EVALUATING GREEN SEA TURTLE (*Chelonia mydas*) NUTRITIONAL NEEDS AND THE

EFFECTIVENESS OF EDUCATION AT A SEA TURTLE REHABILITATION CENTER

by

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(Under the Direction of Sonia M. Hernandez)

ABSTRACT

There are seven species of sea turtles worldwide, and all are of conservation concern. When sea turtles are found stranded, injured or diseased, they are often rescued and brought into rehabilitation care facilities such as the Georgia Sea Turtle Center (GSTC). During rehabilitation, proper nutrition is paramount to the healing process. Green sea turtles are unique among the sea turtles in that hatchlings and young juveniles are carnivorous while later life history stages (juvenile to adult) are primarily herbivorous. Current understanding of this species' dietary requirements is poor and, since proper nutrition is key to recovery, this can significantly impact the rehabilitation process of injured or diseased green sea turtles. One goal of this project was to compare nutritional parameters of rehabilitated green sea turtles to baseline nutritional parameters in healthy free-ranging green sea turtles in order to understand the impact of diet on health and recovery during rehabilitation. A suite of blood nutritional parameters, stable isotope and fatty acid analyses, and gastrointestinal flora (using metagenomics) were evaluated. Because green turtles are an endangered species, rehabilitation and release of healthy animals is important to the status of wild populations. Rehabilitation, however, is a contentious issue. Some people believe it is a diversion of resources, but most people believe rehabilitation of endangered

species is worthwhile. One thing most people agree on is the value of rehabilitation education. It has been shown that rehabilitation centers with public education as a major objective play a critical role in conservation. I proposed that the GSTC and other similar facilities can act as ***boundary organizations for conservation***, translating scientific research in a way the general population can enjoy and get excited about. In order to study this concept, I developed and implemented survey instruments for use within the education department at the GSTC. Information gained from this study will enable rehabilitation centers to understand how they can serve as boundary organizations for conservation as well as how they can make dietary modifications that will enhance the recovery process of green sea turtles.

INDEX WORDS: boundary organization, *Chelonia mydas*, environmental attitudes, green sea turtle, human dimensions, metagenomics, rehabilitation, stable isotopes

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DEDICATION

I would like to dedicate this dissertation to my husband, Matt Bloodgood, and our two

dogs, Blue and Zoey. Without the love and support they have given me over the years, I would

never have dreamed of completing a PhD. Thank you!

ACKNOWLEDGEMENTS

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INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

This interdisciplinary research project was developed through the Integrative Conservation PhD program at the University of Georgia to investigate sea turtle conservation using natural and social science lenses. Interdisciplinary research brings together multiple disciplinary perspectives to gain insights into complex problems (Hirsch et al. 2013). In particular, interdisciplinary research that brings together natural and social sciences has been deemed essential in addressing conservation challenges (MacMynowski 2007). This research focuses on integrating the science of veterinary medicine and sea turtle rehabilitation with environmental education to reinforce the conservation message offered by the Georgia Sea Turtle Center (GSTC) on Jekyll Island, GA. For the veterinary medicine aspect, this research focuses on rehabilitation of a single species, the green sea turtle (GST) (*Chelonia mydas*). Specifically, blood clinical parameters, skin and plasma stable isotopes, and fecal metagenomics are employed to understand how proper nutrition affects overall turtle health. For the environmental education aspect, this research focuses on using in-person surveys to understand how people perceive sea turtle conservation. Boundary organization theory is used to describe how conservation centers work to bridge the gap between science and the general public.

Permits

This research was conducted under the following authorities:

IACUC: GSTC 2013-1

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Georgia Department of Natural Resources Scientific Collecting Permits

- 29-WJH-13-140

- 29-WJH-14-201

- 29-WJH-16-214

Florida Fish and Wildlife Conservation Commission Marine Turtle Permits

- MTP-14-135

- MTP-15-135A

- MTP-16-135A

Institutional Review Board approval ID: STUDY00001134

Study Sites

The GSTC was the site for sample collection from rehabilitation turtles, as well as the site for survey administration. The GSTC is a state-of-the-art rehabilitation, research, and education facility on Jekyll Island, Georgia, USA. Jekyll Island is a barrier island in Glynn County (31°N, 81°W). The Island is approximately 11 km long and 2.4 km wide. The GSTC is the only sea turtle rehabilitation facility in Georgia.

Biological samples used to assess various aspects of nutrition were taken from rehabilitation turtles and were compared to samples collected from free-ranging turtles. The In-Water Research Group, Inc. out of Juniper, Florida assisted with sample collection for the free-ranging component of the study. The In-Water Group captures an average of 175 free-ranging GSTs annually at the St. Lucie power plant on the Florida east central coast, about 50% of which are juveniles in the same size class as those brought into rehabilitation at the GSTC. Turtles coming from the east coast of Florida are thought to feed on algae rather than seagrass, a diet

which is identical to the turtles received for rehabilitation by the GSTC (Allen Foley, personal communication, 1-15-15). However, there is recent evidence that they may eat both seagrass and algae (Gorham et al. 2016).

Green Sea Turtle Conservation Status and Threats

There are seven species of sea turtle worldwide, and all are of conservation concern. The GST is a long-lived migratory species that is listed as endangered or threatened throughout its range, both in the United States and other parts of the world. Threats to sea turtle survival are both natural and anthropogenic in origin. Predators such as raccoons, feral hogs, and fire ants can destroy nests. Fibropapillomatosis (FP), a debilitating, infectious, neoplastic disease, has become panzootic over the last three decades (Page-Karjian et al. 2014). Cold-stunning, a complex hypothermic condition associated with cold water temperatures, is another threat to sea turtle survival (George 1997). Other threats are anthropogenic in origin, including entanglement in fisheries nets and gear, injuries from boat strikes, direct harvest for food or production of commercial items, and decreased recruitment because of coastal development, beach erosion, and artificial lighting (Lutcavage et al. 1997, Witherington 1992).

Rehabilitation of Sea Turtles

Largely as a consequence of the endangered status and population declines of sea turtles, rehabilitation of injured or diseased individuals is considered worth its risks for conservation (Al-Mohanna et al. 2014). Wildlife rehabilitation is defined as "the treatment and temporary care of injured, diseased, and displaced indigenous animals, and the subsequent release of healthy animals to appropriate habitats in the wild" (Miller 2012, page ix). Rehabilitation of individual animals has been criticized for its lack of effect at the population level, its potential to interfere with natural selection, and its potential to increase disease transmission between individuals;

however, many argue that humans have a duty to protect and improve the welfare of animals, especially because so many are harmed as a result of human activity (Sleeman 2008).

When sea turtles are found injured or diseased on the Georgia or Florida coasts, they are rescued and brought into one of the 16 rehabilitation care facilities in Georgia and Florida. The most common species presented to these facilities are green (*Chelonia mydas*), loggerhead (*Caretta caretta*), and Kemp's ridleys (*Lepidochelys kempi*). The combined annual total number of live sea turtles admitted for rehabilitation by these facilities is roughly 500 during an average year without mass stranding events (e.g., oil spill, cold stun, unexplained mortality event) (Florida Fish and Wildlife Conservation Commission, unpublished data).

The GSTC receives an average of 80 sea turtles annually from both Georgia and Florida. Juvenile GSTs, the focal species for this study, represent approximately 33% of the annual caseload. While a wide range of medical issues are encountered in GSTs presenting to the GSTC, many of the problems are nutritionally based or involve the gastrointestinal (GI) tract. These include general debilitation/starvation, GI ileus, grass impactions, lower GI tract obstructions, constipation, obesity, fatty liver, GI microbial flora disruption, and calcium/phosphorous imbalances (Erlacher-Reid et al. 2013). Common medical issues in GSTs maintained in captivity more permanently (e.g. in zoos and aquaria) involve similar issues, such as obesity, constipation, and lower GI obstruction. Erlacher-Reid et al. (2013) proposed that GSTs maintained in captivity may be at an additional risk to obstructive intestinal disease due to obesity, diet, reduced physical activity, chronic intestinal disease, and inappropriate antibiotic administration. Because proper nutrition plays a critical role in the health and eventual release of GSTs presenting for rehabilitation, the first part of this dissertation research focuses on the

unknowns of GST nutrition, and how we can use blood clinical parameters, skin stable isotopes, and fecal metagenomics to better understand how to meet the nutritional needs of these animals.

GREEN SEA TURTLE NUTRITION

<u>Green Sea Turtle Foraging Ecology</u>

      Green sea turtles are unique among the sea turtles in that hatchlings and pelagic juveniles are primarily carnivorous, while later life history stages (coastal juvenile to adult) are primarily herbivorous (Boyle and Limpus 2008). After leaving the nesting beach, young GSTs spend the first part of their life occupying pelagic habitats, often in association with sargassum rafts (Carr 1987). In Atlantic GST populations, once they reach a size of 20 to 25 cm in straight carapace length, they leave pelagic habitats and enter benthic feeding areas, where they shift to a primarily herbivorous diet (Bjorndal and Bolten 1988). It takes a GST anywhere from one to seven years to reach this size (Goshe 2009). In Hawaii and Australia, the shift from the pelagic to benthic stage occurs when the animal is slightly larger, around 35 cm in straight carapace length (Balazs 1980, Limpus et al. 1994).

      Studies on the nutritional requirements of GSTs have primarily focused on the foraging ecology of free-ranging populations (e.g. Bjorndal 1980, Bjorndal 1997, Mortimer 1982, Ogden et al. 1983, Seminoff et al. 2002, Williams 1988). Green sea turtles have been shown to eat both seagrass and algae, and different populations may prefer one, the other or both (Bjorndal 1997, Mortimer 1982). Individuals that eat seagrass prefer young seagrass blades, and they create "grazing plots," areas where they continually crop young shoots and ignore older grasses (Bjorndal 1980). Green sea turtles have also been shown to eat some animal matter, including jellyfish, salps, and sponges (Mortimer 1982). The population on the East Pacific Coast, in particular, may be more carnivorous than other populations (Hays-Brown and Brown 1982,

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Seminoff et al. 2002). In addition to algae, GSTs in Peru have been noted to eat mollusks, polychaetes, jellyfish, fish, fish eggs, and crustaceans (Hays-Brown and Brown 1982).

Specific requirements for calories, protein, fat, and carbohydrates have not been identified in GSTs. Essential amino acids for hatchling GSTs have been identified as lysine, tryptophan, methionine, valine, leucine, isoleucine, phenylalanine, threonine, and histidine; arginine is semi-essential (Wood and Wood 1977a). Requirements for seven of the essential amino acids have been identified as a percent of dry diet, including: lysine (1.7%), tryptophan (0.22%), methionine (1.5%), valine (1.3%), isoleucine (1.0%), leucine (1.6%), and phenylalanine (1.0%) (Wood and Wood 1977a, b).

Gastrointestinal transit time has also been studied in GSTs. Brand et al. (1999) estimated passage time as 6.5 to 13.5 days, but in this study, turtles were fed plastic markers and were later sacrificed to estimate passage time based on the location of the markers in the GI tract at the time of death. Amorocho and Reina (2008) fed plastic markers to captive GSTs and allowed the marker to pass all the way through the GI tract; intake passage time via this method was estimated as 23.3 ± 6.6 days.

