td>571.06</td>
</tr>
<tr>
<td>Silky Dogwood</td>
<td>2013</td>
<td>267.39</td>
<td>2348.04</td>
<td>941.23</td>
<td>4293.63</td>
<td>70.09</td>
<td>1392.00</td>
</tr>
<tr>
<td>Gray Dogwood</td>
<td>2012</td>
<td>0.00</td>
<td>1439.95</td>
<td>983.18</td>
<td>1224.26</td>
<td>1.89</td>
<td>756.61</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>832.66</td>
<td>2287.20</td>
<td>728.58</td>
<td>1931.60</td>
<td>0.53</td>
<td>633.13</td>
</tr>
<tr>
<td>Red Osier Dogwood</td>
<td>2012</td>
<td>0.00</td>
<td>4657.50</td>
<td>5598.20</td>
<td>3471.65</td>
<td>3.51</td>
<td>620.54</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>174.16</td>
<td>2479.90</td>
<td>3160.52</td>
<td>2119.03</td>
<td>0.55</td>
<td>701.17</td>
</tr>
<tr>
<td>Bush Honeysuckle</td>
<td>2013</td>
<td>312.17</td>
<td>2216.38</td>
<td>687.91</td>
<td>1247.22</td>
<td>0.00</td>
<td>406.28</td>
</tr>
<tr>
<td>Arrowwood Viburnum</td>
<td>2013</td>
<td>1266.13</td>
<td>1672.45</td>
<td>0.00</td>
<td>2480.58</td>
<td>1828.75</td>
<td>2531.88</td>
</tr>
<tr>
<td>European Cranberrybush</td>
<td>2012</td>
<td>1066.71</td>
<td>3352.91</td>
<td>59.23</td>
<td>4608.15</td>
<td>70.63</td>
<td>2935.55</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>3891.65</td>
<td>7616.61</td>
<td>59.23</td>
<td>4608.15</td>
<td>70.63</td>
<td>2935.55</td>
</tr>
<tr>
<td>Common Winterberry</td>
<td>2012</td>
<td>3320.01</td>
<td>2174.29</td>
<td>2055.07</td>
<td>5175.47</td>
<td>8.64</td>
<td>1020.02</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>1056.65</td>
<td>1015.09</td>
<td>808.32</td>
<td>2623.54</td>
<td>12.00</td>
<td>1086.66</td>
</tr>
<tr>
<td>Autumn Olive</td>
<td>2012</td>
<td>346.41</td>
<td>439.25</td>
<td>965.69</td>
<td>472.02</td>
<td>5.67</td>
<td>256.41</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>19611.74</td>
<td>1402.60</td>
<td>767.67</td>
<td>3957.01</td>
<td>3.16</td>
<td>482.18</td>
</tr>
<tr>
<td>Common Buckthorn</td>
<td>2012</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>392.02</td>
<td>5845.59</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>559.79</td>
<td>6476.84</td>
</tr>
<tr>
<td>Multiflora Rose</td>
<td>2012</td>
<td>958.42</td>
<td>1616.02</td>
<td>328.08</td>
<td>2274.21</td>
<td>3.78</td>
<td>311.22</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>427.87</td>
<td>559.41</td>
<td>215.09</td>
<td>1597.92</td>
<td>1.05</td>
<td>428.90</td>
</tr>
<tr>
<td>Riverbank Grape</td>
<td>2012</td>
<td>474.94</td>
<td>0.00</td>
<td>2594.77</td>
<td>1623.17</td>
<td>1030.01</td>
<td>1316.39</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>307.37</td>
<td>0.00</td>
<td>3621.12</td>
<td>1678.45</td>
<td>1417.52</td>
<td>1212.40</td>
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Table 2-3: The change in the amount of each PARAFAC component, monomeric anthocyanin content, and total phenol content from 2012-2013. +/- indicates a less than 2-fold change, ++/-- indicates between 2-5 fold change, +++/-- indicates over a 5-fold change. * indicates that the component was not present in the 2012 sample of the fruit. a indicates that the component was not present in the samples from both years.

