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Diet of the Antillean Manatee (Trichechus manatus manatus) in Belize, Central America

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Diet of the Antillean manatee (*Trichechus manatus manatus*) in Belize, Central America

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To those who dedicate their lives to making a difference for endangered species; those who are the voice for the ones who cannot speak.

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Abstract:

Belize has been identified as an important location for Antillean manatees (*Trichechus manatus manatus*), harboring the highest known population density of this subspecies. Information about their dietary habit is important in determining habitat requirements and aiding in conservation efforts. The main objective of this study was to identify the key plant species consumed by manatees in Belize and to establish differences in diet based on location, sex, size classification, and season. Samples were collected from two different locations within Belize where manatees are known to aggregate: Southern Lagoon and the Drowned Cayes off of Belize City. The contents of thirteen mouth, six digestive tract (stomach, duodenum and colon), and 124 fecal samples were analyzed. Five species of seagrasses (*Halodule wrightii, Thalassia testudinum, Ruppia maritima, Syringodium filiforme,* and *Halophila* sp.) made up the highest percentage of plants consumed; undigested rhizome was most prevalent. A vascular plant, the red mangrove (*Rhizophora mangle*), was identified as an important food item of manatees in Belize. Algae (*Chara* sp., *Lyngbia* sp., and *Ulva* sp.) and invertebrates (diatoms and sponges) were represented as well. These items are comparable to other reports of manatee diets in areas near Belize and areas with similar habitat. Variation in the percentage of seagrass, mangrove, and algae consumption was analyzed as a 4-factor factorial Analysis of Variance (ANOVA) with main effects and interactions for locality (Southern Lagoon and the Drowned Cayes), sex, size classification ($>$245 cm & $\leq$245 cm), and season (December – May and June – November). Sex and season did not influence diet composition; differences for locality and size classification were observed. Seagrass was more often consumed in Southern Lagoon ($P = 0.03$), whereas mangroves and algae were more prevalent in the Drowned Cayes ($P = 0.03$). No differences among size classifications in consumption of seagrasses or mangroves were observed. A significantly higher number of samples from adults ($>$245 cm) than juveniles ($\leq$245 cm) contained algae ($P = 0.04$). This is most likely attributed to inexperience in foraging. Findings from these results suggest that diet composition analysis can be used to interpret Antillean manatee habitat and resource utilization and can aid in the conservation of this endangered species.

Keywords: Antillean manatee, Sirenia, microhistological analysis, feeding ecology, dietary habits, Belize, herbivory.

INTRODUCTION

As primary consumers, herbivores play an important role in the food web. Herbivory is well documented in terrestrial and marine ecosystems by both micro- and macroorganisms. Within both environments, primary consumers can have a substantial impact on plant biomass (Penzhorn *et al.* 1974; Thayer *et al.* 1984). In a marine ecosystem, seagrasses comprise an important link in the energy transfer between trophic levels (Heck *et al.* 2008). Marine herbivores include many species of fishes and turtles that feed on algae and seagrass (Thayer *et al.* 1984), but the largest impact on macrophytes is consumption by large mammals belonging to the Order Sirenia (Packard 1984; Aragones and Marsh 2000).

The Order Sirenia encompasses three extant species of manatees in the family Trichechidae and the dugong (*Dugong dugon*) in the Family Dugongidae. All four extant species of sirenians feed in an underwater environment and are known to consume large quantities of vegetation (Husar 1978a, 1978b; Hartman 1979; Johnstone and Hudson 1981; Bengtson 1981, 1983; Best 1981; Marsh *et al.* 1982; Anderson 1984; Etheridge *et al.* 1985; Gallivan and Best 1986; Ledder 1986; Hurst and Beck 1988; Mignucci-Giannoni and Beck 1998; Colares and Colares 2002; Castelblanco-Martinez *et al.* 2009; Alves-Stanley *et al.* 2010; Flores-Cascante *et al.* 2013). Mammalian herbivores have developed several morphological specializations that allow them to feed on plants (Shipley 1999; Clauss *et al*. 2003; Ley *et al.* 2008). In sirenians, these adaptations include prehensile lips and perioral bristles used to grasp food plants (Marshall *et al*. 1998, 2000), a specialized palate and unique dentition that is useful to begin the digestion process (Marsh 1980; Miller *et al.* 1980; Domning 1983; Domning and Hayek 1984;

Fortelius 1985; Marsh *et al.* 1999; Lanyon and Sanson 2006), and an enlarged gastrointestinal tract to accommodate a substantial volume of consumed vegetation (Kenchington 1972; Snipes 1984; Langer 1988; Reynolds and Rommel 1996). The digestive tract of sirenians is similar to that of genetically related orders like Proboscidea (Langer 1988), and other marine megaherbivores such as the green sea turtle (*Chelonia mydas*) (Thayer *et al.* 1984; Reynolds and Rommel 1996). Manatees are hind-gut fermenters like elephants (*Loxodonta africana* and *Elephas maximus*) and horses (*Equus ferus*), with most digestion occurring in the cecum and colon (Murray 1977; Burn 1986). Sirenians have a highly efficient ability to digest plant material due to their slow metabolic rate and long length of colon (Irvine 1983; Lomolino and Ewel 1984; Gallivan and Best 1986; Burn 1986; Lanyon and Marsh 1995; Goto *et al.* 2004a, 2004b; Lanyon and Sanson 2006; Larkin *et al.* 2007), along with anaerobic bacteria within the digestive tract which allows for the breakdown of cellulose (Parra 1978; Burn 1986). The large-scale grazing of these marine mammals is thought to have a positive impact on species biodiversity in seagrass beds (Packard 1984), and a positive effect on the structure and dynamics of these communities (Aragones and Marsh 2000). Knowledge of the diet requirements of sirenians is important for protecting the habitats in which these endangered species reside.

The West Indian manatee (*Trichechus manatus*) occupies coastal fresh, brackish and salt waters within the southeastern United States along the Gulf of Mexico and Atlantic Ocean, ranging throughout the Caribbean and extending south from Central America to Brazil (Husar 1978b; Lefebvre *et al.* 1989; Odell 1982). There are two sub-species, the Florida manatee (*Trichechus manatus latirostris*) and the Antillean manatee

(*Trichechus manatus manatus*). Each subspecies is genetically distinct and considered endangered in their native range (USFWS 2007; Hunter *et al*. 2012). Populations of West Indian manatees have low genetic variation within their populations (Garcia-Rodriguez *et al.* 1998; Vianna *et al.* 2006; Hunter *et al.* 2010; Tucker *et al*. 2012), which along with slow reproductive rates (Rathbun *et al.* 1995), make them vulnerable to extinction. They have also been subjected to hunting pressures since prehistoric times (Gann 1911; Wing and Reitz 1982; McKillop 1985). Although protection for these manatees has increased in countries throughout their range, Antillean manatee populations continue to decline from anthropogenic threats.

Antillean manatees in Belize

The Antillean manatee ranges throughout the Caribbean islands through Central America and south into southern Brazil (Luna 2013). From earlier surveys, the population of manatees within Belize was thought to be stable (O'Shea and Salisbury 1991). However, for such a large region with a current estimated population of 1,000 animals, this number is below what is considered sustainable for a threatened population (Auil Gomez 2011). Belize is a critical habitat that is important for the subspecies' continued survival (Quintana-Rizzo and Reynolds 2007). In 1936, protection for manatees in Belize was first put into place in order to conserve their numbers (McCarthy 1986). Conservation efforts continue to the present day with the Belize Wildlife Protection Act of 1981 (Auil 1998, 2004) in which manatees are included. A Manatee Recovery Plan has been enacted by the Belizean government in order to promote an increase in the manatee population (Auil 1998), but the most recent population trends have seen a decline in what was previously thought to be a stable population (Auil 2004).

In nearby Central American countries, such as Honduras, Nicaragua and Costa Rica, studies show that manatee numbers are relatively low despite having suitable habitat (Rathbun *et al.* 1983; Reynolds *et al.* 1995; Perez 2005). Manatee populations can be used to determine the health of marine ecosystems, as they are a sentinel species for habitat quality (Bonde *et al.* 2004). Because of declining numbers in Central America and low genetic variation in populations (Hunter *et al.* 2010), it is important to conserve these manatees, especially in areas like Belize where they face problems with habitat destruction and urbanization (Auil 1998; Auil *et al*. 2007).

Belize Habitat

Belize, also formerly known as British Honduras, is a small country of 22,960 square kilometers located on the east coast of Central America and shares a border with Guatemala and Mexico. According to a 2010 census, Belize has an estimated human population of 312,698. Belize contains 386 km of coastline along with several rivers, tidal lagoons, and barrier islands (cayes) to the east. The country provides excellent habitat for the Antillean manatee, including the mangrove lagoons bordering the Mesoamerican Barrier Reef. Recent studies observed that manatees were most numerous around the cayes to the east of Belize City, Placencia Lagoon to the south, and Chetumal Bay to the north (Morales-Vela *et al*. 2000; Auil 2004). These areas are lined with seagrass beds extending along the shoreline to the outer-lying cayes and islands, as well as an internal lagoon system that connects to the ocean. Such habitats are an important food source for manatees in Belize. The networks of freshwater rivers are also regions of importance, as manatees require freshwater sources for survival (Castelblanco-Martinez *et al*. 2012). Establishing the dietary preferences of the Antillean manatee can have implications for patterns of habitat use and resource specialization. Therefore, a better understanding of how these animals utilize feeding areas is an important tool that is necessary to adequately protect this endangered species.