<u>Adaptations to Herbivory</u>

Juvenile and adult GSTs are hindgut fermenters and their hindgut microbial flora digests cellulose and hemicellulose with a high degree of efficiency (Bjorndal 1979). As an adaptation to their herbivorous diet, GSTs have proportionally longer GI tracts than carnivorous sea turtle species (Wyneken and Witherington 2001). In addition, their large intestine is approximately 2.5 times the length of the small intestine and the proximal colon is expanded into a functional cecum (Bjorndal 1979, Wyneken and Witherington 2001). One study involving GSTs from several rehabilitation facilities and zoological parks suggests that their unique digestive

adaptations to an herbivorous diet may predispose them to intestinal disease when their intestinal motility is reduced (Erlacher-Reid et al. 2013).

Feeding Green Sea Turtles in Rehabilitation

In rehabilitation and other captive situations, the diet of animals is rarely the same as what that individual would eat in the wild, and a substitute diet must typically be formulated (Hatt 2000). Historically, diet formulation has been based on tradition and an extrapolation of nutritional needs from domestic animals. While wildlife animal nutritionists still rely on domestic animal models, there is increasing support for research specific to individual species (Hatt 2000). Scientifically-based nutrition for captive animals is recognized as imperative in animal management, and is integral to longevity, disease prevention, growth, and reproduction (Dierenfeld 1997).

Sea turtles are typically fed a variety of locally available vegetables and seafood during periods of captivity. Many facilities also use commercial pelleted turtle feed, modified trout chow, and gelatin-based diets. In general, turtles should be fed one to three times per day, and the amount offered should total 1-5% of their body weight (Bluvias and Eckert 2010). This percentage should be adjusted to reflect the turtle's status, with the lower percentage for maintenance and the higher percentage for sick, emaciated and/or younger turtles (Bluvias and Eckert 2010).

There is high variability in what rehabilitation facilities feed GSTs, but they often begin by tube-feeding a fish-based gruel or commercially available formula (e.g. Emeraid Herbivore elemental diet and/or Oxbow Herbivore Fine Grind), or offering a combination of fish, shrimp, and squid if the turtle is eating on its own (Bluvias and Eckert 2010, Norton 2005). These diets are formulated for a high caloric intake and to stimulate eating during the early stages of

rehabilitation, as leafy greens and other vegetables are calorically poor and sometimes not accepted well in the first few weeks after admission (Bluvias and Eckert 2010).

GSTs that present to the GSTC are typically 25-40 cm in curved carapace length, a size indicating that they have recruited to benthic habitats and shifted to the herbivorous feeding stage of older juveniles and adults. This is supported by fecal content analysis in rehabilitation cases and GI contents found during necropsy evaluations. However, at the GSTC, many of these animals seem to prefer seafood over vegetables during the early stages of rehabilitation. Therefore, once a turtle shows interest in eating on its own, it is offered a variety of vegetables (i.e. lettuce, cucumbers, and green peppers) and seafood (i.e. mackerel, herring, shrimp, and squid). If a turtle prefers seafood, it may be fed that until it has reached a normal body condition, and then attempts are made to convert it over to a plant-based diet. In addition, multivitamin (Mazuri® Vita-Zu® Sea Turtle Vitamin for Fish-based diets, 500mg, catalog number 1815523-300, Mazuri, Richmond, IN 47374; recipe in Appendix C) and calcium supplements (Calcium Carbonate 10 gr, 648mg, catalog number 00536-1024-10, Rugby, Livonia, MI 48152) are offered daily. The gel is a mixture of trout chow, seafood, vegetables, vitamins, and gelatin, which was developed for carnivorous loggerhead sea turtle hatchlings, not herbivorous GSTs (recipe in Appendix B). The gel is often unpalatable to the juvenile GSTs, because many of them will not eat it. A future goal based on the results of this research is the development of a more palatable, nutritionally complete, herbivorous gel diet that can be manipulated as needed through additions of fat, protein, and fiber. This is expected to provide GSTs with more ideal nutrition during the rehabilitation process and for long-term maintenance in captivity (Norton, personal communication).

Once GSTs are on a good plane of nutrition, if they continue to be fed an animal-protein rich diet, they have a tendency to become overweight (Norton, personal communication). In addition, a number of facilities have reported elevations in aspartate aminotransferase (AST) and significant calcium/phosphorous (Ca:P) imbalances with animal-based diets (Norton, unpublished data). Liver biopsies have been performed by the GSTC on animals with markedly elevated AST and Ca:P imbalances, revealing hepatic lipidosis (fatty liver) on histopathological examination (Norton, personal communication). In these cases, these values normalized once the turtle was converted to an herbivorous diet. Seafood, in general, has an inverted Ca:P ratio (e.g. Mackerel 1:34; Robbins 1983) and is higher in fat and protein than the typical diet items eaten by free-ranging GSTs (see McDermid et al. (2007) for nutritional composition of common free-ranging GST diet items). Because of this, as soon as a rehabilitation turtle is willing to eat it, the diet is gradually shifted to a plant-based diet.

Even feeding vegetable matter like lettuce may not be equivalent to the seagrass and algae that GSTs eat in the wild. Siegal-Willott et al. (2010) compared the nutritional value of seagrass and algae eaten by free-ranging Florida manatees (*Trichechus manatus latirostris*) to that of romaine lettuce, which is typically fed to captive manatees. The study examined dry matter content, proximate nutrients (crude protein, ether-extracted crude fat, nonfiber carbohydrate, and ash), and the calculated digestible energy of seagrasses and algae compared with those of romaine lettuce. Neutral-detergent fiber, acid-detergent fiber, and lignin were also compared. Results indicated that romaine lettuce and seagrasses and algae are not equivalent forages, and that captive manatees should be provided a diet higher in fiber and lower in fat, protein, digestible carbohydrates, and digestible energy to more closely mimic the diet of free-ranging manatees (Siegal-Willott et al. 2010). These data have direct applicability to GST dietary

analysis and highlight the need to develop a proper diet for GSTs, because the nutritional needs of these animals are likely not being met by feeding them fresh vegetables from terrestrial environments (i.e., lettuces, green peppers, cucumbers, and the gel described earlier). The marine vegetation that GSTs eat in the wild would be more ideal, however, cultivation is expensive and it would take an impractical amount of space to propagate enough vegetation for sea turtle consumption (Butterworth 2010).

Assessing Nutrition through Bloodwork

Hematology and biochemistry profiles are frequently used to assess the health and nutrition of reptiles. Hematology is most valuable in assessing the response of a patient to disease or therapy (Campbell and Ellis 2004). Hematologic evaluation includes examination of erythrocytes, leukocytes, and thrombocytes in the peripheral blood (Campbell and Ellis 2004). This is achieved via a complete blood count (CBC), which includes a packed cell volume (PCV), a measurement of the total proteins (TP), and a total and differential leukocyte count.

A PCV estimates the percentage of red blood cells in the blood and is obtained by microhematocrit centrifugation. Anemia, a reduction in red blood cells or of hemoglobin in the blood, may be found as a result of blood loss, intravascular hemolysis, or chronic inflammatory disease (Thrall et al. 2012). A favorable response in anemic reptiles is an erythrocytic regenerative response, which may be reflected on a blood film by basophilic stippling, an increased number of mitotic figures, increased anisocytosis and polychromasia (Stacy et al. 2011). A nonregenerative anemia may be a result of bone marrow disease, renal failure, inflammatory disease, or nutritional deficiencies (Thrall et al. 2012). An elevated PCV may suggest hemoconcentration (e.g. due to dehydration) (Campbell and Ellis 2004).

Total protein is most accurate when measured via the biuret method (Thrall et al. 2012). A refractometer may be used to estimate total protein, but measurements can be influenced by factors such as hemolysis, lipemia, hyperbilirubinemia, and hyperglycemia (Melillo 2013).

Total leukocyte counts of reptiles may be performed via the Natt-Herrick method or Phloxine B method with a hemocytometer, or through direct cell counts on a blood film. Differential leukocyte counts may also be performed on a blood smear that has been stained (e.g. Wright-Giemsa). Leukocytes in sea turtles include heterophils, eosinophils, basophils, lymphocytes, and monocytes.

The biochemistry panel used in this study included TP, albumin, globulin, cholesterol, triglycerides, AST, creatine kinase (CK), blood urea nitrogen (BUN), glucose, calcium, phosphorus, chloride, potassium, sodium, and uric acid. Total protein and protein fractions (i.e. pre-albumin, albumin, alpha 1 globulins, alpha 2 globulins, beta globulins, and gamma globulins) were also analyzed via electrophoresis, and its interpretation can be found in that section. Cholesterol and triglycerides we also analyzed as part of a lipid panel, and are discussed in that section. Other parameters and their interpretations are below (Table 1.1).

Table 1.1. Selected clinical chemistry parameters and their functions and interpretations. Adapted from Robbins (1983) and Thrall et al. (2006).

Parameter	General Function and Interpretation
AST	Mitochondrial and cytosolic enzyme with high activity in liver, heart, skeletal muscle, and kidney and low activity in the intestines, brain, lung, and testes. Elevations above normal may indicate hepatic disease or muscle injury, although high activity of this enzyme in different tissues makes interpretation difficult.
CK	Enzyme located in skeletal muscle, cardiac muscle, smooth muscle, brain, and nerves; found free in the cytoplasm of muscle cells and leaks from these cells when they are damaged. Levels may be elevated for many reasons, including restraint, physical activity, intramuscular injections, trauma, shivering, and myositis. Because of its sensitivity, this this parameter is generally considered clinically relevant only with large increases (>10,000 U/L) or persistent increases (>2,000 U/L).
BUN	Product of nitrogen metabolism; produced in the liver from the conversion of ammonia and excreted by the kidneys. Elevations in BUN can be caused by recent ingestion of a high protein

meal, gastrointestinal hemorrhage, or decreased glomerular

filtration.

Glucose   Blood glucose is derived from intestinal absorption from meals,

hepatic production, and kidney production (minor).

Hypoglycemia may result from inadequate dietary intake,

excessive cellular utilization, impaired hepatic gluconeogenesis

and glycogenolysis and/or a deficiency of diabetogenic

hormones. Hyperglycemia may result from insulin deficiency,

decreased glucose utilization, and/or increased hepatic

gluconeogenesis and glycogenolysis.

Calcium   Associated with blood clotting, excitability of nerves and

muscles, acid-base balance, eggshell formation, and muscle

contraction. Hypocalcemia may be a result of dietary

insufficiency or endocrine disorders including

hypoparathyroidism and hypovitaminosis D. Hypercalcemia

may be due to hyperparathyroidism, chronic renal failure,

hypoadrenocorticism, and hypervitaminosis D.

Phosphorus   Involved in energy metabolism, muscle contractions, nerve

tissue metabolism, transport of metabolites, nucleic acid

structure, and carbohydrate, fat and amino acid metabolism.