Note: The amount of component 1 in Autumn Olive increased over 56 times in 2013.

<table>
<thead>
<tr>
<th>Fruit</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>Monomeric Anthocyanin Content</th>
<th>Total Phenol Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spicebush</td>
<td>*</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>*</td>
<td>+</td>
</tr>
<tr>
<td>Gray Dogwood</td>
<td>*</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Red Osier Dogwood</td>
<td>*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>European Cranberrybush</td>
<td>+</td>
<td>+</td>
<td>*</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Common Winterberry</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Autumn Olive</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Multiflora Rose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Riverbank Grape</td>
<td>-</td>
<td>0a</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Figure 2-2: Radar plots showing the amount of each component in Spicebush, Gray Dogwood, Red Osier Dogwood, European Cranberrybush, Common Winterberry, Autumn Olive, Multiflora Rose, and Riverbank Grape.
Fluorescence Profiles of Select Fruits

In addition to the PARAFAC scores, variation between years can be observed in the EEMs of fruits. The fluorescence profiles of Autumn Olive for 2012 and 2013 showed substantial variation in the intensity of fluorescence, as well as the chemical composition, and the intensity of fluorescence for Autumn Olive was over 20 times more in 2013 than 2012 (Figure 2-3). In addition, Component 1 is visible in the EEM of the 2012 sample of Autumn Olive, but it is not present in 2013 (Figure 2-3). The EEM of European Cranberrybush did not show as much variation between the two years as did Autumn Olive; however, the intensity of fluorescence was over twice as much in 2013 than in 2012 (Figure 2-3).

Figure 2-3: The Excitation-Emission Matrices of 2012 and 2013 samples of Autumn Olive and European Cranberrybush.
Total Monomeric Anthocyanin Content

The monomeric anthocyanin content of all fruits was lower than 100 mg/L cyanidin-3-glucoside equivalent, with the exception of Arrowwood Viburnum, Riverbank Grape, and Common Buckthorn (Table 2-1). The 2013 sample of Arrowwood Viburnum had the highest monomeric anthocyanin concentration (1828 mg/L cyanidin-3-glucoside equivalent), followed by the 2013 and 2012 Riverbank Grape samples (1417 mg/L cyanidin-3-glucoside equivalent and 1030 mg/L cyanidin-3-glucoside equivalent, respectively; Table 2-1), and the 2013 and 2012 Common Buckthorn samples (560 mg/L cyanidin-3-glucoside equivalent and 392 mg/L cyanidin-3-glucoside equivalent respectively; Table 2-1). The next highest concentrations were observed in 2013 Silky Dogwood (70 mg/L cyanidin-3-glucoside equivalent), 2013 European Cranberrybush (70 mg/L cyanidin-3-glucoside equivalent), and 2012 European Cranberrybush (65 mg/L cyanidin-3-glucoside equivalent). Riverbank Grape, Common Buckthorn, and European Cranberrybush showed an increasing trend in monomeric anthocyanin content in 2013 (Table 2-2).

Total Phenol Content

The total phenol content was highest for 2013 European Cranberrybush (2935 mg/L gallic acid equivalent), and 2013 Arrowwood Viburnum (2531 mg/L gallic acid equivalent), and lowest for 2012 Autumn Olive (256 mg/L gallic acid equivalent) (Table 2-2). The total phenol concentration for all fruits increased from 2012 to 2013, with the exception of Riverbank Grape and Gray Dogwood (Table 2-3).
Correlating Components with Phenol and Monomeric Anthocyanin Content

The data for total phenol content, monomeric anthocyanin and each of the components were not normally distributed, and the Spearman rho test for correlation (for nonparametric data) was conducted. The test indicated a positive correlation between phenol content and component 2 \((r = 0.644, p\text{-value} = 0.013)\) – Figure 2-4. Riverbank Grape (2012 and 2013), Arrowwood Viburnum (2013), Red Osier Dogwood (2012) and Spicebush (2013) were masked from this graph, as they were skewing the correlation (see discussion section).