Objectives

The purpose of this study was to present the first in-depth information on manatee diet in two high-use areas of Belize. This work has significance for determining what habitats are utilized as a necessary resource for these manatees. Belize has the largest population of Antillean manatees in Central America and the conservation of this population is critical to ensuring their survival (Auil 1998, 2004; Morales-Vela *et al.* 2000; LaCommare *et al.* 2008; Hunter *et al.* 2010; Auil Gomez 2011). Identification and protection of the food resources these animals utilize will help ensure their long-term survival in this region. The goals of this study were as follows:

1) To document the diet of Antillean manatees in Belize through microhistological analysis of available mouth, stomach and fecal samples.
2) To determine any differences in diet of manatees sampled in two regions in Belize:
    a. Drowned Cayes in northern Belize.
    b. Southern Lagoon in south central Belize.
3) To determine any differences in diet between sexes or among size classifications.
4) To determine any differences in resource use by season (dry vs. wet).

Hypotheses

Objectives call for testing the following null hypotheses:

1) There is no significant difference in diet between study sites: the Drowned Cayes and Southern Lagoon.
2) There is no difference in diet between sexes.
3) There is no difference in diet among manatee size classifications.
4) There is no change in diet composition by season (dry vs. wet).

Although the habitats in the Drowned Cayes and Southern Lagoon vary, a uniform similarity in the manatee diet is expected. Seagrasses are available to manatees at both sites, and manatees may travel to seek preferred forage (Moore 1951; Hartman 1974, 1979; Montgomery *et al.* 1981; Bengtson 1981; Powell and Rathbun 1984; Rathbun *et al.* 1990; Deutsch *et al.* 2003; Castelblanco-Martinez *et al.* 2012). The variables of sex and size classification can also be assumed to be similar due to the fact that animals feed within the same habitat. Variation in diet preference by sex or size classification has not been documented in any previous studies of sirenian diets and is therefore presumed not to be significant in Belize.

Background Review

Several previous studies have investigated the diet of captive sirenians. The first description of diet came from two Amazonian manatees (*Trichechus inunguis*) kept in captivity and transported from Brazil to France (Devillers 1938). The diet of a pair of dugongs also kept in captivity in India was described in 1959 (Jones 1959). The first publication on the diet of Antillean manatees came in 1975 with a report on the diets of Antillean and Amazonian manatees kept in captivity in Brazil (Pinto da Silveira 1975).

The first comprehensive study of the diet of wild sirenians was conducted in Australia when the stomach contents of wild dugongs were described and included information on dietary shifts from seagrasses to algae after a cyclone destroyed major seagrass beds (Spain and Heinsohn 1973). In the United States, the manatee diet was first described by feeding observations in Crystal River, Florida (Hartman 1971) as well as in the southeastern states of Georgia and South Carolina (Hartman 1974). These publications set the baseline for what is known about the herbivorous diet of sirenians.

In subsequent years, numerous studies have focused on the types of food that sirenians consume in the wild (Spain and Heinsohn 1973; Hartman 1974, 1979; Campbell and Irvine 1977; Johnstone and Hudson 1981; Bengtson 1981, 1983; Best 1981; Marsh *et al.* 1982; Anderson 1984; Gallivan and Best 1986; Ledder 1986; Hurst and Beck 1988; Ames *et al.* 1996; Mignucci-Giannoni and Beck 1998; Colares and Colares 2002; Castelblanco-Martinez *et al.* 2009; Alves-Stanley *et al.* 2010; Flores-Cascante *et al.* 2013). The first comprehensive report on the diet of manatees came in 1981 with a detailed review on the feeding behavior, digestive physiology, consumption and diet of sirenians in wild and captive settings (Best 1981). That same year in Papua New Guinea, mouth samples were taken from 102 dugongs that contained 11 different types of seagrasses, along with algae and mangroves, which were similar to local distributions of vegetative species in close proximity to where the dugongs were captured (Johnstone and Hudson 1981). A similar study on Amazonian manatees identified 24 different species of macrophytes from digestive tract and fecal samples which indicated a higher diversity in types of vegetation consumed during the dry season; manatees were more selective on what they consumed during the wet season (Colares and Colares 2002). In past examinations of dietary habits, some of the best known data derive from Florida manatees (Hartman 1971, 1974; Campbell and Irvine 1977; Best 1981; Bengtson 1983;

Hurst and Beck 1988; Ames *et al*. 1996; Reich and Worthy 2006; Alves-Stanley *et al.* 2010). In Florida, manatees are known to consume over 60 different species of plants (Hartman 1979; Bengtson 1981, 1983; Best, 1981; Etheridge *et al*. 1985; Ledder, 1986, Hurst and Beck, 1988) of varying nutrient values (Siegal-Willott *et al.* 2010). Antillean manatees were documented to consume 10 different species of vegetation in Puerto Rico (Mignucci-Giannoni and Beck 1998). There has been a wide range of reported estimates regarding the amount of food that manatees consume in a given day (kg/day): 9 (Crandall 1964), 12 (Hartman 1979), 28 (Best 1981), 42-56 (Lomolino 1977), 50 (Pinto de Silveira 1975) and 80 (Severin 1955). Manatees have been documented to consume between 4-9% (Bengtson 1983) to 10-15% of their body weight per day (Reep and Bonde 2006). Differences in the proportion of vegetation consumed are dependent on the animals' size, activity level, nutrient value of plants consumed and demands for bodily functions. Consumption is also influenced by the availability of plants which is dependent on season, weather, resources available for plant growth and anthropogenic factors. Correspondingly, as manatees ingest large amounts of vegetation, they can have a profound impact on seagrass beds (Packard 1984). Because food is critical for their survival, it is necessary to detail the forage resources manatees are utilizing.

Manatees select habitat based on the availability of food and freshwater resources (Hartman 1979; Packard and Wetterqvist 1986; O'Shea and Kochman 1990; Gannon *et al*. 2007). Unfortunately, manatees face habitat destruction within many parts of their range which limits the resources necessary for their survival (Smith 1993). There has been a documented decline in seagrass within areas of the Americas (Short *et al.* 2006). Maintaining a food source is necessary for the survival of the Antillean manatee, therefore, it is imperative to learn more about what plant species these manatees consume in parts of the world where their diet is not well documented.

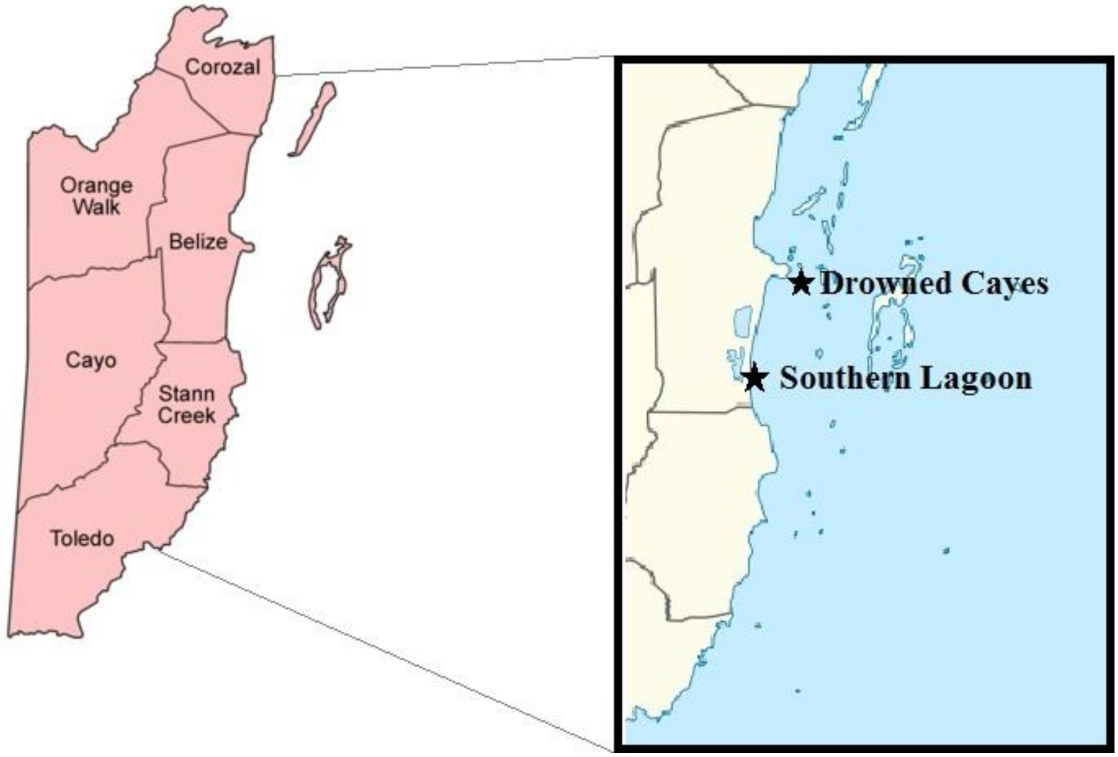
Several methods have been used to study the diet of herbivores, including direct observation and examination of ingesta and fecal samples using microhistological analysis and a microscope point technique (Hurst and Beck 1988). Microhistological analysis is a favored method for the identification of ingesta and fecal samples collected from terrestrial herbivores (Holechek and Vavra 1981). This technique has been used in many studies of terrestrial herbivore diets (Larter 1999; Chapuis *et al.* 2001; Mellado *et al*. 2004; Larter and Nagy 2006) as well as aquatic herbivores (Owen 1975; Black *et al.* 1994; Carriere *et al*. 1999; Castelblanco-Martinez *et al.* 2009; Flores-Cascante *et al.* 2013). A modified microscope point technique has been employed in previous studies of the diet of sirenians (Channels and Morrissey 1981; Hurst and Beck 1988; Mignucci-Giannoni and Beck 1998). This technique is an effective, yet inexpensive, method to gather information on herbivore diets. The methodology outlined in Hurst and Beck (1988) was followed for this study.

