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Of turtles and trees: Nutritional analysis of tree heliotrope (*Heliotropium foertherianum*) leaves consumed by green turtles (*Chelonia mydas*) in Hawai‘i[*]

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Abstract— Fallen, senescent leaves of tree heliotrope (*Heliotropium foertherianum*), a ubiquitous shoreline tree from the Indian Ocean, tropical Asia, Australia, and the western and central Pacific, are a novel food item for green turtles (*Chelonia mydas*) in Hawai‘i. Leaves were collected in two seasons and analyzed for proximate nutrients, including dry matter, ash, crude fat, crude protein, carbon:nitrogen (C:N) ratio, gross energy, as well as dietary fibers (ADF and NDF), lignin, and total phenol content. The senescent leaves showed a high C:N ratio, and measurable phenolic compounds, but were low in protein ($\bar{x}$ = 5.5%) and fat ($\bar{x}$ = 2.2%) based on dry weight. Gross energy averaged > 4500 Kcal/kg ash-free dry weight; therefore, *H. foertherianum* could provide more calories than other marine food sources for Hawaiian green turtles. Green turtles elsewhere within the broad geographic range of tree heliotrope’s distribution may also consume senescent leaves that fall into coastal waters.

Introduction

Green turtles (*Chelonia mydas* L.) in the Hawaiian Islands have eclectic diets composed of over 275 species of macroalgae, two seagrass species (Balazs 1980, Balazs et al. 1987, Russell & Balazs 1994a, 1994b, 2000, 2009, Russell et al. 2003, Arthur & Balazs 2008), one species of terrestrial turfgrass (McDermid et al. 2015), sponges, and other animal food (Russell et al. 2011). However, we observed green turtles repeatedly consuming senescent leaves (Fig. 1) of tree heliotrope (*Heliotropium foertherianum* Diane & Hilger) that had fallen into the nearshore waters on Hawai‘i Island. Although leaves and other parts of black mangrove (*Avicennia schaueriana*) and gray mangrove (*A. marina*) trees growing intertidally can be significant components of green turtle diets

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in parts of Australia, Brazil, Galápagos Islands, Colombia, and Baja California (Pritchard 1971, Pendoley & Fitzpatrick 1999, Limpus & Limpus 2000, López-Mendilaharsu et al. 2005, Amorocho & Reina 2007, Arthur et al. 2009, Carrión-Cortez et al. 2010, Guebert-Bartholo et al. 2011, Nagaoka et al. 2012), no other study on green turtle foraging ecology has reported leaves of the terrestrial tree, *H. foertherianum* as a food item (Bjorndal 1980, Mortimer 1981, 1982, Thayer et al. 1982, 1984, Mendonça 1983, Bjorndal 1985, Garnett et al. 1985, Ross 1985, Forbes 1996, Bjorndal 1997, Seminoff et al. 2002, Fuentes et al. 2006, Sterling et al. 2013).

Tree heliotrope, *Heliotropium foertherianum* (Family Boraginaceae) (Hilger & Diane 2003), originally described as *Messerschmidia argentea*, renamed *Tournefortia argentea,* and then *Argusia argentea,* grows as a shrub or small tree (up to about 6 m tall) near the littoral zone along shorelines (Fig. 2), and tolerates shallow, infertile, and saline soils (Manner & Elevitch 2006). The tree is distinguished by an umbrella-shaped silhouette; light green, oblong leaves with a silvery-gray sheen (7.5 -18 cm long and 2.5-6 cm wide); and small white flowers crowded in curved or coiled clusters (Manner & Elevitch 2006). As *H. foertherianum* leaves age, chlorophyll degradation occurs, the leaves yellow, and nutrients, especially nitrogen, are recycled to other parts of the plant.

Tree heliotrope, well-known under a variety of common names, is native to tropical Asia, tropical Australia, and islands of the western Indian Ocean, Polynesia, and Micronesia (Falanruw et al. 1990, Whistler 1992a, Merlin et al. 1994, Thaman 1994, Little & Skolmen 2003, Manner & Elevitch 2006). In the Hawaiian Islands, *H. foertherianum* was a “post-Cook” introduction (after Captain James Cook’s 1778 arrival in the Hawaiian Islands), and was first noted by the botanist Hillebrand in 1871 (Little & Skolmen 2003). Tree heliotrope is now a naturalized species on the shorelines of some of the high islands of the Main Hawaiian Islands and on the low atolls of the Northwestern Hawaiian Islands.