Hypophosphatemia is associated with hyperparathyroidism and

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diabetic ketoacidosis. Common causes of hyperphosphatemia are renal failure, vitamin D toxicosis, and hypoparathyroidism.

Ca:P	Plasma concentration of ionic Ca and inorganic phosphate tend to be related reciprocally.
Chloride	Principle anion of body fluids; involved in acid-base relations, gastric acidity (hydrochloric acid), and digestion. Hypochloremia may result from excess water retention or loss of chloride from the body (e.g. diarrhea). Hyperchloremia may result from chloride retention or water loss from the body in excess of chloride loss.
Potassium	Occurs primarily within cells and functions in nerve and muscle excitability, carbohydrate metabolism and enzyme activation, tissue pH, and osmotic regulation. Hypokalemia may be due to anorexia or decreased intake, translocation of potassium to intracellular fluid, or excessive excretion via vomiting, diarrhea, or urinary loss. Hyperkalemia may be due to increased intake, translocation of potassium to extracellular fluid, or decreased urinary excretion.
Sodium	Principle cation in the extracellular fluid; important in regulation of body fluid volume and osmolarity, acid-base balance and

tissue pH, muscle contraction, and nerve impulse transmission. Hyponatremia may result from excess water retention or loss of sodium from the body (e.g. diarrhea). Hypernatremia may result from sodium retention or water loss from the body in excess of sodium loss.

Uric Acid	Product of nitrogen metabolism; excreted by the kidneys. Increases in uric acid may reflect impaired renal function, but this is not specific as it may also be raised after a high protein meal, during starvation, or with tissue necrosis.

Normal biochemistry and hematology parameters have been established for several free-ranging GST populations (e.g., Bolten and Bjorndal 1992, Flint et al. 2010, Jacobson et al. 2007, Osborne et al. 2010, Samour et al. 1998). In addition, blood biochemical reference values for debilitated GSTs associated with specific health problems, such as FP or cold-stunning have been described (Aguirre and Balazs 2000, Anderson et al. 2011). Throughout this study, we used reference ranges established by Bolten and Bjorndal (1992) from a population of 100 juvenile GSTs in the southern Bahamas (Table 1.2). These reference ranges were not considered absolute, as animals included in reference range studies may not all be healthy and environmental (e.g. temperature, location) and individual characteristics (e.g. age, sex) may affect these ranges (Thrall et al. 2012).

Table 1.2. Reference ranges for a population of 100 juvenile green sea turtles in the southern Bahamas (Bolten and Bjorndal 1992). Values are presented as mean (range).

Parameter	Mean (range)
PCV (%)	35.2 (26.4-42.0)
Total Protein (g/dl)	5.1 (2.6-6.9)
Glucose (mg/dl)	114 (87-167)
Sodium (meq/l)	172 (157-183)
Potassium (meq/l)	5.3 (4.1-6.9)
Chloride (meq/l)	113 (100-130)
BUN (mg/dl)	14 (3-107)
Creatinine (mg/dl)	0.5 (0.3-0.9)
Uric Acid (mg/dl)	1.5 (0.5-3.5)
Calcium (mg/dl)	9.1 (1.6-12.2)
Phosphorus (mg/dl)	6.7 (3.8-10.9)
Cholesterol (mg/dl)	217 (73-365)
Triglycerides (mg/dl)	172 (43-413)

Protein Electrophoresis

While hematology and chemistry panels are frequently performed as part of a routine physical exam, many other more specific assays for evaluation of the nutritional status of animals are available. One of these is protein electrophoresis, which not only provides a measure of the total plasma protein, but breaks down total protein into its separate fractions including: pre-albumin, albumin, alpha 1 globulins, alpha 2 globulins, beta globulins, and gamma globulins. Protein electrophoresis involves separating plasma proteins on an agarose gel with an applied electric current based on their charge. The migrations of the proteins are represented graphically, and peaks correspond to the various fractions.

The two major types of proteins in plasma are albumin and globulin. Albumin is a carrier protein and plays a role in the transport of free fatty acids, bile acids, bilirubin, calcium, hormones, and drugs. Albumin also plays an important role in oncotic pressure (preventing water from diffusing from the blood into the tissues) (Thrall et al. 2012). A pre-albumin fraction has been observed in healthy chelonian species; the significance of this fraction is unclear, but likely represents proteins with similar carrier functions as albumin (Zaias and Cray 2002). Globulins are a heterogeneous group of proteins classified by their electrophoretic separation as either alpha, beta, or gamma. Alpha globulins include acute-phase proteins alpha-lipoprotein, alpha-1-antitrypsin, and alpha-2-macroglobulin; beta globulins are acute-phase proteins and include fibrinogen, transferrin, beta-lipoprotein, and complement; gamma globulin represents the circulating immunoglobulins (e.g. antibodies) (Zaias and Cray 2002). After quantifying these proteins, an albumin:globulin (A:G) ratio may be used to identify the relative proportion of albumin and globulin. This is calculated as (pre-albumin + albumin) / (alpha-1 + alpha-2 + beta + gamma globulins).

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Many of the plasma proteins (e.g. acute phase proteins and immunoglobulins) change in disease (Zaias and Cray 2002). A summary of parameters and their interpretations can be found in Table 1.3.

Table 1.3. Total protein and protein fractions and their interpretations (table from Zaias and Cray 2002).

Parameter	Interpretation
Total Protein	Hyperproteinemia: dehydration
	Hypoproteinemia: overhydration
Albumin	Hyperalbuminemia: rare outside of dehydration
	Hypoalbuminemia:
	Loss of albumin: kidney, liver, gastrointestinal disease, internal parasites
	Decreased synthesis of albumin: liver disease, malnutrition, chronic inflammatory disease
Alpha-1 Globulins	Increased: acute inflammation, parasitism
Alpha-2 Globulins	Increased: acute inflammation, hepatitis, nephritis/nephrotic syndrome

Beta Globulins	Increased: acute and chronic inflammation, hepatitis, nephritis
Gamma Globulins	Increased: chronic inflammatory and infectious disease, immune-mediated disease, tumors of the reticuloendothelial system
A:G	May decrease because of elevation of globulins, decrease in albumin, or both

Zaias and Cray (2002) cited unpublished data of birds and reptiles, where the concentration of globulins is minimal compared to albumin. However, in a study of 41 wild, healthy loggerhead turtles, Gicking et al. (2004) found that gamma globulin levels were significantly elevated compared to avian species. The reason for this was unknown, however the authors proposed that it could be due to subclinical infection with digenetic trematodes (e.g. Spirorchiidae), a common infection in loggerhead turtles (parasitism would increase antibody production and raise the gamma globulin level) (Gicking et al. 2004). This study also revealed a large number of individuals (11 out of 41) with beta-gamma bridging, which was also attributed to spirorchiid infection. Plasma protein fractions for 29 turtles in this study without beta-gamma bridging are shown in Table 1.4 (Gicking et al. 2004).

Table 1.4. Plasma protein fractions in 29 Atlantic loggerhead sea turtles. Table adapted from Gicking et al. (2004).

	Total Protein	Albumin	Alpha Globulins	Beta Globulins	Gamma Globulins	A:G
All (N=29)	4.3 ± 0.72	1.0 ± 0.17	0.48 ± 0.10	0.80 ± 0.20	1.94 ± 0.62	0.33 ± 0.10
Adult males (N=7)	4.6 ± 0.33	1.1 ± 0.11	0.54 ± 0.12	0.99 ± 0.17	1.97 ± 0.27	0.32 ± 0.05
Adult females (N=7)	4.4 ± 0.75	0.97 ± 0.13	0.49 ± 0.05	0.81 ± 0.14	2.1 ± 0.64	0.30 ± 0.62
Juvenile males (N=8)	4.1 ± 0.66	0.96 ± 0.19	0.46 ± 0.11	0.78 ± 0.13	1.8 ± 0.61	0.33 ± 0.10
Juvenile females (N=7)	3.9 ± 0.78	1.0 ± 0.17	0.44 ± 0.06	0.60 ± 0.07	1.9 ± 0.76	0.38 ± 0.15

Lipoprotein Chemistry Panel

Another useful indicator of nutrition is the lipoprotein chemistry panel, which includes total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL). Cholesterol is important in cell membrane fluidity and serves as a precursor to steroid hormones, bile acids, and vitamin D. Cholesterol can be acquired through the diet only if the diet contains animal tissue. It can also be created endogenously; the major site of cholesterol synthesis is the liver (Thrall et al. 2012).

Triglycerides are made up of three fatty acids esterified to a glycerol molecule (Pond 1998).
Triglycerides are synthesized primarily in adipose tissue, the liver, small intestine, and mammary
gland (Thrall et al. 2012). Lipoproteins are transport molecules made up of lipid and proteins that
carry triglycerides and cholesterol through the blood. Different lipoproteins were originally
identified by centrifugation, and are thus named after their relative densities: HDL has the
highest proportion of protein and lowest of lipid; LDL and VLDL have higher percentages of
lipid.

Plasma triglyceride concentrations in the blood rise after feeding and then decrease as
they are cleared from the blood by peripheral tissues (Price et al. 2013). Triglyceride levels are
generally low when fasting (Price et al. 2013). Triglycerides and cholesterol may be elevated in
cases of hepatic lipidosis, and in adults may be elevated during normal vitellogenesis
(Hernandez-Divers and Cooper 2006).

Fatty Acid Panel

Lipid and fatty acid panels can be interpreted together. The fatty acid panel includes
measurements of total fat, and a breakdown of the relative proportion of each fatty acid:
saturated fatty acids, monounsaturated fatty acids, trans fatty acids, and polyunsaturated fatty
acids.

Fatty acids consist of a chain of carbon atoms attached to hydrogen atoms. One end of
this chain has a –COOH group (characteristic of an acid), and the other has a methyl group (–
CH_3). Fatty acids with less than eight carbons are called short-chain fatty acids (also volatile
fatty acids/VFAs), those with 8-12 are called medium-chain, and those with more than 12 are
called long-chain (Pond 1998). Fatty acids with single carbon-carbon bonds are called saturated
(because they are saturated with hydrogens), while those with double carbon-carbon bonds are

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called unsaturated (monounsaturated have one of these bonds and polyunsaturated have two or more). The number of carbon atoms and the ways they are joined together determine the biological roles of different fatty acids. Fatty acids are typically esterified to glycerols, forming larger molecules such as triglycerides and phospholipids. Free fatty acids (non-esterified) move through the blood via carrier molecules such as albumin.

Animal and plant cells can synthesize fatty acids, and most fatty acids pass unaltered from plant to herbivore (Pond 1998). In terrestrial mammals and birds, the most common saturated fatty acids are palmitic, stearic, and myristic, and the most common monounsaturated fatty acid is oleic. Essential fatty acids are those that the body cannot synthesize and therefore must be acquired through the diet (Pond 1998). In most animals, these include linoleic (18:2ω6) and α-linolenic (18:3ω3).