MATERIALS AND METHODS

Subjects

Manatees utilized in this study were Antillean manatees (*Trichechus manatus manatus)* captured in two locations within the country of Belize (Fig. 1): Southern Lagoon (17°12'40"N, 88°20'17"W) near Gales Point, Belize District from 1997-2008, 2010 and 2012, and the Drowned Cayes (17°29'25"N, 88°08'10"W) off Belize City, Belize District from 2004-2007, 2012, and 2013. Digesta also was obtained at necropsy from manatee carcasses collected near Belize City in 1999, 2002-2003, 2008 and 2013.

Figure 1. Map of collection locations in Belize



Collection

A project to capture and release wild manatees for routine health assessments and intermittent radio-tagging has been ongoing in Belize since 1997. Manatees are captured by an experienced crew from Florida and Belize. The capture process begins by encircling a target manatee(s) with a large net to prevent escape and reduce stress. Individual manatees are then selected and extracted using a smaller net and pulled onto land or on the deck of a boat for a detailed physical examination (Wong *et al*. 2012; Bonde *et al*. 2012). Routine measurements and biological samples are collected, including fecal and mouth content samples when available. Through such health assessments, baseline knowledge of manatees' health can be obtained and studied. Mouth and fecal samples collected through this method were used for this study.

Within Belize, manatees face natural and anthropogenic threats that may cause mortality. When reported to local authorities, manatee carcasses are examined to document cause of death. Digesta samples also are collected when present. For this study, internal contents from the stomach, duodenum, and large intestine of deceased manatees were obtained for analysis.

Specimens for this dietary analysis (n = 143) were obtained from 134 live manatees and 9 samples from carcasses. Samples from live manatees were derived from the mouth (n = 13) and as fecal samples (n = 124) collected during capture events. Internal samples were collected from the stomach (n = 4), duodenum (n = 1) and large intestine (n = 1) of carcasses. Of these samples, 111 (n = 111) were obtained from Southern Lagoon and 32 (n = 32) were acquired in the Drowned Cayes. Fecal and gut content samples were preserved in 70% EtOH upon collection and stored until ready for examination.

Microhistological Analysis

All samples were examined using microhistological characters of visible plant fragments and analyzed using techniques developed by Owen (1975), Holechek *et al*. (1982a, 1982b), and Hurst and Beck (1988). A modified microscope point technique was employed; a large fragment size was preferred to enable easier identification. Therefore, the samples were not processed to achieve a uniform size as has been done in other diet studies, but, prior to examination, each sample was rinsed with tapwater over a 30-mesh (0.52 mm) screen to remove sand, dirt and other fine particulate matter that might obscure microscopic observation of plant cellular structures. After rinsing, a subsample of the digesta was placed on a 2 x 3 inch glass slide to which several drops of Hertwig's solution was added. The slide was then held over an alcohol flame to facilitate the clearing of pigments from the plant cells, allowing for easier viewing of cellular structures. After clearing, the subsample was divided onto 5 additional 2 x 3 inch glass slides for microhistological examination.

During microscopic observation, each slide was first observed at 40X for the purpose of scanning the contents. Samples were then observed at 100X and analyzed for content. Each slide was analyzed through the microscope by identifying 5 points visible in an eyepiece micrometer grid (see Appendix A) along a prescribed transect sequence at 20 different coordinates on the stage; observations were recorded at each coordinate (see Appendix B). This allowed for 100 different points of identification on each slide and was repeated five times for each sample (see Appendix C). A total of 500 points was identified for each sample as recommended by Hurst and Beck (1988) (see also Beck and Clementz 2012).

For identification purposes, plant fragments were compared with reference voucher slides and photomicrographs available at the USGS Sirenia Project laboratory along with illustrations from Hurst and Beck (1988) which described leaf, stem, flower, root and rhizome fragments of over 100 plant species cataloged for the study of manatee diet through microhistological examination. Some species of algae were referenced using field-guides and outside expert analysis through electron microscopy. Observations for each slide were recorded on paper (see Appendices B & C) and simultaneously recorded on a Microsoft Excel spreadsheet. Photographs were also obtained of the most common digesta items for confirmation of identification.

Data Analysis

The results of microhistological analysis on diet samples from manatees in Belize were characterized to determine the potential similarities and differences in items consumed by manatees. Differences in two locations (Drowned Cayes and Southern Lagoon), manatee size classification (>245 cm for adult, 200 – 245 cm for subadult and <200 cm for calf), sex (male and female) and season (December – May for dry season, June – November for wet season) were defined by two methods. Percent frequency of species observed was determined by adding the total count of each species type seen in the samples, dividing by the total number of samples analyzed for each group, and then dividing by 500 (see Microhistological Analysis). Diet composition was also described through percent occurrence of samples analyzed across four categories: seagrass, mangrove (*Rhizophora*), algae and invertebrate. Variation in percent seagrass, mangrove, and algae was further analyzed statistically as a 4-factor factorial Analysis of Variance (ANOVA) with main effects and interactions for locality, sex, size classification and season. Prior to analysis, the data were transformed to ranks to adjust for the non-normal distribution of percentages. All statistical analyses were carried out using the SAS PROC GLM (SAS Institute Inc. 2013). This is the most comprehensive study of manatee diet within Belize to date.

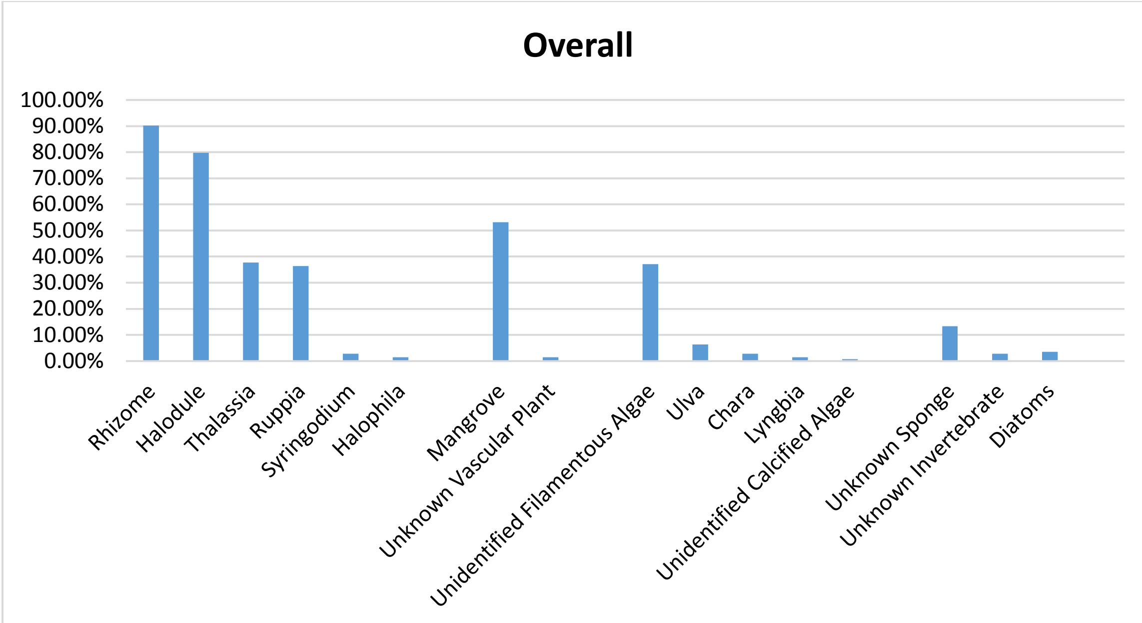
RESULTS

Microhistological analysis revealed the contents (ingesta and digesta) of each sample by identifying plant fragments through microscopic investigation. The fragment sizes in mouth and stomach samples were larger than those found in fecal samples. Mouth samples contained almost complete, intact pieces of plant material, facilitating identification. As samples progressed further along the digestive tract, advancing from the stomach to large intestine, sample quality and the ease of identifying fragments decreased due to digestion. When possible, ingesta were identified to genus as well as fragment type (e.g., leaf, stem, rhizome, etc.). Contents were then summarized based on frequency of appearance.