Although previously unknown in green turtle diets, island peoples use tree heliotrope as food, fodder, and traditional medicine (Manner & Elevitch 2006). Green *Heliotropium* leaves are eaten raw in Kiribati and India, and used as a cooking spice in Tokelau (Thaman 1993, Manner & Elevitch 2006). In the past, the leaves were used as famine food in the Maldives (Romero-Frias 2013). In Nauru and Tokelau, tree heliotrope leaves are used as pig fodder (Thaman 1993). Throughout the Pacific islands, tree heliotrope leaves are highly valued for their medicinal properties. In the Marshall Islands and Kosrae, the leaves are used in steam baths, medicinal teas, and poultices (Merlin et al. 1993, 1994). In Tonga, an infusion of the leaves is drunk after eating tainted fish or is spread on infected cuts and stings (Whistler 1992b). In French Polynesia, especially the Tuamotus, yellow leaves, ready to fall, are cleaned, boiled, and served as a hot or cold beverage to combat the symptoms of ciguatera fish poisoning (Kumar-Roiné et al. 2011, Louis Malardé Institute 2014).

The yellowed, senescent leaves of *Heliotropium foertherianum* are known to contain rosmarinic acid, a biologically active phenolic compound, which Rossi et al. (2012) reported as active against ciguatoxin, the chemical involved in ciguatera fish poisoning in humans. Many novel, bioactive, phenolic compounds have been isolated from other *Heliotropium* species (Lin et al. 1999, 2002). However, the nutritional value or health benefits of senescent, deciduous, yellowed *H. foertherianum* leaves to turtles is unknown. This research tests the hypothesis that senescent *H. foertherianum* leaves have nutritional value comparable to macroalgae and seagrasses consumed by green turtles in Hawai‘i. Information on food resources and foraging ecology of green turtles is valuable for developing plans to sustain populations of this protected species.

Figure 1. Green turtle consuming floating, senescent tree heliotrope leaves in the Hilton Waikoloa Lagoon, Kona, Hawai'i. Photo by M. Rice

Figure 2. Tree heliotrope growing along a seawater canal at Nan Madol, Pohnpei, FSM. Photo by Gregory A. Koob, US FWS, 2016

Materials and Methods

Senescent, yellow *Heliotropium foertherianum* leaves were collected by hand from Kona, Hawai'i at the Hilton Waikoloa Lagoon, which connects to the ocean with free access by turtles (19.9240° N, 155.8875° W) on October 9, 2016 and January 4, 2017, which represent the two seasons known in Hawai'i: *kau*, the warm season (May-October) and *ho'oilo*, the season of cooler temperatures (November to April) (Sanderson 1993). Leaves were placed in 4 L plastic bags and frozen within 30 minutes of collection to preserve chemical composition. Voucher specimens were prepared and identified using taxonomic references (Wagner et al. 1990, Little & Skolmen 2003). Additional senescent leaves were collected on January 12, 2018.

Samples from 2016 and 2017 were subsequently freeze-dried, ground into a fine powder using a mortar and pestle, and stored in labeled, air-tight glass jars in a refrigerator at 4°C. The ground samples were divided into two, three, or ten sub-samples depending on the assay, and were analyzed for their proximate nutrients along with acid detergent fiber (ADF), neutral detergent fiber (NDF), and lignin according to the Association of Official Analytical Chemists (AOAC) standard procedures (AOAC 2006) with specific methods as follows: dry matter was determined according to AOAC method 930.15 (135°C for 2 h), ash (AOAC 942.05), crude protein (CP) by determining nitrogen (N) by dry combustion using a LECO analyzer (LECO CN-2000, Leco Corp., St. Joseph, MI) (AOAC 976.05, CP = N × 6.25), crude fat via ether extract (AOAC 920.39, using Soxhlet apparatus and petroleum ether), ADF (AOAC 973.18) and NDF (AOAC 2002.04) were determined using Ankom200 Fiber Analyzer (Ankom Technology, Macedon, NY), and lignin by the direct method using an alcohol-benzene solution (AOAC 932.01). Gross energy of the leaves was determined using an oxygen bomb calorimeter (Parr 6200, Parr Instrument Company, Moline, IL) and expressed as Kcal/kg. Leaves from 2018 were divided into three ~30 g sub-samples, dried at 60°C for 4 days in an air oven, and re-weighed. Water content of the fresh material available to green turtles was calculated by subtracting the dried sample weight from the fresh sample weight.

To calculate C:N ratio, samples were prepared by folding 5.0-6.0 mg of fine-powdered, dried sample into a Costech tin capsule using a method to assure that no sample would be lost, and no air pockets formed within the capsule. Samples were placed in a Costech 410 Elemental combustion system to determine carbon and nitrogen content.