Joseph et al. (1985) showed that fatty acid composition in GSTs varies with dietary fatty acids consumed. The authors looked at rendered oils from wild GSTs from Panama and Somalia, and fat depots from wild GSTs in Hawaii and the Caribbean, and found that the most common fatty acids were lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), and oleic acid (18:1ω9) (Joseph et al. 1985). Somewhat higher percentages of 16 and 20 carbon polyenes in Hawaiian than Caribbean turtle fat was thought to reflect the inclusion of algae and/or jellyfish in the diet of Hawaiian turtles. This study also included pen-reared turtles fed a diet of a "commercial extruded ration" (undescribed composition), and an aquarium-reared turtle fed primarily herring. Pen-reared turtles had a fatty acid profile dominated by 16:0, 18:1ω9, and 18:2ω6, similar to the turtle feed they consumed; the aquarium-reared turtle had a significant amount of 20 and 22 carbon monoenes, similar to the fatty acid composition of carnivorous marine fishes, and thus reflective of the herring-based diet of this individual (Joseph et al. 1985).

Microbial fermentation in the hindgut of GSTs produces volatile fatty acids (VFAs) such as acetic, butyric, and proprionic acids (Bjorndal 1979). Levels of VFAs are highest in the cecum and colon, indicating that these are the primary sites of fermentation. Levels decrease from the mid-colon to the rectum, indicating that most of the VFAs are absorbed in the cecum and large intestine (Bjorndal 1979).

Vitamin D Panel

A vitamin D profile includes 25-hydroxyvitamin D (calcifediol), parathyroid hormone (PTH), and ionized calcium (iCa). While total calcium is routinely measured on a biochemical panel, it represents both iCa and calcium bound to albumin and other molecules. Ionized Ca is the physiologically active form and is a better measurement of calcium homeostasis (Stringer et al. 2010). Parathyroid hormone and vitamin D_3 are calcium-regulating hormones (Adkesson and Langan 2007). Parathyroid hormone is secreted by the parathyroid gland in response to decreased plasma calcium; PTH stimulates bone demineralization to increase circulating calcium levels (Adkesson and Langan 2007). Calcifediol is a metabolite of vitamin D_3 (cholecalciferol). Vitamin D_3 is obtained through the diet or through skin exposure to UV-B radiation. In one study comparing rehabilitated and wild GSTs, 25-hydroxyvitamin D values did not differ, but iCa values were significantly lower and PTH significantly higher in rehabilitation turtles compared to wild turtles (Stringer et al. 2010). The authors suggested this was due to improper diets in captivity.

Vitamins A and E

Vitamins A and E are both fat-soluble vitamins (along with vitamins D and K) that are required by vertebrates for survival (Robbins 1983). Vitamin A is expressed as retinal, retinol, or retinoic acid, and is acquired directly from the diet or by conversion of dietary carotenoids.

Herbivores acquire vitamin A by biosynthesis from ingested plant carotenoids, such as β-carotene; omnivores and carnivores acquire vitamin A from ingestion of animal tissues with preformed vitamin A (Underwood 1984). Vitamin A is a major constituent of the visual pigment rhodopsin, and is required for growth and integrity of epithelial tissue (Robbins 1983). Deficiencies may be characterized by nervous disorders, reproductive disorders, impaired eyesight, and bone and teeth abnormalities; toxicity is rare (Robbins 1983). Retinol is stored in the liver, and plasma levels are held relatively constant, so a drop in plasma retinol is not seen unless there is a considerable drop in total concentration (Frutchey 2004). Contaminants (e.g. polychlorinated biphenyls) have been shown to disrupt normal vitamin A physiology, and thus the use of retinol as a biomarker for contaminant exposure has been explored (Simms 2000).

Vitamin E is expressed as the most biologically active form, α-tocopherol. Vitamin E is acquired through the diet and occurs naturally in nuts, seeds, oils, fruits, vegetables, and grasses; animal tissues are poor sources of vitamin E (Bauernfeind 1980). Tocopherols are the primary natural lipid-soluble antioxidants, and serve to protect cell membranes through preventing lipid peroxidation (McCay and King 1980). There is also some evidence of vitamin E providing a protective effect on retinol at a cellular level (Underwood 1984). Vitamin E deficiencies may be characterized by myopathy; lesions of reproductive system, central nervous system, and cardiovascular system; hematopoietic disorders; hepatic necrosis; and excessive accumulation of lipopigment (a product of oxidation of unsaturated fatty acids; Nelson 1980).

Few studies examine vitamin A and E concentrations in GSTs. Frutchey (2004) examined plasma concentration of vitamins A and E in nesting GSTs in the Archie Carr National Wildlife Refuge in Melbourne Beach, Florida. Vitamin A (measured as µg/ml retinol) concentrations averaged 0.52 ± 0.04 µg/ml (mean ± 1 SE) and ranged between 0.04 – 1.74 µg/ml. Vitamin E

(measured as µg/ml α-tocopherol) concentrations averaged 6.08 ± 0.40 µg/ml and ranged between $0.90 – 25.31$ µg/ml.

Frutchey (2004) also examined plasma concentrations of vitamins A and E in juvenile GSTs at three different capture sites on the Atlantic coast of Florida (Indian River Lagoon, Nearshore Reef, and Trident Basin). Across sites, vitamin A (measured as µg/ml retinol) concentrations averaged 0.61 ± 0.01 µg/ml (mean ± 1 SE) and ranged between $0.05 – 1.31$ µg/ml. Vitamin E (measured as µg/ml α-tocopherol) concentrations averaged 3.87 ± 0.13 and ranged between $0.64 – 12.41$ µg/ml. Differences in vitamin concentrations in turtles from the three sites as well as differences between healthy individuals and those with FP were examined. Degree of FP was categorized on a scale of 0-3 where 0 = no sign of FP, 1 = mild FP, 2 = moderate FP, and 3 = severe FP. Size differences at the three capture sites was also factored in the analysis—the average straight carapace lengths were 41.6 cm at Indian River Lagoon, 42.6 cm at Nearshore Reef, and 30.9 cm at Trident Basin. Plasma concentrations of both vitamins A and E varied significantly between the three study sites (Table 1.5). This was attributed to potential differences in intake due to vegetation biomass/food availability and diversity of forage species among sites; however this was speculation and a call was made for further investigation into the vitamin content of diet items.

Table 1.5. Vitamin A (measured as µg/ml retinol) and vitamin E (measured as µg/ml α-tocopherol) levels in 282 green sea turtles from three sites in Archie Carr National Wildlife Refuge, FL (Frutchey 2004). Values are presented as least-squares mean ± 1 SE.

Vitamin A (µg/ml)			Vitamin E (µg/ml)		
Indian River Lagoon	Nearshore Reef	Trident Basin	Indian River Lagoon	Nearshore Reef	Trident Basin
0.65 ± 0.03	0.50 ± 0.04	0.51 ± 0.04	0.61 ± 0.03	0.69 ± 0.04	0.52 ± 0.05

Plasma concentrations of vitamin A did not vary with the size of the turtle; however, vitamin E concentrations were significantly higher in smaller turtles. Smaller animals may have to mobilize more vitamins for growth than larger animals, however, it is unclear why this would be true for vitamin E and not vitamin A (Frutchey 2004).

Plasma concentrations of vitamin A did not vary with FP status. Vitamin E concentration varied significantly with degree of FP at one study site, Indian River Lagoon. This was the site of highest prevalence of FP (50-70%). Turtles with an FP category of 2 had significantly lower concentrations of Vitamin E than those with a category of 0; turtles classified as category 1 and 3 were not significantly different, but this may have been due to small sample sizes. This is relevant to our research in that some turtles coming into rehabilitation at the GSTC have clinical signs of FP, and may have a depressed immune status and lower circulating concentrations of vitamin E as a result.

In this research, vitamin E represents α-tocopherol and vitamin A represents retinol. We also tested several carotenoids, including lutein and zeaxanthin concentrations.

Trace Mineral Panel

Minerals are required by the body for many functions. Minerals represented in the body in relatively large amounts (milligrams per gram) are termed macroelements; these include calcium, phosphorus, sodium, potassium, magnesium, chlorine, and sulfur. Those minerals required in smaller amounts are called trace minerals. These include iron, zinc, manganese, copper, molybdenum, iodine, selenium, cobalt, fluoride, and chromium. Carbon, hydrogen, oxygen, and nitrogen are usually grouped as organic constituents and are not included in mineral analyses (Robbins 1983). A mineral panel measures the concentration of specific minerals in plasma. Our panel includes calcium, copper, iron, magnesium, phosphorus, potassium, selenium, and zinc. The functions of calcium, phosphorus, and potassium were described in Table 1.1. Other minerals included in our panel are described below in Table 1.6.

Table 1.6. Elements used in our study and their functions in the body (adapted from Robbins 1983).

Element	Function
Copper	Necessary for hemoglobin and melanin formation; component of several blood proteins and enzyme systems

Iron	Metal chelate of hemoglobin, myoglobin, and oxidizing enzymes
Magnesium	Essential constituent of bone and teeth formation; important in enzyme activation
Selenium	Interacts with vitamin E to maintain tissue integrity
Zinc	Essential for synthesis of DNA, RNA, and proteins; component or cofactor of many enzyme systems

Few studies have analyzed trace elements in sea turtle plasma (e.g. Suzuki et al. 2012a, b). For 25 hawksbill sea turtles in Japan, Suzuki et al. (2012b) found no correlation between carapace size and trace element concentration. When comparing plasma from adult wild (N=9) and captive (N=5) GSTs in Japan, Suzuki et al. (2012a) found that phosphorus and sulfur were the only significantly different elements in these two populations. Phosphorus was significantly lower in wild (93.50 ± 39.80 µg/ml) than in captive animals (305.6 ± 227.1 µg/ml). Sulfur was also significantly lower in wild (428.3 ± 107.5 µg/ml) than in captive individuals (696.0 ± 171.7 µg/ml). They attributed these differences to diet; wild animals were assumed to be herbivorous (all animals in the study were adults), while captive animals were fed vegetables but were also fed fish and squid, which are high in phosphorus and sulfur (Table 1.7).

Table 1.7. Summary of plasma trace mineral concentrations for selected minerals found in five captive and nine wild green sea turtles (Suzuki et al. 2012a). Concentrations are reported as mean µg/ml.

Element	Concentration (µg/ml) in captive turtles (N=5)	Concentration (µg/ml) in wild turtles (N=9)
Calcium	191.43 ± 162.6	73.74 ± 13.02
Copper	0.351 ± 0.124	0.276 ± 0.135
Iron	1.328 ± 0.333	1.311 ± 0.826
Magnesium	32.47 ± 15.75	23.69 ± 12.35
Phosphorus	305.6 ± 227.1	93.50 ± 39.80
Potassium	120.0 ± 28.90	139.6 ± 23.60
Selenium	0.128 ± 0.036	0.177 ± 0.185
Zinc	2.721 ± 2.077	1.359 ± 0.423

Stable Isotopes in Relation to Nutrition

Stable isotope analysis (SIA) is an emerging technique that has applications ranging from global biogeochemistry to animal migration and dietary studies (Peterson and Fry 1987). The proportions of stable isotopes of the elemental building blocks (e.g. C, N, H, O, and S) that

comprise the bulk of the biosphere, hydrosphere, and atmosphere are utilized (Hobson and Wassenaar 2008). All of these elements have a common "light" isotope and a less common "heavy" isotope; ratios of these isotopes in nature vary due to physical and chemical processes that result in isotopic fractionation, and these variations can tell us much about the natural history of an animal (Peterson and Fry 1987). A mass spectrometer is used to detect the minute differences in isotopic composition due to fractionation.