Overall Percent Frequency of Manatee Diet

The frequency of items was identified for the overall diet of manatees in Belize by identifying the number of times each item was found in a sample and dividing by the total number of samples studied (Fig. 2). Most samples contained more than one type of item. Of 143 samples, rhizome was found most frequently in 129 (90.21%) samples. The seagrass *Halodule wrightii* was the second most frequently detected in 114 (79.72%) samples, followed by red mangrove, *Rhizophora mangle*, in 76 (53.15%) samples. Of seagrass species, *Thalassia testudinum* was represented in 54 (37.76%) samples, followed by *Ruppia maritima* (52, 36.36%), *Syringodium filiforme* (4, 2.80%) and *Halophila* sp. (2, 1.40%). Algae represented a large portion of species in samples: an unidentified filamentous alga was seen in 53 (37.06%) samples, followed by *Ulva* sp. (9, 6.29%), *Chara* sp. (4, 2.8%), *Lyngbia* sp. (2, 1.40%), and an unidentifiable calcified algae found in one sample (0.70%). Invertebrates were also found in samples with an unknown species of sponge frequently seen in 19 samples (13.29%), followed by an unknown invertebrate in 4 (2.80%) samples. An unknown vascular plant was observed in 2 (1.40%) samples, and diatoms or diatomaceous earth were observed in 5 (3.50%) samples.

Figure 2. Percent Frequency: Overall



All 143 samples were examined to identify percent occurrence relative to 500 points of identification in each sample (Table 1) to classify the average composite make-up of each specimen. Classifications were defined as seagrass (*Halodule wrightii, Thalassia testudinum, Ruppia maritima, Syringodium filiforme, Halophila* sp.), mangrove (*Rhizophora mangle)*, algae (e.g., *Ulva sp*., unidentified filamentous algae) and invertebrates (e.g., unknown Poriferan, unknown invertebrate). Overall, seagrass averaged 81.06% (SD = 21.34) of 500 points observed within 143 samples. Mangrove comprised a mean of 6.72% (SD = 11.99) points in the samples, algae constituted a mean of 2.48% (SD = 5.32) points, and invertebrates made up a mean of 1.60% (SD = 5.13) points. Within several samples, there were some fragments that were unidentifiable at the time of observation. Since it was not possible to classify these fragments, they were disregarded for analytical purposes.

Table 1. Belize Manatee Diet Sample Composite: Overall

Type	n	Mean	Min.	Max.	Std. Dev.	Variance	Std. Error	Coeff. Of Variation
SEAGRASS	143	81.06	0.00	100.00	21.34	455.31	1.78	26.32
MANGROVE	143	6.72	0.00	84.00	11.99	143.84	1.00	178.54
ALGAE	143	2.48	0.00	33.80	5.32	28.31	0.45	214.22
INVERTEBRATE	143	1.60	0.00	33.20	5.13	26.32	0.43	319.79

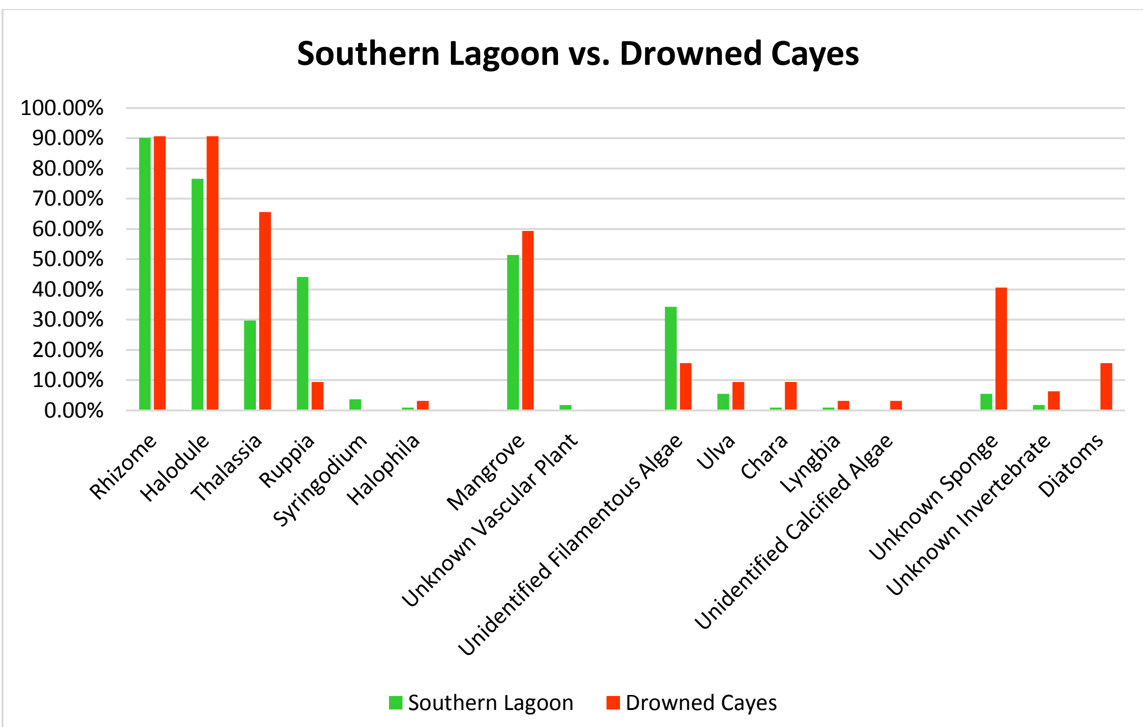
Percent Frequency by Location

Microhistological analysis revealed the contents (ingesta and digesta) of manatees' diet in two locations studied within Belize: Southern Lagoon and the Drowned Cayes (Fig. 3). For each location (Southern Lagoon, n = 111; Drowned Cayes, n = 32), rhizome was the predominant finding for each sample: 90.09% frequency in Southern Lagoon and 90.63% in the Drowned Cayes. Of 111 samples from Southern Lagoon and 32 samples from the Drowned Cayes, *Halodule wrightii* had the highest frequency (Southern Lagoon 85, 76.58%; Drowned Cayes 29, 90.63%).

In samples from Southern Lagoon, *Rhizophora mangle* was the third most common species detected in 57 (51.35%) samples, followed by seagrasses: *Ruppia maritima* (50, 44.14%), *Thalassia testudinum* (33, 29.73%), *Syringodium filiforme* (4, 3.60%) and *Halophila* sp. in one sample (0.90%). Algae was frequently seen in samples with 38 (34.23%) containing an unidentifiable filamentous algae, followed by *Ulva* sp. (6, 5.41%), *Chara* sp. (1, 0.90%) and *Lyngbia* sp. (1, 0.90%). Invertebrates persisted in Southern Lagoon diet samples with a frequency of 5.41% (6 samples) containing an unknown species of sponge (Porifera), followed by an unidentified invertebrate in 2 samples (1.80%). An unknown vascular plant was also observed in 2 samples (1.8%) from Southern Lagoon.

In the Drowned Cayes, *Thalassia testudinum* was the third most frequent species, observed in 21 (65.63%) samples, followed by *Rhizophora mangle* (19, 59.38%); the seagrasses *Ruppia maritima* were observed in 3 (9.38%) of the samples and *Halophila* sp. in 1 sample (3.13%). Invertebrates in samples consisted of an unknown Poriferan observed in 13 (40.63%) samples and in 2 samples (6.25%) as an unknown invertebrate. Algae was detected in 13 (40.63%) samples, commonly with more than one type present: 5 (15.63%) samples as an unknown filamentous algae, 3 (9.38%) samples with *Ulva* sp., 3 samples (9.38%) with *Chara* sp., 1 (3.13%) sample with *Lyngbia* sp., and 1 (3.13%) sample with an unidentified calcified algae. Samples from the Drowned Cayes also consisted of diatoms or diatomaceous earth in 5 (15.63%) of the samples, most likely ingested incidentally while foraging.

Figure 3. Percent Frequency: Location



The average percent occurrence per 500 points observed from samples obtained in

Southern Lagoon and the Drowned Cayes was also investigated (Table 2). Specimens from Southern Lagoon consisted of an average 83.14% (SD = 20.87) seagrass. Mangrove consisted of a mean 5.65% (SD = 9.63), algae a mean 1.55% (SD = 3.94) and invertebrates a mean 0.43% (SD = 1.99). Within the Drowned Cayes, samples included an average 73.18% seagrass, 11.11% (SD = 18.02) mangrove, 6.05% (SD = 9.38) invertebrates and 5.03% (SD = 6.32) algae. These were further evaluated with an analysis of variance (see statistical analysis results) in order to investigate statistical differences in diet between these two collection sites.