Total phenol content was analyzed following the procedure described by Makkar (2000). For each sample, 200 mg of plant powder were transferred to a 15 ml conical tube; 10 ml of 70% acetone (aq.) were added. Each sample was vortexed to homogenize the acetone extracts, sonicated in a sonicate bath at room temperature for 20 min, and centrifuged at 4°C for 10 min at a speed ≤ 6,000 rpm. The supernatant was transferred to a new 15 ml conical tube. Aliquots of 20, 50, and 100 µl were mixed with distilled water to bring the total volume to 0.5 ml. Then 250 µl of Folin-Ciocalteau reagent (Folin-Ciocalteau, 1N, from SigmaAldrich, St. Louis, MO) were added, followed by 1.25 ml of sodium-carbonate (20%, aq.). Each sample was vortexed for 40 min, and its UV absorbance at 725 nm was recorded using a PG Instruments T60U (UV-Vis, UK) spectrophotometer.

An unpaired Student T-test was used to assess significant differences for each of the parameters.

Results

Senescent *Heliotropium foertherianum* leaves collected in October 2016 and January 2017 contained consistent levels of all eleven components tested (Table 1). There were no significant differences in the results of chemical analyses of Oct 2016 and January 2017 samples (N $p = 0.93$, $t = 0.85$, $df = 18$; C:N $p = 0.21$, $t = 1.31$, $df = 18$; ash $p = 0.79$, $t = 0.29$, $df = 3$; ADF $p = 0.25$, $t = 1.61$, $df = 2$; NDF $p = 0.083$, $t = 3.25$, $df = 2$; lignin $p = 0.58$, $t = 0.65$, $df = 2$; energy $p = 0.16$, $t = 1.75$, $df = 4$; phenol $p = 0.43$, $t = 0.87$, $df = 4$). Based on this lack of variation among seasons, all *H. foertherianum* data were combined to obtain overall mean values. Only dry matter showed a significant difference between the Oct 2016 and January 2017 values ($p = 0.029$, $t = 3.95$, $df = 3$).

The leaves were low in nitrogen ($\bar{x}$ = 0.65%), crude protein ($\bar{x}$ = 5.5%), and crude fat ($\bar{x}$ = 2.2%), and had a high C:N ratio ($\bar{x}$= 47.5). Fiber analyses showed high values for ADF ($\bar{x}$ = 31.5%), NDF ($\bar{x}$ = 41.5%), and lignin ($\bar{x}$ = 13.8%). Gross energy averaged more than 4500 Kcal/kg ash-free dry weight in each month. Amounts of phenolic compounds ranged from 556.8-594.7 mg/g dry weight. Fallen, senescent leaves available to foraging green turtles had an average water content of 87.9% (± .62 SE).

Discussion

The fallen, senescent *Heliotropium foertherianum* leaves contained less nitrogen, lower crude protein, greater lignin, and higher C:N values on a dry weight basis than other known food items, such as macroalgae, seagrasses, and a terrestrial grass, reported in Hawaiian green turtle diets (Table 2) (McDermid et al. 2007, 2015). On Palmyra Atoll, Young et al. (2010) analyzed mature, non-senescent, green *H. foertherianum* leaves, which contained five times more nitrogen and a C:N ratio three times lower than the senescent leaves in Hawaiʻi. Although green, growing *H. foertherianum* leaves would be more nutritious, turtles can forage only on low nitrogen, yellowed *H. foertherianum* leaves that have fallen into the water. Fat content of *H. foertherianum* leaves was similar to values for macroalgae, seagrass, and turfgrass eaten by green turtles in the Hawaiian Islands; however, the energy content of the senescent *H. foertherianum* leaves was higher than in other turtle food sources (McDermid et al. 2007, 2015). Senescent *H. foertherianum* leaves could provide more energy per gram ingested than other marine food sources for Hawaiian green turtles, especially if the turtles harbored microflora adapted to metabolize the complex structural carbohydrates in the leaves.