Ratios of stable isotopes are expressed relative to a standard in a delta notation:

$$\delta X = (R_{sample} / R_{standard} - 1) \times 1000$$

The standards for the elements used in this study, carbon (C) and nitrogen (N), are PeeDee Belemnite and N gas in the atmosphere, respectively (Fry 2007).

Stable isotope analysis in animal tissues depends on the turnover rate for the particular tissue of interest. Fixed tissues like keratinous hair and feathers are metabolically inert after synthesis and therefore reflect the location or diet of an animal when that tissue was synthesized; metabolically active, or dynamic, tissues reflect integration ranging from a few days in the case of blood plasma, to several weeks or months in the case of muscle and skin, to a lifetime in the case of bone collagen (Hobson and Wassenaar 2008). During the digestive process there is usually some degree of isotopic enrichment from prey to predator (Lemons et al. 2011). Carbon (^{13}C) enrichment is minimal (-1‰ to 1‰), but nitrogen (^{15}N) enrichment has been estimated at 3‰ to 5‰ per trophic level (DeNiro and Epstein 1978, DeNiro and Epstein 1981). Because of this isotopic enrichment, it is useful to compare the C and N from prey and predator in order to elucidate the diet composition of the predator (DeNiro and Epstein 1978).

Carbon SIA can tell us about feeding location and about the types of plants an animal consumes. Isotopically distinct systems can tell us about where an animal has been feeding, for

example: terrestrial versus marine and inshore versus offshore. Marine systems tend to be more enriched in ^{13}C relative to terrestrial biomes, and inshore food webs tend to be more enriched than offshore (Arthur et al. 2008, Hobson 1999, Rau et al. 1992). Within terrestrial systems, carbon isotopic fractionation, due to different photosynthetic pathways, can elucidate differences in consumption of C3 versus C4 or Crassulacean acid metabolism (CAM) plants (Peterson and Fry 1987). Within marine systems, plants farther offshore are more negative (-15‰) than those near shore (-11‰) (Arthur et al. 2008, Rau et al. 1992).

Unlike with carbon, there is no process that causes large fractionation of nitrogen isotopes in plants (Kelly 2000). Nitrogen primarily acts as a "trophometer," allowing researchers to estimate trophic levels of individuals. ^{14}N is excreted and metabolized faster than ^{15}N, leaving animals with higher $\delta^{15}N$ values (Fry 2007). Thus, an herbivore will be enriched 3‰ to 5‰ compared to a plant, and a carnivore will be enriched 3‰ to 5‰ compared to an herbivore.

Caveats to Stable Isotope Analysis

One variable that can influence carbon values is the lipid content of the sample. Lipids are ^{13}C depleted compared to proteins (DeNiro and Epstein 1978). Therefore, if a sample is particularly fatty, the tissue may appear more depleted than it really is. Lipids would not affect ^{15}N given their low nitrogen content (Habran et al. 2010). Overall, a fatty sample would lead to an increased C:N ratio (Rau et al. 1992). For this reason, in samples that are expected to be fatty, the fat is typically extracted prior to analysis.

Two variables other than diet that can influence nitrogen values are fasting and pollution of the animal's environment. Fasting can cause protein catabolism, leading to an increase in body ^{15}N values (Hobson and Wassenaar 2008). Hobson et al. (1993) first demonstrated this in an experiment using nutritionally stressed captive Japanese quail (*Coturnix japonica*) and wild

Ross's geese (*Chen rossii*). Captive quail were divided into two groups: one was fed a rationed diet designed to maintain, but not increase, body mass, while the other group was fed the same food *ad libitum*. After 17 days, animals were sacrificed and tissues were taken for SIA. Liver and bone collagen from quail raised on a rationed diet were enriched in ^{15}N by more than 1‰ compared to the same tissues from animals fed *ad libitum*. In wild Ross's geese, liver and muscle tissues collected from geese that fasted during egg incubation (average of 22 days) had 2.2 and 1.2‰ more enriched levels of ^{15}N, respectively, than tissues collected from non-fasted animals prior to the start of incubation (Hobson et al. 1993). Hobson et al. (1993) also noted that the extent of ^{15}N enrichment in tissues depends on the turnover rates in those tissues. More metabolically active tissues (e.g. liver) would more readily show effects of protein catabolism than tissues with slower turnover (e.g. bone collagen). They also noted that no evidence was found for changes in carbon due to nutritional stress. While some studies have sought to measure ^{15}N changes in relation to fasting to provide an index of nutritional stress, other studies have found no relationship (Hatch 2012).

Nitrogen values can also be affected by human contributions; nitrogenous waste can increase environmental nitrogen and, in turn, increase levels in an animal (Fry 2007). This was suggested as a possible cause of high ^{15}N values in GSTs in an urbanized bay in San Diego, CA; animals in this study had the highest levels of ^{15}N (16.9‰) reported for this species (Lemons et al. 2011). Stable isotope analysis of an animal's environment, analyzed along with environmental contaminant data, may be useful in interpreting these cases (Lemons et al. 2011).

Stable Isotope Analysis in Sea Turtles

Stable isotope technology has been applied in marine turtles to investigate many different questions surrounding sea turtle life history. Among those are migration (Allen et al. 2013),

recruitment (López-Castro et al. 2013, Reich et al. 2007), and diet (Lemons et al. 2011, Revelles et al. 2007). Several different tissues of GSTs have been used in this type of analysis, including whole blood, plasma, liver, muscle, skin, and scute (Reich et al. 2007, Cardona et al. 2010, Dodge et al. 2011, Lemons et al. 2011). Turnover rates in plasma and skin are not available for GST (Prior et al. 2015). Average residence time of stable isotopes in in various tissues from small juvenile loggerheads (9.0 to 13.1 cm straight carapace length) have been estimated (Reich et al. 2008). Skin and plasma, as are used in our current study, are estimated to have average residence times (days) for $\delta^{13}C$ of 46.1 ± 8.9 and 39.6 ± 9.1 and for $\delta^{15}N$ of 44.9 ± 3.1 and 22.5 ± 5.1, respectively. As these residence times were in rapidly growing individuals, the estimated turnover time in larger juveniles is several months (Vélez-Rubio et al. 2016).

One area of stable isotope research currently receiving attention focuses on the timing of the shift in GSTs from a carnivorous to an herbivorous diet. Traditionally, this shift has been thought to occur upon recruitment to neritic (near-shore) habitats (Bjorndal 1997). Stable isotope technology, however, indicates that this shift in feeding preference may be asynchronous with the arrival to neritic habitats in some areas, including California (Lemons et al. 2011), NW Africa (Cardona et al. 2009), and the Mediterranean (Cardona et al. 2010, Godley et al. 1998). Stable isotope technology is thus an important tool for discovering the dietary composition and needs of GSTs.

Metagenomics in Relation to Nutrition

Metagenomics is an emerging field that attempts to describe and quantify the genomes of entire communities of microbes in various tissues (e.g. skin, the gastrointestinal and respiratory tracts). This is an important area of research, as prior to this ability, scientists largely relied on the identification and quantification of microbes based on culture. It is estimated that less than

one percent of microbes can be cultured, and thus the vast majority of the microbial community was undetected. Utilizing molecular methodologies, the field of metagenomics has advanced to allow scientists to access a community's genome without relying on cultures (Handelsman et al. 2007). The most common use of metagenomics involves shotgun sequencing of 16S ribosomal RNA (rRNA).

In humans, the practical significance of gastrointestinal (GI) bacterial communities is rapidly becoming apparent. Intestinal microbiome composition has been associated with problems such as obesity, inflammatory disease, diabetes, and cancer (Robinson et al. 2010). Studies in GI metagenomics have typically been applied in humans, or in other vertebrate mammals, in order to better understand the coevolution of mammals and their microbial communities (Ley et al. 2008). In humans, the microbial community of the lower GI tract is predominately composed of *Bacteroidetes* and *Firmicutes* (Robinson et al. 2010). Normal intestinal bacterial flora has not been definitively identified in GSTs. The reptilian microbiome is the least studied of all taxa (for review see Colston and Jackson 2016). Gastrointestinal bacterial metagenomes of reptiles have been studied in American alligators (*Alligator mississippiensis*; Keenan et al. 2013), Burmese pythons (*Python molurus*; Costello et al. 2010), cottonmouths (*Agkistrodon piscivorus*; Colston et al. 2015), Galapagos marine iguanas (*Amblyrhynchus cristatus*) and land iguanas (*Conolophus subcristatus* and *C. pallidus*; Lankau et al. 2012 and Hong et al. 2015), and timber rattlesnakes (*Crotalus horridus*; McLaughlin et al. 2015). Only two studies focus on chelonians, including one on herbivorous gopher tortoises (*Gopherus polyphemus*) and one on carnivorous loggerhead sea turtles (*Caretta caretta*) (Yuan et al. 2015, Abdelrhman et al. 2016). Costello et al. (2010) found that the GI microflora of fasting Burmese pythons is dominated by the same two bacterial phyla that dominate human GI tracts—

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Bacteroidetes and *Firmicutes*. After the animals ate, however, the overall species-level diversity increased significantly. This could have important implications for our study, as GSTs often come into the GSTC anorexic and may not have eaten for an extended period of time (Norton, personal communication).

Host diet also influences GI bacterial diversity (Ley et al. 2008). In humans, emerging research suggests that it may be possible to use our diet to manipulate our GI microbiota to improve health (Umu et al. 2013). Bjorndal (1985) suggested that microbial communities in digestive tracts of sea turtles may play a role in diet selection. This suggestion was based on three lines of evidence: 1) GSTs typically eat algae or seagrass and not a mixture, even when both are available (Bjorndal 1980, Mortimer 1982), 2) in turtles which feed primarily on algae, seagrass appears undigested in feces, and vice versa (Bjorndal 1980), and 3) structural carbohydrates differ in seagrasses and algae, thus requiring different microbial communities for digestion (Bjorndal 1985). These differences could play a role in diet selection because turtles with gut microbes adapted to either algae or seagrass would digest that food item more efficiently (Bjorndal 1985). This diet selection is only one component of foraging strategy; when food is limited or of greater diversity, GSTs may ingest a more varied diet because the costs of searching for an all-algae or all-seagrass diet would outweigh the energy gained from more efficient digestion (Bjorndal 1997).