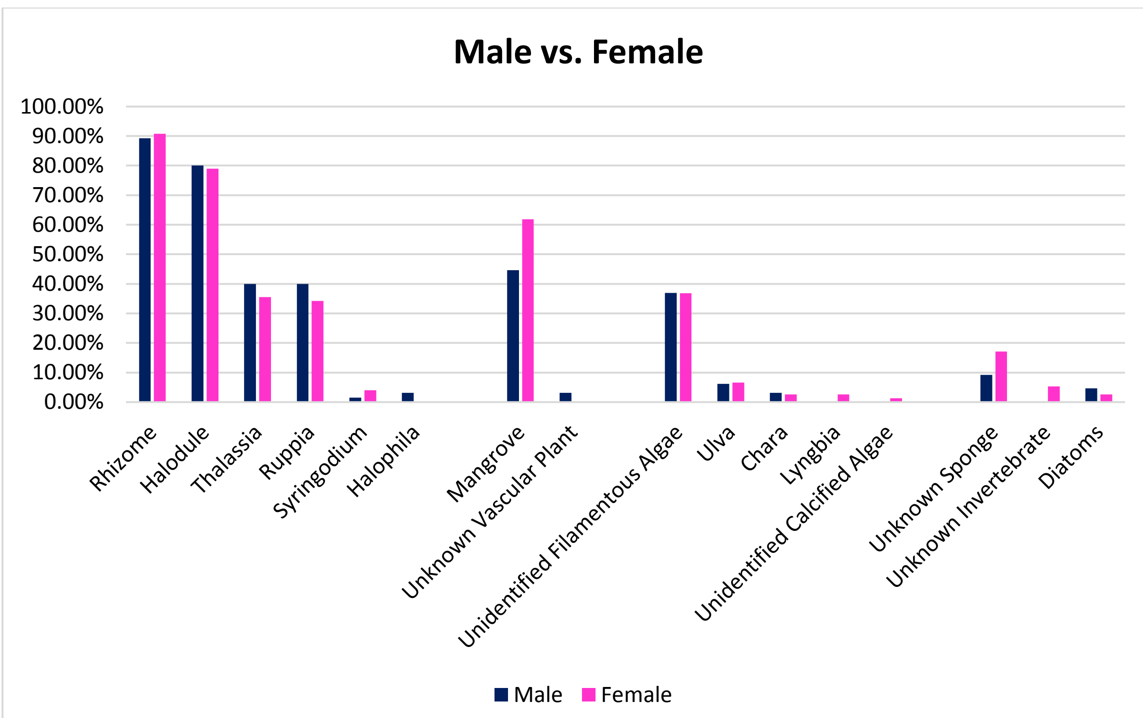
Table 2. Belize Manatee Diet Sample Composite: Location

Locality	Type	n	Mean	Min.	Max.	Std. Dev.	Variance	Std. Error	Coeff. Of Variation
SOUTHERN LAGOON	SEAGRASS	111	83.14	14.20	100.00	20.87	435.42	1.98	25.10
SOUTHERN LAGOON	MANGROVE	111	5.65	0.00	53.80	9.63	92.81	0.91	170.49
SOUTHERN LAGOON	ALGAE	111	1.55	0.00	33.80	3.94	15.51	0.37	253.53
SOUTHERN LAGOON	INVERTEBRATE	111	0.43	0.00	13.00	1.99	3.96	0.19	460.16
DROWNED CAYES	SEAGRASS	30	73.18	0.00	100.00	21.89	478.99	4.00	29.91
DROWNED CAYES	MANGROVE	30	11.11	0.00	84.00	18.02	324.84	3.29	162.18
DROWNED CAYES	ALGAE	30	5.03	0.00	20.80	6.32	39.94	1.15	125.55
DROWNED CAYES	INVERTEBRATE	30	6.05	0.00	33.20	9.38	87.99	1.71	155.13

Percent Frequency by Sex

Percent frequency was examined to detail potential differences in diet between sexes, male (M) and female (F) (Fig. 4). In both males (n = 65) and females (n = 76), rhizome was observed most frequently (M = 58, 89.23%, F = 69, 90.79%), followed by *Halodule wrightii* (M = 52, 80.00%, F = 60, 78.95%), and *Rhizophora mangle* (M = 29, 44.62%, F = 47, 61.84%). In males, seagrasses *Ruppia maritima* and *Thalassia* *testudinum* were seen in 26 (40.00%) samples, followed by an unidentified filamentous algae (24, 36.92%), an unknown Poriferan (8, 9.23%), algae species *Ulva* sp. (4, 6.15%) and *Chara* sp. (2, 3.08%), an unknown vascular plant (3.08%) and seagrasses *Halophila* sp. (2, 3.08%) and *Syringodium filiforme* (1, 1.54%). In females, unidentified filamentous algae was observed in 28 (36.84%) samples, followed by seagrasses *Thalassia testudinum* (27, 35.53%) and *Ruppia maritima* (26, 34.21%), an unknown Poriferan (13, 17.11%), *Ulva* sp. (5, 6.58%), an unknown invertebrate (4, 5.26%), *Syringodium filiforme* (3, 3.95%), algae *Chara* sp. (2, 2.63%) and *Lyngbia* sp. (2, 2.63%) and an unidentified calcified algae (1, 1.32%). Diatoms were also observed in 3 (4.62%) samples from male manatees, and 2 (2.63%) samples from females.

Figure 4. Percent Frequency: Sex



Samples from male and female manatees were also compared across an observation of 500 points (Table 3). Male manatee samples contained an average of

83.41% (SD = 19.05) seagrass, mangrove 5.02% (SD = 8.22), algae 2.06% (SD = 3.93) and invertebrates 1.30% (SD = 5.27). Female manatee samples were comprised of an average of 78.97% (SD = 23.15) seagrass, mangrove 8.35% (SD = 14.43), algae 2.49% (SD = 5.36), and invertebrates 1.90% (SD = 5.09). These samples were further compared statistically to examine differences in the average diet between males and females (see statistical analysis results).

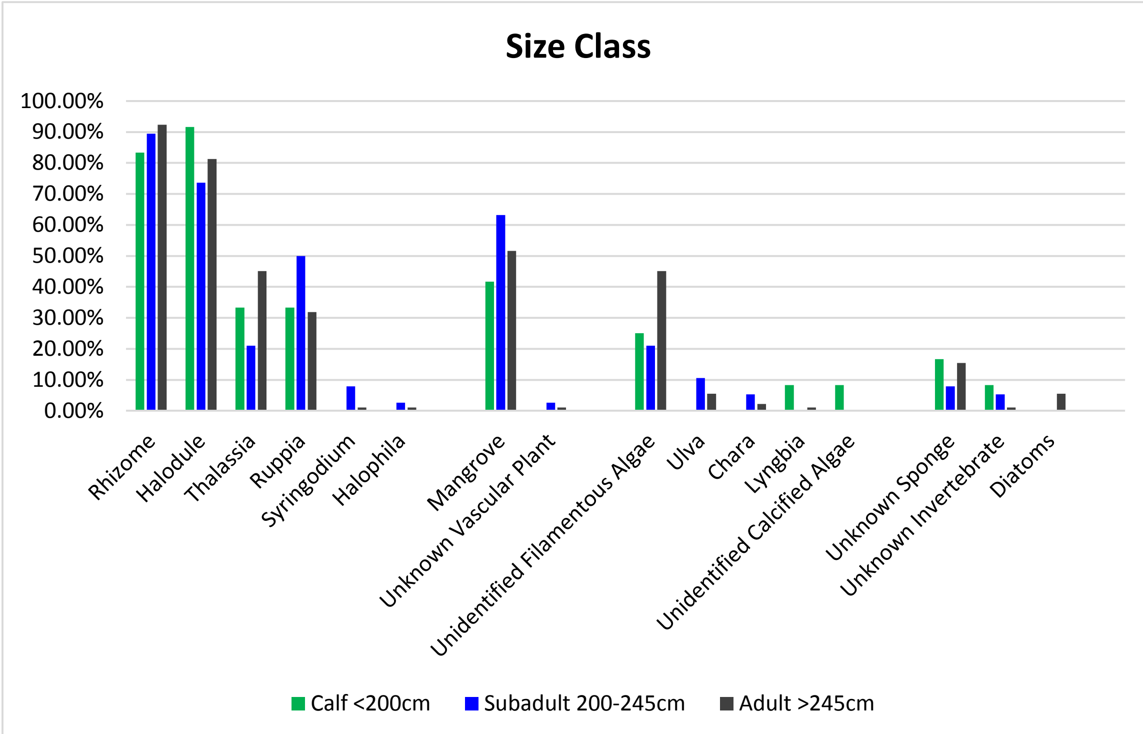
Table 3. Belize Manatee Diet Sample Composite: Sex

Sex	Type	n	Mean	Min.	Max.	Std. Dev.	Variance	Std. Error	Coeff. Of Variation
MALE	SEAGRASS	65	83.41	27.40	100.00	19.05	363.09	2.36	22.84
MALE	MANGROVE	65	5.02	0.00	39.60	8.22	67.64	1.02	163.98
MALE	ALGAE	65	2.06	0.00	20.80	3.93	15.47	0.49	191.08
MALE	INVERTEBRATE	65	1.30	0.00	33.20	5.27	27.81	0.65	404.22
FEMALE	SEAGRASS	76	78.97	0.00	100.00	23.15	536.02	2.66	29.32
FEMALE	MANGROVE	76	8.35	0.00	84.00	14.43	208.21	1.66	172.81
FEMALE	ALGAE	76	2.49	0.00	33.80	5.36	28.71	0.61	214.77
FEMALE	INVERTEBRATE	76	1.90	0.00	29.20	5.09	25.86	0.58	267.27