Figure 2-4: Spearman rho correlation between component 2 and total phenol content, \(r = 0.644\), p-value < 0.05. AO indicates Autumn Olive, EC indicates European Cranberrybush, GD indicates Gray Dogwood, MFR indicates Multiflora Rose, RH indicates Red Honeysuckle, S indicates Spicebush, SD indicates Silky Dogwood, W indicates Common Winterberry. 12 indicates the fruit sample was from 2012; 13 indicates the fruit sample was from 2013.

The monomeric anthocyanin data did not correlate with component 3 \((r = -0.055, p\text{-value} > 0.05)\), or any other component (p-value > 0.05 for all).
Discussion

Identifying Components

Visual analysis of the characteristic peaks of component 2 at 275nm (excitation peak) and 316nm (emission peak) suggests that this component may be phenolic, and resembles the spectrum of catechin (Airado-Rodríguez et al., 2011). This component also correlates with the total phenol content data obtained using the Folin-Ciocalteu assay (Figure 2-4). It is, however, important to note that Riverbank Grape (2012 and 2013), Arrowwood Viburnum (2013), Red Osier Dogwood (2012) and Spicebush (2013) were masked from the data in order to obtain the graph shown in Figure 2-4. Riverbank Grape and Arrowwood Viburnum had a low amount of component 2 for the amount of phenol content detected by the Folin-Ciocalteu assay. If component 2 is catechin-like, this discrepancy could be explained if PARAFAC is only detecting catechin-like fluorophores as component 2, while the Folin-Ciocalteu assay is detecting multiple reducing agents that are phenolic in nature, but may not be catechin (Magalhães et al., 2010). Spicebush and Red Osier Dogwood, on the other hand, had a high amount of component 2, but lower total phenol contents. Different groups of fluorophores represented in the PARAFAC model have varying quantum yields and antioxidant capacities. It is conceivable that a molecule with a strong quantum yield could have a weaker antioxidant capacity- skewing the data to show a strong component score with a lower relative corresponding assay phenol concentration.

Visual analysis of the spectral profile of component 3 indicates that it peaks at 265nm (excitation) and approximately 350nm (emission). The excitation and emission peaks for component 3 are not clear and well-defined, and is therefore difficult to identify with certainty. However, this component might be attributed to anthocyanin-like components, as it is similar to the anthocyanin spectrum identified by Airado-Rodriguez (2009), and also the UV-Vis
absorption spectrum of anthocyanins studied by Andersen et al. (2004). The Riverbank Grape samples had high amounts of monomeric anthocyanin (Table 2-2), which is characteristic of many fruits of the Vitis genus and its hybrids (Zhao et al., 2010), and it also had a relatively high amount of component 3 (Table 2-2). However, the results obtained from the monomeric anthocyanin assay did not correlate with component 3. This may be attributed to several causes. The monomeric anthocyanin assay is a pH differential assay, which was modified from a cuvette to a microplate and the modified microplate method used does not account for a limit of detection. While this allowed us to run several samples at a time, it also decreased the path length of each sample by almost 20%, which may have reduced the sensitivity of the test. For example, the fruit with the highest amount of monomeric anthocyanin is Arrowwood Viburnum (Table 2-2), which is consistent with published literature (Bolser et al., 2013). However, the Arrowwood Viburnum sample had no component 3 according to the PARAFAC results. This discrepancy can be explained if PARAFAC is detecting the presence of polymeric anthocyanin, and the Arrowwood Viburnum samples only contained monomeric anthocyanin. However, this does not explain the presence of component 3 in anthocyanin-deficient fruits such as Red Osier Dogwood and Spicebush. The sample size for fruits which are known to be rich in anthocyanin was not large enough in this study to obtain a viable correlation. Three fruits had a monomeric anthocyanin concentration higher than 100 mg/L cyaniding-3-glucoside (Arrowwood Viburnum, Riverbank Grape, and Common Buckthorn). Of these, Common Buckthorn was not included in the PARAFAC model as it was skewing the data. This further limited the sample size to obtain an effective correlation between component 3 and monomeric anthocyanin content. Therefore, these discrepancies may be a topic for future studies.
Component 1 has an excitation peak at < 240 nm, and an emission peak at 324nm, which resembles protein-like fluorophores, such as tyrosine and tryptophan, which exhibit peak emission between 300 – 305 nm and 340 – 350nm respectively, when excited at 220 – 275nm (In: Coble, 1996). Component 4 exhibits peak excitations at <240nm and 310nm, and peak emission at 420nm, which resembles the spectra of humic-like substances formed as a result of the degradation of dead organic matter in studies of dissolved organic matter in water bodies (Pagano et al., 2012; Coble 1996). While these fruits may not contain degraded components of humics, their biomass may contain the same starting material (such as lignin) of the degradation process.