Caveat to Gastrointestinal Metagenomics

One caveat of interest for our study is the use of antibiotics and their effect on microbial communities. In humans, antibiotics consumed at therapeutic doses have been shown to disturb the GI ecosystem, and may lead to overgrowth of pathogenic bacteria (Rafii et al. 2008). In reptiles, indiscriminant use of antimicrobials can lead to intestinal bacterial dysbiosis and

disruption of the fermentative cycle, potentially causing anorexia, bloating, and diarrhea (Mitchell 2006). Sea turtles presenting to rehabilitation hospitals often require antibiotic treatment, and its effect on the GI microbiota composition has not previously been described.

Conclusions

The potential effects of feeding captive, herbivorous GSTs a predominately carnivorous diet are unknown, and commercial diets for these unique animals have not been formulated. We utilized a suite of parameters, including blood chemical parameters, skin stable isotopes, and fecal metagenomics to describe the dietary requirements of GSTs in rehabilitation. To accomplish this, comparisons across three timepoints in rehabilitation, as well as comparisons to wild turtles, were made. The three timepoints during rehabilitation (i.e. admission, mid-rehabilitation, and recovery) were defined according to the diet consumed. At admission, individuals should reflect their wild foraging habits. From admission to mid-rehabilitation, turtles consumed a mixture of vegetables and seafood. Animals were gradually transitioned throughout rehabilitation to a predominately vegetable-based diet. We defined mid-rehabilitation as the point when an individual was consuming 25% vegetables for two weeks, and recovery as the point when an individual was consuming 75% vegetables for two weeks.

Information gained from this study will significantly improve the management of GSTs in rehabilitation. This is critically needed information in order to facilitate the successful recovery of injured and ill GSTs.

HYPOTHESES AND OBJECTIVES

Hypothesis 1: Clinical pathology parameters, body condition scores, and nutritional parameters of rehabilitated sea turtles will change over time in rehabilitation with changes in diet,

and will differ from those of similarly sized, free-ranging sea turtles that forage on a
natural diet.

> *Objective 1:* Compare a suite of clinical pathology and plasma nutritional
> parameters for GSTs in rehabilitation over three timepoints: admission,
> mid-rehab, and recovery.

> *Objective 2:* Compare the clinical pathology and plasma nutritional parameters
> from each timepoint in rehabilitation to the same parameters in wild GSTs
> at a single timepoint (i.e. time of capture).

Hypothesis 2: Skin and plasma stable isotope composition of rehabilitated sea turtles will
change over time in rehabilitation with changes in diet, and will differ from those of
similarly sized, free-ranging sea turtles that forage on a natural diet. For example,
admission samples will have lower $\delta^{15}N$, reflective of the herbivorous wild diet (eaten 4-
6 months prior) than the recovery sample point (reflective of the predominantly
carnivorous diet they are fed in the early stages of rehabilitation).

> *Objective 1:* Analyze stable isotope composition of the seafood, vegetables, and
> the standard gel-diet formulas typically fed to GSTs undergoing
> rehabilitation at the GSTC.

> *Objective 2:* Compare stable isotope analysis of skin biopsies taken from GSTs in
> rehabilitation at admission to skin biopsies taken from wild GSTs at time
> of capture.

> *Objective 3:* Compare stable isotope analysis of skin biopsies taken from GSTs in
> rehabilitation at admission and compare it to skin biopsies taken from
> GSTs in rehabilitation at recovery.

Objective 4: Compare stable isotope analysis of plasma samples taken from GSTs in rehabilitation at admission to plasma samples taken from wild GSTs at time of capture.

Hypothesis 3: Plasma fatty acid composition of rehabilitated sea turtles will change over time in rehabilitation with changes in diet, and will differ from those of similarly sized, free-ranging sea turtles that forage on a natural diet.

Objective 1: Analyze fatty acid composition of the seafood, vegetables, and the standard gel-diet formulas typically fed to GSTs undergoing rehabilitation at the GSTC.

Objective 2: Compare fatty acid analysis of plasma samples taken from GSTs in rehabilitation at admission to plasma samples taken from wild GSTs at time of capture.

Hypothesis 4: The GI microbial diversity and composition of rehabilitated sea turtles will change over time in rehabilitation with changes in diet.

Objective 1: Evaluate the bacterial community composition in feces from GSTs in rehabilitation over three timepoints: admission, mid-rehab, and recovery.

ENVIRONMENTAL EDUCATION CENTERS AS BOUNDARY ORGANIZATIONS

To maximize the impact of wildlife rehabilitation, treatment and release of animals should be combined with educational and research initiatives (Sleeman 2008). Studies have shown that rehabilitation activities best promote conservation when paired with education (Ballantyne et al. 2007, Feck and Hamann 2013). Including public education and allowing for a multidisciplinary approach to wildlife conservation is important for reaching conservation goals, as ultimately, wildlife conservation requires the support of the public (Sleeman 2008). For this

reason, this study aims to use an interdisciplinary approach and tie together the veterinary and education aspects of rehabilitation.

The GSTC has adopted a multidisciplinary approach to sea turtle conservation by integrating rehabilitation, education, and research teams. In fact, the Center's mission statement states: "Through sea turtle rehabilitation, research and educational programs, Georgia Sea Turtle Center staff work to increase awareness of habitat and wildlife conservation challenges, promote responsibility for ecosystem health and empower individuals to act locally, regionally, and globally to protect the environment" (http://gstc.jekyllisland.com/about-us/mission/).

As part of the education mission of the GSTC, the facility recognizes that individual sea turtle patients and their stories can have a profound impact on visitors. To take advantage of this, many educational programs are offered in conjunction with the medical treatment and rehabilitation of individual turtles. Daily programs include: behind-the-scenes, gallery education programs, meet the patients, patient feedings, and puppet shows. Other educational experiences offered include field trips, homeschool events, distance learning, summer camps, beach walks, and ride-alongs with the turtle patrol, among others.

Environmental Education

The ultimate goals of environmental education (EE) programs include fostering responsible environmental behavior, effecting long-term changes of attitudes toward conservation and nature, and providing basic ecological knowledge (Ballantyne and Packer 2005, Bogner 1998). Environmental educators engage the public and can help foster enthusiasm towards conservation (Athman and Monroe 2001). Effective EE programs empower learners to address environmental issues and instill in learners a sense of conservation stewardship (Athman and Monroe 2001).

Environmental education could benefit from greater attention to program evaluation (Athman and Monroe 2001, Carleton-Hug and Hug 2010). The goal of the evaluation is to ascertain the value of the program, decide whether the goals of the program are being met, and determine how to specifically improve it, if needed (Monroe 2010). One criticism of EE programs is they often lack clear objectives, a critical component needed for evaluation (Carleton-Hug and Hug 2010). When programs do not have formative evaluation approaches, opportunities for modification and improvement are often missed (Loomis 2002).

There are many challenges to evaluating EE programs. Several have been outlined by Carleton-Hug and Hug (2010), including dealing with a compressed time frame and institutional resistance to evaluation. Program evaluation is often subjected to short time frames for a variety of reasons, from logistical to budgetary. Institutional resistance may stem from a lack of understanding about program evaluation, concern about the consequences of negative evaluations, or a lack of incentive or desire to perform evaluations (Carleton-Hug and Hug 2010).

Currently, evaluations exist for many of the programs offered at the GSTC, including but not limited to school field trips and classroom outreach, nest walks, and ride-alongs with the turtle patrol. Existing evaluation methods have focused on measuring whether a visitor's knowledge base and awareness of conservation concerns increases to assure meeting performance standards for various funding organizations (i.e. NOAA, AmeriCorps). Surveys and customer feedback via social media platforms such as Facebook and Travelocity have further helped to measure customer satisfaction used for marketing purposes. However, little is known about the GSTC's overall effectiveness in reference to the attitudes and behaviors of the guests towards conservation. Hence, the objective of this study was to measure this aspect of the

educational programming using more rigorous scientific methodology. In-person survey

instruments were developed and implemented within the education department at the GSTC.

Surveys were developed for two of their main educational programs, "Behind-the-Scenes" (BTS)

and "Sea Turtle Releases." All GSTC visitors can view veterinary treatments through a window

and explore the gallery of sea turtle exhibits, but visitors who participate in the BTS program are

led on an hour-long tour of the "behind-the-scenes" operations of the hospital, and participants

meet the veterinarian and view treatments up-close. Sea Turtle Releases are public events held

on the beach during the release of a successfully rehabilitated patient. A short program about the

patient and its time in rehabilitation is offered before the release. These events are announced in

advance, and many people plan trips to attend specific turtle releases. Other people happen to be

on the beach and see the release opportunistically.

Boundary Objects and Organizations

One concept these surveys will measure is whether rehabilitation centers may act as

boundary organizations for conservation by translating scientific research in a way the general

population can enjoy and get excited about. The idea of a boundary organization stems from the

theory of 'boundary work,' which seeks to demarcate science and non-scientific intellectual

pursuits (Gieryn 1983). Guston (1999) first coined "boundary organization" to refer to

organizations which work at the interface of two different social worlds. Boundary organization

theory has traditionally been applied to the theoretical boundary between politics and science

(Guston 2001). In fact, in 2001, *Science, Technology, & Human Values* ran an issue devoted to

boundary organizations in policy and science; topics ranged from climate change (Agrawala et al. 2001, Miller 2001) to air quality and public health (Keating 2001), to agriculture (Cash 2001).

This concept can been applied in more diverse situations where the perceived boundary lies between two areas more general than politics and science, such as that between science and the general public. Breuer et al. (2010) touched on this idea when they deemed the Florida Cooperative Extension Service a boundary organization because of the Extension's role as an intermediary between a research consortium producing climate data and farmers using the data. The GSTC acts as a boundary organization by interpreting science for the general population. Each year, approximately 130,000 people visit the Center. Once inside, visitors have the opportunity to walk through a gallery of sea turtle exhibits with information on conservation. Visitors may look through the treatment window and see the veterinary team treating current patients; visitors may also walk through the Rehabilitation Pavilion and view the holding tanks with all current patients.

The concept of medical treatment for animals is something that most people have only experienced with their dog or cat; but when visiting the turtle hospital, visitors get to see and understand that physical exams, diagnostics, and state-of-the-art medical care are also needed and applied to an endangered species. The kind of understanding gained when someone watches a shell wound caused by a boat strike being treated in a similar way to what would be performed in a human emergency room, or having the veterinarian explain how to assess pain in a turtle, is invaluable. Experiences offered by places like the GSTC make science understandable and exciting, and encourage people to donate to wildlife hospitals or rehab centers, support rescue of

turtles, and start or maintain behaviors that reflect a conservation ethic (e.g. recycling, turning lights off if they live on the beach).

A thorough literature search has been performed, and to my knowledge, the concept of a boundary organization has never been applied to a rehabilitation center. If these facilities can use the theory of a boundary organization as a framework, they may be empowered to create a stronger conservation-education initiative. Knowledge gained from this study will be applicable to rehabilitation facilities, aquaria, museums, and other conservation-education facilities worldwide.

HYPOTHESES AND OBJECTIVES

Hypothesis 1: Cronbach's alpha reliability coefficients and principal components analysis will reveal that the survey instrument developed will be both reliable and valid.