Two red macroalgae eaten by green turtles, *Ahnfeltiopsis concinna* and *Pterocladiella capillacea,* were reported to contain 59.8% and 43.5% total dietary fiber on a dry weight basis, respectively (McDermid et al. 2005). Senescent *Heliotropium foertherianum* leaves contained $\bar{x}$ = 41.5% NDF, a close estimate of "total" fiber. NDF is the fiber fraction that comprises plant cell wall components, i.e., cellulose, hemicelluloses, lignin, silica, tannins, and cutins. Although lignin is indigestible, green turtles can efficiently digest cellulose and hemicelluloses via hind-gut fermentation that creates short-chain fatty acids, which the turtle may use for energy (Bjorndal 1979). ADF is the least digestible fiber portion of forage, and includes cellulose, lignin, and silica. Senescent *H. foertherianum* leaves contained $\bar{x}$ = 31.5% ADF, which is comparable to very early, pre-bloom, fine-stemmed, leafy alfalfa (ADF < 29%) (Putnam et al. 2008).

Although green turtle foraging habits can reflect the availability and abundance of food resources (Forbes 1996, Guebert-Bartholo et al. 2011), some selectivity in food choice is seen in green turtles (Bjorndal 1985; Nagaoka et al. 2012). Green turtles are known to shift diets and exploit new food sources (Russell and Balazs 1994a, b, 2009, Russell et al. 2003, Bell & Ariel 2011, McDermid et al. 2015). However, why turtles on the Kona coast of Hawaiʻi Island are now eating *H. foertherianum* leaves, is not known. Their normal food resources may be limited (Wabnitz et al. 2010), or these turtles may be culinary pioneers, opportunistically foraging on floating leaves.

Table 1. Results of chemical analysis of senescent leaves of *Heliotropium foertherianum* from Kona, HI. All values are mean values. C:N = carbon:nitrogen ratio, ADF = acid detergent fiber, NDF = neutral detergent fiber. SE represents the standard error of the mean. Values of carbon, nitrogen, ash, protein, fat, fiber, lignin, energy and phenol are based on dry matter. n=number of sub-samples.

	% Dry Matter (SE)	**% Nitrogen (SE)**	**C:N (SE)**	**% Ash (SE)**	**% Crude Protein**	**% Crude Fat**	**% ADF (SE)**	**% NDF (SE)**	**% Lignin (SE)**	**Energy, Kcal/kg (SE)**	**Total Phenol, mg/g (SE)**
Oct 2016	97.38	0.65	46.03	2.46	5.25	2.43	31.26	40.70	13.91	4556.4	584
	(0.29)	(0.003)	(1.58)	(0.07)			(0.26)	(0.21)	(0.22)	(42.0)	(6.7)
	n=2	n=10	n=10	n=2	n=1	n=1	n=2	n=2	n=2	n=3	n=3
Jan 2017	96.49	0.64	48.94	2.48	5.65	2.00	31.82	42.35	13.60	4649.8	573
	(0.05)	(0.35)	(1.56)	(0.04)			(0.22)	(0.47)	(0.42)	(32.9)	(10.5)
	n=3	n=10	n=10	n=3	n=1	n=1	n=2	n=2	n=2	n=3	n=3

Table 2. Comparison of nutritional content of senescent leaves of *Heliotropium foertherianum* and some other foods reported in Hawaiian green turtle diets. C:N = carbon:nitrogen ratio. Values are mean values. Carbon, nitrogen, protein, fat, and lignin values are based on dry weight of the plant material. Energy values are based on ash-free dry weight. NM = not measured.

	% H_2O content	% Nitrogen	C:N	% Crude Protein	% Fat	% Lignin	Energy, Kcal/kg	Source
H. foertherianum, senescent leaves, seasons combined	87.9	0.645	47.5	5.45	2.22	13.76	4603	This paper
Ahnfeltiopsis concinna thalli	68.0	1.7	21.5	10.8	1.9	0.62	2846	McDermid et al. 2007, 2015
Pterocladiella capillacea thalli	77.8	2.7	14.2	16.9	2.3	3.7	3220	McDermid et al. 2007, 2015
Paspalum vaginatum leaves	77.5	2.2	23.2	17.2	NM	11.5	4006	McDermid et al. 2015
Halophila hawaiiana leaves	90.3	2.3	NM	14.4	3.8	NM	1696	McDermid et al. 2007

Senescent tree heliotrope leaves may be "an acquired taste" or a high calorie snack food. Green turtles elsewhere within the broad geographic range of tree heliotrope's distribution may also consume senescent leaves that fall into coastal waters.

Senescent *H. foertherianum* leaves could provide more calories than their normal foods: macroalgae and seagrasses. Diet and nutrition are strong determinants of green turtle growth rate, reproductive maturity, fecundity, and population dynamics (Balazs 1982, Bjorndal 1982), and as Nagaoka et al. (2012) emphasized, "understanding feeding habits of this endangered species is essential to its conservation."

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