Annual Variation in Fluorophore Concentration of Fruits

Many studies conducted on the impact on climate change on fruits have focused on nutrition related to humans. For example, a study conducted on grapes for the production of Pinot Noir wines reports that summer warming is likely to have a negative impact on the phenolic content of grapes (Nicholas et al., 2011). Another study conducted on pomegranate fruits indicates an inverse relationship between seasonal temperatures and anthocyanin content (Borochov-Neori et al., 2011). However, not all studies indicate this trend – strawberries produce less phenolic acid, flavonols and anthocyanins when grown at cooler temperatures (Wang and Zheng, 2001). Few studies have researched phenol and anthocyanin content of wild berries in the context of migratory bird nutrition (Bolser et al., 2013), and none, to my knowledge, have studied the impact of climate change on the phenol and anthocyanin concentration of these berries. Additionally, empirical differences in the Folin-Ciocalteu method for quantifying total phenol content, and the pH differential method for quantifying total monomeric anthocyanin
content makes direct comparisons to other studies challenging. However, the study conducted by Bolser et al., explains that spatial variation in antioxidant concentrations in fruits may be attributed to factors such as soil moisture content, and microclimate, which may also explain some of the temporal variation in the concentration of fluorophores in our samples for the two years (2013).

The fluorescence profile of each fruit species changed from 2012 to 2013. Few trends were seen in the fluorophore composition of fruits when grouped by taxonomic relatedness. For example, three fruits in this study that belong to the Cornaceae family are Gray Dogwood, Silky Dogwood and Red Osier Dogwood. Of these, only Gray Dogwood and Red Osier Dogwood were collected in 2013. Both these fruits lacked component 1 during 2012, but not in 2013, and showed a decrease in component 3 in 2012. However, they showed opposing trends for all other analytes (Table 2-2 and 2-3). Additionally, Autumn Olive, Multiflora Rose, and Riverbank Grape belong to the clade Rosid, but show different trends in the change in concentration of each component between the two years. Autumn Olive showed the greatest change – the total concentration of fluorophores increased over 10-fold, with component 1 increasing by over 56 times in 2013. Riverbank Grape, on the other hand, showed the least amount of change in fluorophore concentration. Unlike Autumn Olive and Riverbank Grape, Multiflora Rose showed a decreasing trend in the sum of PARAFAC scores in 2013. This suggests that temperature and precipitation are likely not the only factor influencing the fluorophore concentration of fruits. Other environmental factors, such as microclimate during growing seasons and ripening, soil chemistry, and foliar nutrients may be impacting the biochemical composition of these fruits, as is the case for the leaves of *Myrcia tomentosa*, a flowering plant in the Rosid clade (Borges et al., 2013).
The sum of PARAFAC scores of most, but not all, fruits increased in 2013, which is consistent with the original hypothesis. Multiflora Rose, Common Winterberry, and Red Osier Dogwood showed a decrease in the total concentration of fluorophores. Component 3 in Riverbank Grape increased in 2013, which is believed to be an indicator of anthocyanin content. This is consistent with the results from the monomeric anthocyanin assay, which indicates an increase in the monomeric anthocyanin content of Riverbank Grape (Tables 2-3 and 2-4). The monomeric anthocyanin content of Common Buckthorn also showed an increasing trend in 2013. The total phenol content of all but two species was higher in 2013 than in 2012. Riverbank Grape and Gray Dogwood showed a slight decrease in phenol content. These results are consistent with the prediction in the study conducted by Barnuud et al. (2013) and Nicholas et al. (2011), and indicate that anthocyanin content and total phenol content may be negatively correlated with higher temperatures.