> *Objective 1:* Develop a survey based on existing questions and scales in current literature to measure the knowledge, attitudes, and behaviors of GSTC visitors towards sea turtle conservation, and complete a pilot test to check construct reliability (i.e. using Cronbach's alpha) and validity (i.e. using principal components analysis).

Hypothesis 2: Individuals who attend the Behind-the-Scenes program will demonstrate more knowledge of sea turtles, more positive attitudes and behaviors towards sea turtles, and more conservation ethics in general than people who only visit the gallery.

> *Objective 1:* Administer surveys to 1) people who have finished touring the gallery and 2) people who have completed a Behind-the-Scenes tour. The

survey instrument will assess the effect of the BTS program on visitors'

perceptions, attitudes, and behaviors towards sea turtles and conservation.

Hypothesis 3: The GSTC, through education programming, may serve as a *boundary*

organization for conservation.

      Objective 1: Utilize boundary organization theory literature, combined with

      information gained in Objectives 1 and 2, to determine the potential of

      conservation entities to act as boundary organizations.

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CHAPTER 2

HEALTH PARAMETERS OF HEALTHY AND REHABILITATING ATLANTIC GREEN SEA TURTLES (*CHELONIA MYDAS*)

INTRODUCTION

The green sea turtle (*Chelonia mydas*) is listed as endangered or threatened throughout its range, both in the United States and other parts of the world (NOAA 2016). Studies on the nutritional requirements of this species are lacking, and are primarily focused on the foraging ecology of free-ranging populations (e.g., Bjorndal 1980, 1997, Mortimer 1982, Ogden et al. 1983, Seminoff et al. 2002, Williams 1988). Green sea turtles are unique among the sea turtles in that hatchlings and pelagic juveniles are thought to be primarily carnivorous while coastal juveniles and adults are primarily herbivorous (Boyle and Limpus 2008). In Atlantic populations, green sea turtles leave pelagic habitats and enter neritic feeding areas once they reach a size of 20 to 25 cm in straight carapace length (Bjorndal and Bolten 1988). Once in these neritic environments, they eat primarily seagrass and algae, and different populations may prefer one or the other or both (Bjorndal 1997, Mortimer 1982). This shift to an herbivorous diet can be gradual, and even some adult greens have been noted to eat animal matter opportunistically, including jellyfish, salps, and sponges (Mortimer 1982).

Understanding this unique foraging ecology is critical to green sea turtle conservation for various reasons, including habitat protection. However, this is especially true when considering the implications for feeding injured and ill sea turtles that are often rescued and brought into

rehabilitation facilities where they are cared for until they are deemed healthy enough for release. Not including mass stranding events (e.g. oil spill, cold stun), roughly 500 sea turtles are admitted yearly to the 16 sea turtle rehabilitation hospitals in Georgia and Florida (Florida Fish and Wildlife Conservation Commission, unpublished data). Proper nutrition during rehabilitation plays a critical role in the health and eventual release of an individual, however, the natural foraging ecology of green sea turtles makes prescribing a diet for them in rehabilitation settings especially challenging. Food items high in animal protein (e.g. fish, squid, shrimp) are often offered early in rehabilitation to combat poor appetite and emaciation, however this may result in gastrointestinal pathologies and potential obesity. A number of facilities have reported elevations in aspartate aminotransferase (AST) and significant calcium/phosphorous (Ca:P) imbalances with animal diets (Stringer et al. 2010, Norton, unpublished data). Seafood (e.g. fish, squid, shrimp) generally has an inverted Ca:P ratio (e.g. Mackerel 1:34 (Robbins 1983)) and is higher in fat and protein than the typical diet items consumed by free-ranging green sea turtles (see McDermid et al. (2007) for nutritional composition of common free-ranging green sea turtle diet items). As a result, it is recommended that rehabilitation facilities gradually shift green sea turtles to a more natural, plant-based diet as soon as an individual is willing to eat it.

The health and nutrition of sea turtles are often assessed with hematology and blood biochemistry profiles. Reference clinical chemistry and hematology parameters have been established for several free-ranging green sea turtle populations (e.g. Bolten and Bjorndal 1992, Flint et al. 2010, Osborne et al. 2010, Samour et al. 1998, Page-Karjian et al. 2015). Biochemical analyses that appear most useful in chelonians are levels of aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium, cholesterol, creatine kinase (CK), electrolytes (sodium,

potassium, and chloride), glucose, phosphorus, total protein (TP) and protein fractions, triglycerides, and uric acid.

Blood biochemical reference values for debilitated green sea turtles with specific health problems, such as fibropapillomatosis and cold-stunning, have been also previously been described (Aguirre and Balazs 2000, Anderson et al. 2011). However, this information is rarely extended to include other nutritional panels, and few studies exist on changes in health parameters in green sea turtles due to dietary composition. One such study compared health parameters in green sea turtles in Barbados that were supplemented as part of a tourist attraction to a nearby population that was not supplemented (Stewart et al. 2016). The supplemented group was found to have significantly different biochemical, hematologic, vitamin and mineral parameters compared to the unsupplemented group, and many of these differences could be directly linked to diet (Stewart et al. 2016).

To understand the effect of diet on health and recovery, nutritional parameters in green sea turtles undergoing rehabilitation at the Georgia Sea Turtle Center (GSTC) on Jekyll Island, GA were compared to those of healthy, free-ranging turtles in St. Lucie County, Florida. Hematology and biochemistry panels, as well as plasma protein electrophoresis (PEP), levels of selected vitamins, trace minerals and lipoproteins were evaluated. Free-ranging turtles were evaluated at a single timepoint. Rehabilitated turtles were monitored at admission, mid-rehabilitation, and recovery. These three timepoints represented a shift from a primarily carnivorous, seafood-based diet at admission to the rehabilitation facility, to a primarily herbivorous diet at recovery. Rehabilitation turtles at the point of admission were expected to have blood parameter values similar to free-ranging animals, with potential differences related to the cause of presentation. Mid-rehabilitation values were expected to differ significantly from

free-ranging animals because of the high protein diet fed at admission. We expected that values obtained from turtles that had recovered would closely resemble those of free-ranging turtles.

MATERIALS AND METHODS

Rehabilitation Diets

Food items fed to green sea turtles at the GSTC include romaine lettuce and leafy lettuce (*Lactuca sativa*), cucumber (*Cucumis sativus*), green bell pepper (*Capsicum annuum*), mackerel (*Scomber scombrus*), herring (*Clupea harengus*), shrimp (*Penaeus* spp.), squid (*Loligo opalescens*), and a custom gel diet consisting of vegetables, seafood, and vitamins (recipe in Appendix B). In addition, multivitamin (Mazuri® Vita-Zu® Sea Turtle Vitamin for Fish-based diets, 500mg, catalog number 1815523-300, Mazuri, Richmond, IN 47374; recipe in Appendix C) and calcium supplements (Calcium Carbonate 10 gr, 648mg, catalog number 00536-1024-10, Rugby, Livonia, MI 48152) were offered daily. In order to understand the impact of the typical rehabilitation diet on health, these diet items were not altered for this study; instead, three timepoints during rehabilitation were defined according to the diet consumed. At admission, individuals should reflect their wild foraging habits. From admission to mid-rehabilitation, turtles consumed a mixture of all items listed above. Animals were gradually transitioned throughout rehabilitation to a predominately vegetable-based diet. We defined mid-rehabilitation as the point when an individual was consuming 25% vegetation for two weeks, and recovery as the point when an individual was consuming 75% vegetation for two weeks.

Turtles

Green sea turtles that present to the GSTC are typically 25-40 cm in curved carapace length, a size consistent with turtles that have recruited to neritic habitats and shifted to the herbivorous feeding stage of older juveniles and adults (Bjorndal and Bolten 1988). This is

supported by fecal content analysis in rehabilitation cases and gastrointestinal contents found

during necropsy evaluations, which indicate that these animals are herbivorous, and that they

feed primarily on algae (Norton, personal communication).

Samples from 34 rehabilitating green sea turtles were collected from turtles presenting to

the GSTC from January 2014 – May 2016. Samples were collected at each of the three

timepoints. At each timepoint, turtles were weighed and physical exams were performed. A

subjective body condition score (BCS) on a scale of 1 – 5 was recorded. Straight carapace length

(SCL) was taken at admission and recovery time points. When both weight and SCL were

available, body condition index (BCI) was calculated according to the following formula from

Bjorndal et al. (2000):

$$BCI = [mass\ (kg)/straight\ carapace\ length\ (cm)^3] * 10,000$$

Samples from 34 free-ranging green sea turtles were collected by the In-Water Research

Group, Inc. out of Juniper, Florida from March 2015 – February 2016. The In-Water Group

captures an average of 175 free-ranging green sea turtles annually at the St. Lucie power plant;

animals are incidentally entrained in cooling water and transported through the intake pipes to an

enclosed canal where they must be manually removed. Animals were weighed and morphologic

measurements were taken at the time of capture, and BCI was calculated from this information.

Turtles coming from the this area of Florida are thought to feed on algae rather than seagrass, a

diet which is identical to the turtles received for rehabilitation by the GSTC (Allen Foley,

personal communication, 1-15-15). However, there is recent evidence that they may eat both

seagrass and algae (Gorham et al. 2016).

Blood was collected from the external jugular vein (dorsal cervical sinus) using a sodium heparinized syringe. Collections were performed at each of the three timepoints for rehabilitation turtles, and at time of capture for free-ranging turtles. The total volume of blood collected each time was no more than 0.5% of the total body weight, well within the established safe collection volume of up to 1% of the total body weight (Mader 2006). A suite of clinical pathology and plasma nutritional parameters were tested to assess nutritional health from both whole blood and separated plasma. A small amount of whole blood was transferred to a microhematocrit tube and centrifuged to measure packed cell volume (PCV). Plasma total solids (TS) were measured by refractometer. Two blood smears were made, stained with Wright-Giemsa, and an estimated white blood cell (WBC) count was performed by Dr. Nicole Stacy at the University of Florida using the following formula from Weiss (1984):

$$\text{WBC estimate/}\mu l = \text{average of WBC in 10 HPF x objective power}^2$$

where HPF is high power field and the objective power used was 50x. A 200-cell differential as well as red and WBC morphology were assessed from the blood smear.

The remaining amount of whole blood was placed in a 1.5 mL microcentrifuge tube and centrifuged at 3,500 RPM for 5 min. Plasma was separated for submission to various laboratories specializing in sea turtle hematology, biochemistry, and other nutritional panels described below. Plasma for chemistry panels was refrigerated and submitted the same day; the remaining plasma was stored at -80 ºC until submission.