Percent Frequency by Size Classification

Antillean manatees in Belize were examined for percent frequency of samples compared by age, approximated by size classification (Fig. 5). Size classifications were defined as calves (<200cm), subadults (≥200 – ≤245cm), and adults (>245cm). Within samples from calves (n = 12), *Halodule wrightii* was seen in 11 (91.67%) samples followed by rhizome observed in 10 (83.33%) samples, and *Rhizophora mangle* in 5 (41.67%) samples. Seagrasses *Thalassia testudinum* and *Ruppia maritima* were observed in 4 (33.33%) samples, followed by an unknown filamentous algae (3, 25.00%), and an unknown Poriferan (2, 16.67%). *Lyngbia* sp., an unknown calcified algae, and an unknown invertebrate were found in one (8.33%) sample from a manatee calf. From sampled subadults (n = 38) rhizome was observed in 34 (89.47%) samples, followed by *Halodule wrightii* (28, 73.68%), *Rhizophora mangle* (24, 63.16%), and *Ruppia maritima* (19, 50.00%). *Thalassia testudinum* and an unknown filamentous algae were seen in 8 (21.05%) samples, followed by *Ulva sp*. (4, 10.53%); *Syringodium filiforme* and an unknown Poriferan were seen in 3 (7.89%) subadult samples followed by *Chara* sp. (2, 5.26%), an unknown invertebrate (2, 5.26%), *Halophila* sp. and an unknown vascular plant in one (2.63%) sample. In adult manatee samples (n = 91), rhizome was observed in 84 (92.31%) samples, then *Halodule wrightii* (74, 81.32%), *Rhizophora mangle* (47, 51.65%), and *Thalassia testudinum* and an unknown filamentous algae were observed in 41 (45.05%) samples. *Ruppia maritima* was observed in 29 (31.87%) adult samples, an unknown Poriferan in 14 (15.38%) samples, followed by diatomaceous earth (5, 5.49%), algae *Ulva* sp. (5, 5.49%) and *Chara* sp. (2, 2.20%). Seagrasses *Syringodium filiforme, Halophila* sp., alga *Lyngbia* sp., an unknown invertebrate and an unknown vascular plant were also observed in one (1.10%) sample obtained from an adult.

Figure 5. Percent Frequency: Size Classification



Size classifications also were analyzed as a function of 500 points to examine percent occurrence between each type (Table 4). Percent occurrence among manatee calves contained a mean of 85.02% (SD = 17.86) seagrass, and an average of 3.25% (SD = 5.45) mangrove, 2.20% (SD = 5.32) algae, and 1.10% (SD = 2.10) invertebrates. Subadult manatees averaged 79.26% (SD = 25.24) seagrass, 10.91% (SD = 18.45) mangrove, 1.10% (SD = 3.73) invertebrates and 1.07% (SD = 2.59) algae. Adult samples consisted of a mean of 82.23% (SD = 20.20) seagrass, 5.57% (SD = 8.49) mangrove, 2.82% (SD = 5.28) algae, and 1.92% (SD = 5.91) invertebrates. For means of statistical analysis, results from calf and subadult samples were combined (≤245cm) and compared with adult manatees (>245cm) to determine differences in diet by size classification (see statistical analysis results).

Table 4. Belize Manatee Diet Sample Composite: Size Classification

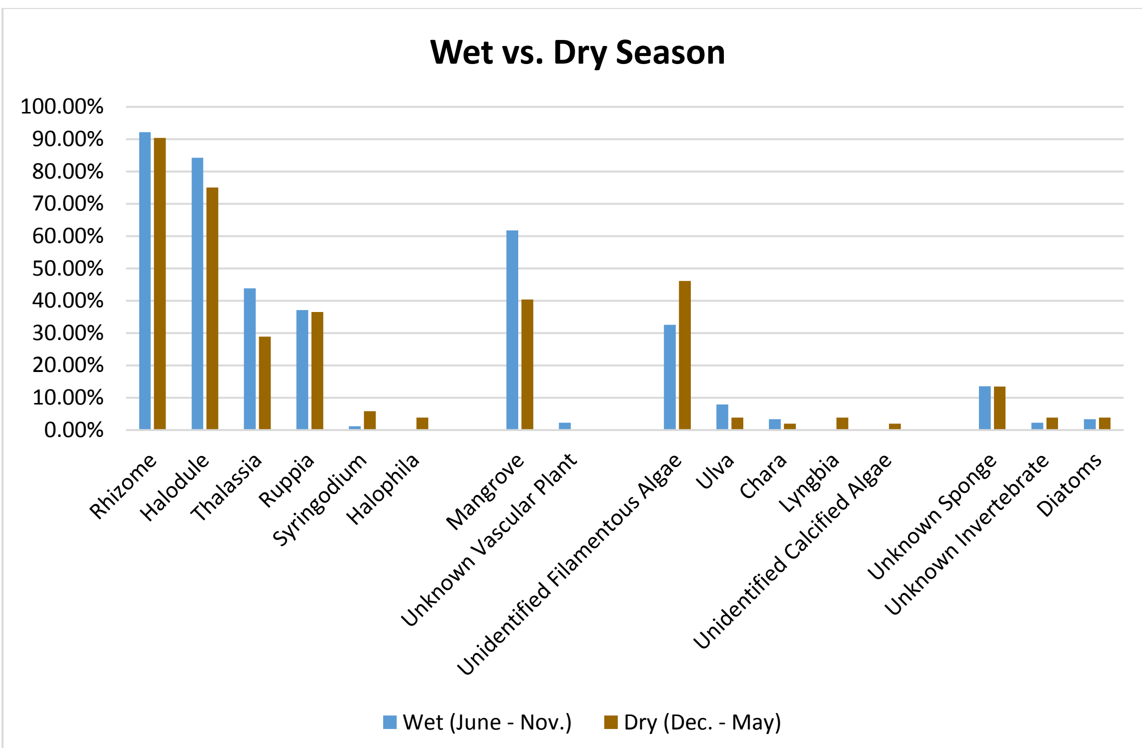
Size	Type	n	Mean	Min.	Max.	Std. Dev.	Variance	Std. Error	Coeff. Of Variation
CALF	SEAGRASS	12	85.02	47.40	100.00	17.86	318.85	5.15	21.00
CALF	MANGROVE	12	3.25	0.00	15.20	5.45	29.65	1.57	167.55
CALF	ALGAE	12	2.20	0.00	18.40	5.32	28.31	1.54	241.86
CALF	INVERTEBRATE	12	1.10	0.00	5.60	2.10	4.42	0.61	191.09
SUBADULT	SEAGRASS	38	79.26	0.00	100.00	25.24	637.20	4.09	31.85
SUBADULT	MANGROVE	38	10.91	0.00	84.00	18.45	340.31	2.99	169.08
SUBADULT	ALGAE	38	1.07	0.00	12.60	2.59	6.70	0.42	241.14
SUBADULT	INVERTEBRATE	38	1.10	0.00	16.20	3.73	13.94	0.61	339.38
ADULT	SEAGRASS	91	81.23	23.40	100.00	20.20	408.14	2.12	24.87
ADULT	MANGROVE	91	5.57	0.00	53.80	8.49	72.06	0.89	152.37
ADULT	ALGAE	91	2.82	0.00	33.80	5.28	27.88	0.55	187.54
ADULT	INVERTEBRATE	91	1.92	0.00	33.20	5.91	34.96	0.62	308.50

Percent Frequency by Season

Ingesta samples were also examined to detail seasonal influences on manatee diet (Fig. 6). Belize experiences a wet [rainy] season from June through November, with peak rainfall in July and August; the dry season is therefore defined as December through May. Samples for the wet (n = 89) and dry season (n = 52) contained rhizome in the majority of samples (wet = 82, 92.13%, dry = 47, 90.38%), followed by *Halodule wrightii* (wet = 75, 84.27%, dry = 39, 75.00%). During the wet season, samples containing *Rhizophora mangle* comprised 55 (61.80%) samples, and the seagrasses *Thalassia testudinum* (39, 43.82%) and *Ruppia maritima* (33, 37.08%), along with an unknown filamentous algae (29, 32.58%) were also observed in samples. Unknown Poriferians were present in 12 (13.48%) samples during the wet season, followed by algal species *Ulva* sp. (7, 7.87%) and *Chara* sp. (3, 3.37%); diatoms were also identified in 3 (3.37%) samples, as well as an unknown invertebrate and unknown vascular plant in 2

(2.25%) samples, and *Syringodium filiforme* in one (1.12%) sample. Of samples collected in the dry season, an unidentified filamentous algae was detected in 24 (46.15%) samples, followed by *Rhizophora mangle* (21, 40.38%), the seagrasses *Ruppia maritima* (19, 36.54%) and *Thalassia testudinum* (15, 28.85%), and an unknown Poriferan persisted in 7 (13.46%) samples. Other items recorded in samples during the dry season were *Syringodium filiforme* (3, 5.77%), *Halophila* sp. (2, 3.85%), an unknown invertebrate (2, 3.85%), diatomaceous earth (2, 3.85%), and algal species *Ulva* sp. and *Lyngbia* sp. in 2 (3.85%) samples each; *Chara* sp. and an unknown calcified algae were each also present in one (1.92%) dry season sample.