**PARAFAC as a Tool for Determining the Nutritional Quality of Fruits**

The results indicate that fluorescence spectroscopy with PARAFAC is a useful tool in determining the phenolic and antioxidant potential of fruits available for migratory birds during fall migration. Unique fluorescence profiles can be obtained for fruits of the same or different species, and PARAFAC is able to display small changes in the fluorescent molecules in fruits between years. This procedure is able to provide a broad description of the fluorophore composition of fruits which may be correlated with phenol and anthocyanin content. Therefore, it may be a comprehensive and more practical method of detecting the presence of certain fluorescent chemicals in fruits than other methods such as High-Performance Liquid Chromatography (HPLC) (See Bugden et al., 2008). However, further studies are needed to
demonstrate the best parameters for the application of PARAFAC in wild fruit extracts. Fluorescence spectroscopy coupled with techniques such as HPLC have been used in previous studies (Reynolds, 2003), and a similar approach may help definitively identify components present in PARAFAC samples of wild fruits. Expanding to additional years to the dataset would provide a better understanding of the impact of climate change on fruit biochemical composition. One disadvantage of the model used in this study is that it contained several unrelated species, with various temperature and drought tolerance levels, and PARAFAC models with more similar fruits may provide more information on the chemical composition of these fruits.

**Conclusion**

In this study, we investigated the usefulness of multidimensional fluorescence spectroscopy and parallel factor analysis for identifying and quantifying the fluorescent molecules present in wild fruits. Some fruits showed an increase in the sum of PARAFAC scores between years (Spicebush, Gray Dogwood, European Cranberrybush, Riverbank Grape, and Autumn Olive) whereas others showed a decrease in the sum of PARAFAC scores from 2012 to 2013 (Red Osier Dogwood, Common Winterberry, Multiflora Rose). The four components in the PARAFAC model were identified as protein-like, catechin-like, anthocyanin-like, and humic-like fluorophores. The total phenol content data obtained correlated with the catechin-like component 2, but the monomeric anthocyanin data for fruits did not correlate with anthocyanin-like component 3, which may be attributed to two factors – the reduced sensitivity of the pH differential method that was modified from a cuvette to a microplate in this study, and the small sample size of fruits high in anthocyanin (5 fruits samples, of which 2 were not a part of the PARAFAC dataset). Fruits that had a higher anthocyanin content (Riverbank Grape and
Common Buckthorn) showed an increasing trend in 2013, which was generally a cooler year than 2012. This is consistent with published literature that predicts a decline in anthocyanin content of grape with high temperatures due to climate change.
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Appendix

Figure A-1: EEMs for Gray Dogwood (collected in 2012) showing corrected EEM (top), a PARAFAC modelled EEM (middle), and a PARAFAC residuals EEM (bottom). The fluorescence intensity is in Raman units (R.U.)
Figure A-2 (continued on next page): Pie charts depicting the relative percent of each component in the 2012 and 2013 samples of Spicebush, Gray Dogwood, Red Osier Dogwood, European Cranberrybush, Common Winterberry, Autumn Olive, Multiflora Rose, and Riverbank Grape.