Figure 6. Percent Frequency: Season



Wet and dry seasons were compared by percent occurrence of each type (seagrass, mangrove, algae, invertebrate) per 500 points (Table 5). Wet season samples were documented to contain 79.86% (SD = 22.04) seagrass, 8.53% (SD = 14.22) mangrove, 2.11% (SD = 3.99) algae, and 1.71% (SD = 5.55) invertebrates. From samples acquired during the dry season, the composite contained 83.00% (SD = 20.31) seagrass, 3.87% (SD = 5.97) mangrove, 2.60% (SD = 5.84) algae, and 1.48% (SD = 4.46) invertebrates. As with results obtained by location, sex, and size classification, these percent occurrences were also analyzed in an ANOVA to statistically validate the findings (see statistical analysis results).

Table 5. Belize Manatee Diet Sample Composite: Season

Season	Type	n	Mean	Min.	Max.	Std. Dev.	Variance	Std. Error	Coeff. Of Variation
WET (June – Nov.)	SEAGRASS	89	79.86	0.00	100.00	22.04	485.93	2.34	27.60
WET (June – Nov.)	MANGROVE	89	8.53	0.00	84.00	14.22	202.30	1.51	166.74
WET (June – Nov.)	ALGAE	89	2.11	0.00	20.80	3.99	15.93	0.42	188.73
WET (June – Nov.)	INVERTEBRATE	89	1.71	0.00	33.20	5.55	30.83	0.59	323.86
DRY (Dec. – May)	SEAGRASS	52	83.00	27.40	100.00	20.31	412.64	2.82	24.47
DRY (Dec. – May)	MANGROVE	52	3.87	0.00	18.20	5.97	35.67	0.83	154.21
DRY (Dec. – May)	ALGAE	52	2.60	0.00	33.80	5.84	34.13	0.81	224.69
DRY (Dec. – May)	INVERTEBRATE	52	1.48	0.00	21.60	4.46	19.93	0.62	302.28

Statistical Analysis Results

Analysis of variance (ANOVA) was utilized to investigate 4-factors of interactions between diet and effect of locality (Southern Lagoon vs. Drowned Cayes), sex (male vs. female), size classification ($\leq$ 245cm vs. > 245cm), and season (wet vs. dry). Separate analyses were run for three food categories: seagrass, mangrove and algae. Variations among diets within these categories were examined by ANOVA results.

The full 4-factor ANOVA analysis did not detect any significant effects among sexes ($P > 0.09$) for seagrass, mangrove or algae. To account for this and to allow the other categories to be examined in more detail, the models were reduced to a 3-factor design (location x size x season) by pooling the sexes. In the reduced 3-factor analysis, locality had a significant effect on seagrass consumption ($P = 0.03$) due to a higher estimated mean in Southern Lagoon (83.5 ± 2.12) compared to the Drowned Cayes (70.5 ± 4.58). However, percent consumption of both mangrove and algae was significantly higher ($P = 0.03$) in the Drowned Cayes (mangrove: 14.7 ± 2.35, algae 4.6 ± 1.00) when compared to Southern Lagoon (mangrove: 5.4 ± 1.08, algae 1.5 ± 0.46). Percent algae consumption was also significantly affected by size classification ($P = 0.04$) due to a higher estimated mean for manatees >245cm (3.8 ± 0.65) compared to manatees ≤245cm (2.3 ± 0.89). No other main effects or interactions were statistically significant ($P > 0.30$).

DISCUSSION

Seagrass was the predominant type of food item consumed by manatees at both sites in Belize. Of the seagrasses, *Halodule wrightii* was determined to be the primary food source in Southern Lagoon and the Drowned Cayes. In samples from Southern Lagoon, *Ruppia maritima* was the second most prevalent type of seagrass present followed by *Thalassia testudinum*. In the Drowned Cayes, *Thalassia testudinum* was the second most common species. Mangroves made up a significant portion of manatee diet in both locations as well; algae and invertebrates were also detected in small quantities among samples.

Species present in manatee diet samples from Belize have been previously reported in other manatee diets (Best 1981; Ledder 1986; Hurst and Beck 1988, Mignucci-Giannoni and Beck 1998; Borges *et al*. 2008; Castelblanco-Martinez *et al*.

2009). The results obtained here are similar to studies from nearby areas (Castelblanco-Martinez *et al*. 2009; Flores-Cascante *et al.* 2013) and compare similarly to studies conducted in similar habitats (Mignucci-Giannoni and Beck 1998). With manatees feeding predominantly on seagrasses, as determined in previous studies, the species reported here are in similar orders of importance (Mignucci-Giannoni and Beck 1998; Castelblanco-Martinez *et al*. 2009; Flores-Cascante *et al.* 2013). The findings are also congruent with results from stable isotope analysis of manatee tissues from Belize (Alves-Stanley *et al.* 2010). Mangrove made up a significant portion of the samples, similar to findings in Puerto Rico, Mexico and Belize (Mignucci-Giannoni and Beck 1998; Castelblanco-Martinez *et al*. 2009; Flores-Cascante *et al.* 2013). Three specific types of algae were identified, but instances of unidentified algal remains were frequent. Of these three discernable species, two have been reported in other locations: *Ulva* sp. in Puerto Rico (Mignucci-Giannoni and Beck 1988) and *Chara* sp. in Mexico (Castelblanco-Martinez *et al.* 2009). All three algal species have been observed in content samples from Florida (Hurst and Beck 1988). Manatee diet samples in Belize also contained small amounts of invertebrates, primarily identified as an unknown Poriferan. Invertebrates can be epiphytic on seagrasses, and incidental ingestion by manatees has been described in additional studies (Hartman 1979; Best 1981; Ledder 1986; Hurst and Beck 1988; Courbis and Worthy 2003), although manatees and dugongs are known to be omnivorous when resources are limited (Powell 1978; Preen 1995a). Algal and animal material have been observed in the dugong diets as well (Lipkin 1976). A previous small scale study of manatees in Belize did not report any invertebrate or algal components (Flores-Cascante *et al.* 2013). The presence of sand grains and detritus has also been described in diet samples of manatees (Best 1981). Additionally, diatoms are certainly a result of incidentally ingesting diatomaceous earth, or these organisms were present on the plant material when ingested. Sand particles were also present, but not counted for purposes of this analysis. This study reinforced that manatees are obligate generalist herbivores utilizing a wide range of resources found in each environment from algae to vascular plants to seagrasses.

From these samples examined from Belize, the number of species identified in each sample ranged from 1-6 different types. Mouth samples consisted of 1-2 species of seagrass, GI-tract samples consisted of 1-4 species, and fecal samples ranged from 1-6 different species. In previous studies, several plant species have been documented as occurring interspersed within the digestive tract of Florida manatees (Best 1981; Ledder 1986; Hurst and Beck 1988), Amazonian manatees (Colares and Colares 2002) and the dugong (Heinsohn and Birch 1972; Lipkin 1976). Since plants are digested at different rates, mouth and stomach samples represented the most identifiable fragments, whereas fecal samples showed the most variation and the greatest challenge for identification. Because manatees have long digestive passage times ranging from 4 to 10 days (Larkin *et al*. 2007), fecal samples are not able to precisely reflect individual feeding habits. Fecal samples contained a higher amount of unknown or unidentifiable points. Further, the actual composite percentage of what species made up the diet as a whole could not be attained. Courbis and Worthy (2003) discussed this for animal tissues in manatee diet, and the same can be assumed similarly for types of algae. Filamentous algae was determined to be the most common type of alga ingested by manatees in Belize. Exact species identification was limited due to lack of recognizable structures, most likely attributed to digestive processes. By obtaining and analyzing diet samples of manatees, the nutritional requirements of manatees can be better understood in order to further protect the important resources they utilize that is integral to conservation efforts.

Location Diet Differences

There have been several different studies focusing on manatee diet in various locations throughout the world. The diet of manatees has shown variation with regard to location. As for samples from Belize, the diet composition is similar to areas nearby with similar habitats, with seagrass being the primary dietary focus. There was some variation within two locations from Belize. Seagrass consumption was significantly higher within Southern Lagoon ($P = 0.03$), whereas mangrove and algae were higher in the Drowned Cayes ($P = 0.03$). Therefore, the null hypothesis for diet differences between locations is rejected. Likewise, Alves-Stanley *et al.* (2010) observed a difference in $\delta^{15}N$ values between manatees sampled in the Drowned Cayes and Southern Lagoon. This cannot simply be attributed to differences in environment between each location. As most of the specimens observed in this study were fecal samples, and due to a manatees' long gut transit time, an accurate snapshot of manatee diet differences cannot be assumed. Furthermore, manatees are transient and have been documented moving between the two sampling sites within the same day (pers. comm., R.K. Bonde, U.S. Geological Survey); manatees in Belize have also been known to show seasonal changes in habitat use (Morales-Vela *et al.* 2000; Auil *et al.* 2007).

Differences between regions illustrated dietary differences. Manatees in Florida have a high variability for differences in diet between regions (Alves-Stanley *et al.* 2010). Florida manatee diets often contain the same seagrass types as manatees in the Caribbean

(Best 1981; Ledder 1986; Hurst and Beck 1988; Mignucci-Giannoni and Beck 1998; Castelblanco-Martinez *et al*. 2009; Flores-Cascante *et al.* 2013) but also contain several other species. Antillean manatee diets in Brazil contain 21 different types of algae, seagrass and invertebrates, but predominantly contain rhodophytes (Borges *et al*. 2008), while Amazonian manatees consumed 24 different plant species within the central Amazon (Borges *et al*. 2008). As manatees are able to move between fresh, salt and brackish waters, they have been documented to make use of dominant plant types in each type of environment.

Diet Differences between Sexes

There were no significant differences observed in diet preferences between male and female manatees within Belize, comparable to previous studies (Castelblanco-Martinez *et al*. 2009). In each environment, manatees of both sexes were subjected to the same availability of food items for each location, season and year. Observations of diet between sexes accepted the null hypothesis of this study. No other studies of manatee diet have determined diet differences between sex.

Diet Differences between Size Classifications

In Belize, no difference in manatee diet between size classifications was detected for seagrass or mangrove consumption. In samples that contained algae, a higher estimated mean for adult animals >245cm (3.8 ± 0.65) compared to juveniles $\leq$245cm (2.3 ± 0.89) identified that there was a statistical difference between these size classifications (P = 0.04), where larger animals consumed a greater proportion of algae. No previous studies have analyzed the relationship between animals' size (length) and diet. The null hypothesis for differences between size classifications and diet is accepted for seagrass and mangrove, but rejected for algae. This may be attributed to the inexperience in foraging by juvenile animals. Further, influencing factors may be the size difference between an adult and juvenile manatee, the amount of ingesta taken up during feeding, and/or the duration of time spent feeding.

Seasonal Diet Differences

Manatees in Belize have been observed to utilize certain habitat types between the wet (June through November) and dry (December through May) seasons (Morales-Vela *et al.* 2000; Self-Sullivan *et al*. 2004; Auil *et al.* 2007). This study took into account the potential changes in diet with regard to season. There were no statistical differences observed in diet samples by season in Belize, thus accepting the null hypothesis. Stable-isotope analysis of manatee diet in Belize observed seasonal changes in $\delta^{13}C$ and/or $\delta^{15}N$ (Alves-Stanley *et al.* 2010). In the Amazon, manatees have been observed to be more selective during the rainy season, but fed on a more diverse number of plants during the dry season (Colares and Colares 2002). Like in Belize, no seasonal differences were observed nearby in Chetumal Bay, Mexico (Castelblanco-Martinez *et al*. 2009); also, no seasonal differences have been observed in dugongs (Preen 1995b). Although seasonal habitat use may change within Belize, the uniform similarity of food plants available for consumption may account for the little diet differences reported for this study.

CONCLUSIONS AND FUTURE RECOMMENDATIONS

As Belize is one of the central locations for areas in the Caribbean populated by manatees (O'Shea and Salisbury 1991), it represents a habitat that is important for the future survival of the subspecies (Quintana-Rizzo and Reynolds 2007; Auil Gomez 2011). Manatees in Belize face an increasing threat from anthropogenic and natural causes of mortality (Auil Gomez 2011). Identification of the types of plants that manatees feed upon is crucial for the conservation of habitats where manatees reside.

Conclusions

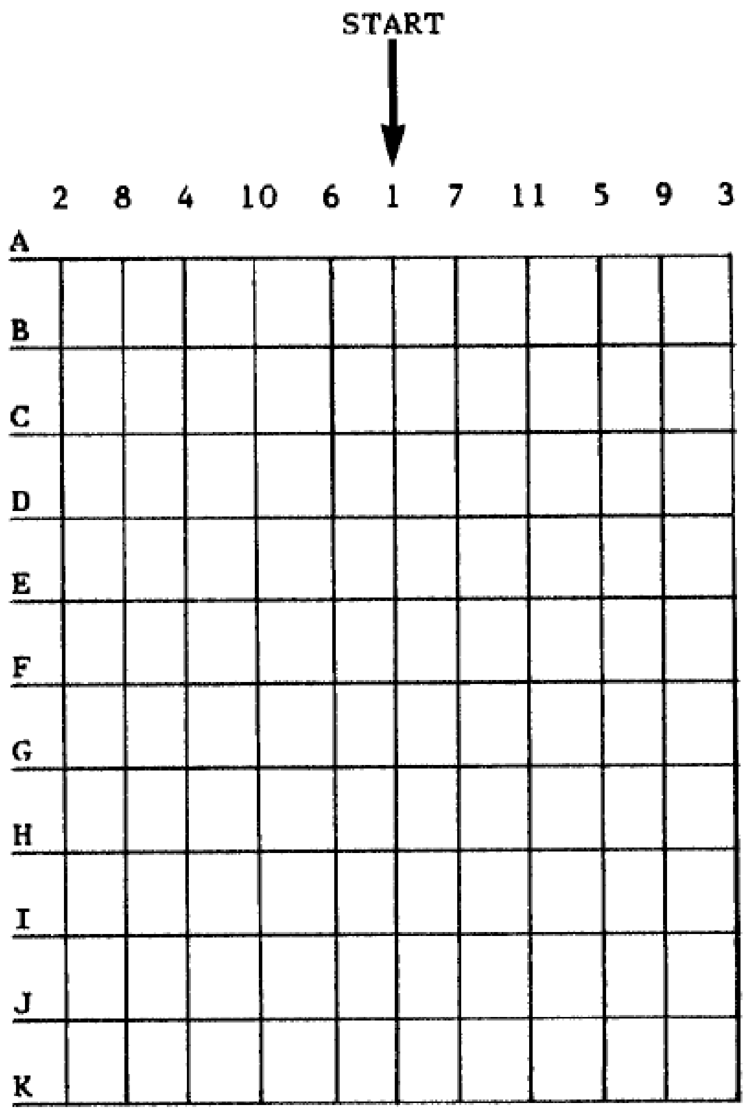
This study concluded that seagrass is the predominant food item consumed by manatees in Belize. Red mangrove, a vascular plant, also made up a significant portion of the manatee diet; algae and invertebrates were detected in small quantities. For microhistological analysis of manatee diet, mouth and stomach samples represented the greatest opportunity for positive identification, while fecal samples showed the highest variation in species present. This data can be used for future conservation efforts of manatees in Belize.

Future Recommendations

Findings from this study recommend an increased effort to preserve seagrass and mangrove habitats in Belize. The species identified by this study provide essential nutrients to manatees. Locations where manatees feed and common food items flourish should be identified as regions of significance to prevent degradation of these valuable resources. Placing an importance on protection of these areas and working directly to preserve the manatees' feeding grounds will support the sustainability of the species. This will further reduce anthropogenic causes of mortality, and promote a positive outcome for continued growth of the regional manatee population in Belize.

APPENDIX A

Transect sequence on eyepiece grid of microscope (from Hurst and Beck 1988) used for microhistological analysis of manatee diet samples in Belize. Identification sequence begins at column 1, row A, and proceeds down the column (1A, 1B, 1C, etc.) until five points of identification are recorded. If five points cannot be identified at the end of each column, sequence is continued in column 2, proceeding down (2A, 2B, 2C, etc.) until five recorded observations are completed. Columns 3-11 are used as needed, also starting at row A, until five points are identified within the eyepiece grid.



APPENDIX B

Datasheet and coordinate system used to record species observed on each slide (from Hurst and Beck 1988). Five points from eyepiece grid (see Appendix A) were identified at 20 coordinate locations, totaling 100 points of identification for each slide.

FIELD ID ____________ SLIDE # ____________

TYPE ____________ START ____________

DATE ____________ END ____________

OBSERVER ____________

SPECIES OBSERVED

FIELD	COORDINATES													
1	55/125													
2	45/125													
3	35/125													
4	25/125													
5	25/120													
6	35/120													
7	45/120													
8	55/120													
9	55/115													
10	45/115													
11	35/115													
12	25/115													
13	25/110													
14	35/110													
15	45/110													
16	55/110													
17	55/105													
18	45/105													
19	35/105													
20	25/105													
100 TOTAL POINTS														

APPENDIX C

Summary datasheet used to record species observed and percent occurrence in 5 slides analyzed for each sample (from Hurst and Beck 1988).

DIET SUMMARY

FIELD ID ____________________ ANALYSIS DATE ______________

TYPE ______________________ ANALYSIS TIME ______ MINUTES

INGESTA COLOR ______________ OBSERVER __________________

COLLECTION DATE ____________ TOTAL LENGTH ___________ cm

LOCATION ________________________________ SEX M / F

	SPECIES											
SLIDE #1												100
SLIDE #2												100
SLIDE #3												100
SLIDE #4												100
SLIDE #5												100
TOTAL POINTS												500
% OCCURRENCE												100%

COMMENTS ____

____

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