Chemistry panels were performed by IDEXX Laboratories (Westbrook, Maine 04092, USA). The following values were measured: Asparate aminotransferase (AST), creatine kinase (CK), albumin, total protein (TP), globulin, blood urea nitrogen (BUN), cholesterol, glucose,

calcium, phosphorus, chloride, potassium, sodium, uric acid, and triglycerides. Plasma vitamin D (25-hydroxyvitamin D), ionized calcium (iCa), and parathyroid hormone (PTH) were measured by radioimmunoassay at Michigan State University Diagnostic Center for Population & Animal Health (Lansing, Michigan 48910, USA). Plasma Vitamin A (as aldehyde, palmitate, and retinol), Vitamin E (α-tocopherol), lutein and zeaxanthin concentrations were determined by high performance liquid chromatography at The McGraw Lab at Arizona State University (Tempe, Arizona 85281, USA). Protein fractions (i.e. pre-albumin, albumin, alpha 1 globulins, alpha 2 globulins, beta globulins, and gamma globulins) were evaluated by plasma electrophoresis (PEP) at the University of Miami (Coral Gables, Florida 33124, USA). Lipoprotein panels, including cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL), were also completed at the University of Miami. Cholesterol, triglycerides, and HDL were directly analyzed (Ortho Vitros 250 Chemistry Analyzer, Rochester, NY) and LDL and VLDL were calculated using the Friedewald formula (Friedewald et al. 1972). Trace mineral panels (calcium, copper, iron, magnesium, phosphorus, potassium, selenium, and zinc) were conducted by inductively coupled plasma - mass spectroscopy at the University of Pennsylvania Animal Diagnostic Laboratory System (PADLS), New Bolton Center Toxicology Laboratory (Kennett Square, Pennsylvania 19348, USA).

Statistical Analyses

Statistical analyses were performed using R 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria). Mean and ranges were determined for all parameters. For any values that were less than the limit of detection (LOD), a value of LOD/sqrt(2) was used. Non-normally distributed data were square root transformed for all analyses.

Comparisons among the three rehabilitation timepoints were performed using linear mixed effects models with the nlme package in R; this method takes into account the repeated measures nature of the design (Pinheiro et al. 2016). Dependent variables that were correlated biologically were combined. Parameters that were repeated in different panels were not repeated for statistical analyses (e.g. levels of albumin and other protein fractions were determined from PEP and not from the chemistry panel); details of which parameters were evaluated for which panels can be seen in the table of statistical results in Appendix A.

Turtle was included as a random effect and length of time in rehabilitation was included in the autocorrelation structure of the models, because turtles went through rehabilitation at different rates. Fixed effects included timepoint, size class, month of sample collection, and binary variables for debilitation, cold-stunning, and fibropapillomatosis (FP). Debilitation was a subjective assessment of whether a turtle was debilitated or not, cold-stunning or not was based on presentation of the animal, and FP was based on whether the animal had visible fibropapillomas. If the combined dependent variable was significant, separate linear models were run for each dependent variable, and Bonferroni adjustments were made to the alpha values (i.e. $0.05/N$, where N is the number of dependent variables; Tabachnick and Fidell 2013). Fixed effects that were not significant in the combined model were removed for the separate analysis.

Comparisons between admission and free-ranging turtle samples and between recovery and free-ranging turtle samples were performed with MANOVA. The same variables used in the analysis of rehabilitation turtles described above were used.

RESULTS

<u>Turtles</u>

A total of 34 green sea turtles presenting for rehabilitation at the GSTC were included in this study. Of these, 28 had samples taken at all three timepoints. The remaining six were only sampled at admission and recovery due to a rapid recovery time. Reasons for admission varied among rehabilitation animals, and the duration in captivity ranged from 25 – 233 days (mean = 100); however, all animals were successfully rehabilitated and either released or placed at another facility for the duration of their lives. A total of 34 free-ranging green sea turtles captured at the St. Lucie power plant were included for comparison.

Weight, SCL, BCI, and BCS increased throughout rehabilitation for turtles admitted to the GSTC (Table 2.1). Rehabilitation turtles at admission (N=34) weighed significantly less (W = 232, $p < 0.001$), were significantly smaller in SCL (W = 246, $p < 0.001$), and had significantly lower BCI (W = 321.5, $p < 0.01$) than free-ranging turtles (N=34; Table 2.2).

Table 2.1. Physical exam parameters for green sea turtles (*Chelonia mydas*) in rehabilitation (admission, mid-rehabilitation, and recovery) at the Georgia Sea Turtle Center and free-ranging turtles at time of capture. Parameters include weight (kg), straight carapace length (SCL; cm), body condition index (BCI = (mass/SCL^3) * 10,000 kg/cm^3) and body condition score (BCS) on a scale of 1 – 5. At mid-rehabilitation, SCL was not recorded and therefore BCI could not be calculated. Body condition scores were not recorded for free-ranging turtles.

Physical Exam	Admission					Mid-Rehab					Recovery					Free-Ranging				
	Mean	SD	Min	Max	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max	N
Weight (kg)	3.17	1.46	1.60	7.90	28	3.47	1.49	1.85	7.85	28	4.26	1.52	2.15	8.65	28	9.62	7.18	2.00	26.60	34
SCL (cm)	29.70	4.28	23.10	40.70	26	NA	NA	NA	NA	NA	31.68	3.78	25.50	41.00	26	39.92	10.52	25.50	60.00	34
BCI	1.18	0.11	0.98	1.43	25	NA	NA	NA	NA	NA	1.30	0.09	1.13	1.46	26	1.24	0.07	1.07	1.37	34
BCS	2.14	0.34	1.50	3.00	23	3.94	4.73	2.00	25.00	22	3.49	0.34	2.75	4.00	26	NA	NA	NA	NA	NA

Table 2.2. Month and year of sample collection, straight carapace length (SCL), and body condition index (BCI) for rehabilitation turtles at admission to the Georgia Sea Turtle Center (N=34) and free-ranging turtles at time of capture (N=34). NA values mean SCL was not available and therefore BCI could not be calculated.

| | | Rehabilitation Turtles | | | | | Free-Ranging Turtles | | |
| | | Weight | SCL | | | | Weight | SCL | |
Turtle	Mo-Yr	(kg)	(cm)	BCI	Turtle	Mo-Yr	(kg)	(cm)	BCI
C14001	Jan-14	3.00	30.2	1.1	032757	Jul-15	14	48.6	1.2
C14002	Jan-14	1.85	25.3	1.1	LLA378	Jul-15	15.8	48.7	1.4
C14003	Jan-14	5.20	34.5	1.3	LLH610	Mar-15	4.1	31.7	1.3
C14004	Jan-14	3.90	33.8	1.0	LLH617	Mar-15	3.9	31.0	1.3
C14008	Jan-14	6.25	37	1.2	LLH634	Apr-15	9.9	43.0	1.2
C14009	Jan-14	3.30	31.4	1.1	LLH794	Jul-15	3.1	28.8	1.3
C14010	Jan-14	2.10	27	1.1	LLP203	Sept-15	2.9	29.6	1.1
C14011	Jan-14	3.95	32.5	1.2	LLP210	Oct-15	14	48.7	1.2
C14012	Feb-14	4.15	33.2	1.1	LLP214	Oct-15	12.1	46.2	1.2
C14023	Mar-14	3.70	33.6	1.0	883792	Oct-15	2.4	26.9	1.2
C14027	Apr-14	1.60	NA	NA	LLP218	Oct-15	3.7	31.1	1.2
C14059	May-14	7.90	40.7	1.2	LLP229	Oct-15	2.9	29.7	1.1
C14063	May-14	10.95	45.4	1.2	LLP238	Oct-15	3.6	30.5	1.3
C14389	Oct-14	2.25	26.5	1.2	LLP248	Oct-15	13.9	46.7	1.4
C14396	Oct-14	2.00	25.6	1.2	843473	Oct-15	2	25.5	1.2

C14405	Nov-14	2.55	27.2	1.3	LLP262	Oct-15	26.60	60.0	1.2
C15004	Jan-15	1.85	24.7	1.2	938249	Nov-15	2.60	27.7	1.2
C15005	Jan-15	2.40	NA	NA	LLP272	Nov-15	10.20	44.1	1.2
C15009	Feb-15	2.75	28.4	1.2	LLA141	Nov-15	16.50	50.1	1.3
C15010	Feb-15	3.25	28.5	1.4	LLP303	Dec-15	8.30	40.0	1.3
C15026	Mar-15	2.55	27.3	1.3	938198	Dec-15	2.50	27.3	1.2
C15029	Mar-15	8.70	41.3	1.2	LLP328	Dec-15	10.00	43.2	1.2
C15030	Mar-15	3.15	30.4	1.1	LLP330	Dec-15	19.90	53.6	1.3
C15051	Apr-15	3.50	30.2	1.3	LLP350	Dec-15	13.40	48.2	1.2
C15100	May-15	3.50	31.8	1.1	LLP357	Dec-15	21.80	55.6	1.3
C15363	Aug-15	1.95	26.4	1.1	LLP383	Jan-16	22.60	56.2	1.3
C15379	Sept-15	1.80	25.1	1.1	LLP390	Jan-16	4.20	32.9	1.2
C15383	Sept-15	3.75	29.7	1.3	473052	Jan-16	13.60	46.6	1.3
C15412	Dec-15	3.25	29.7	1.2	LLP418	Jan-16	3.50	30.8	1.2
C15413	Dec-15	2.30	27.2	1.1	LLP423	Jan-16	8.40	40.9	1.2
C15414	Dec-15	1.60	23.1	1.3	LLP427	Jan-16	22.60	57.4	1.2
C15418	Dec-15	1.60	23.6	1.2	LLP429	Jan-16	4.50	32.4	1.3
C15423	Dec-15	3.25	29.8	1.2	LLS252	Feb-16	3.00	28.8	1.3
C16005	Jan-16	3.50	30.5	1.2	LLS304	Feb-16	4.50	34.8	1.1
Average		**3.51**	**30.4**	**1.2**			**9.62**	**39.9**	**1.2**

<u>Samples</u>

Appendix A represents the results for all statistical analyses; only significant results are highlighted here.

Hematology

The total WBC decreased throughout rehabilitation ($p < 0.001$), heterophils decreased throughout rehabilitation ($p < 0.001$), and monocytes increased until mid-rehabilitation and then decreased at recovery ($p < 0.01$). The total WBC and heterophil proportions were significantly higher at admission than for free-ranging turtles (both $p < 0.001$). At recovery, the only significant difference found was that the monocyte count was significantly elevated in recovery compared to free-ranging turtles ($p < 0.01$) (Table 2.3).

Table 2.3. Hematology parameters for green sea turtles (*Chelonia mydas*) in rehabilitation (admission, mid-rehabilitation, and recovery) at the Georgia Sea Turtle Center and free-ranging turtles from St. Lucie County Florida at time of capture.

Hematology Parameter	Admission			Mid-Rehab			Recovery			Free-Ranging		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
PCV %	30.18	5.16	16	27.91	4.84	27	30.46	4.70	28	31.91	3.89	34
TS g/dL	2.19	0.60	28	3.75	0.86	27	3.99	0.89	28	3.64	0.68	34
White Blood Cell estimate/µl	14177.78	5846.91	28	11935.71	4024.11	28	9634.62	3059.27	26	8712.12	2076.47	33
Heterophils %	65.26	16.12	27	46.61	14.20	28	44.11	12.73	27	49.58	10.28	33
Absolute Heterophils/µl	9369.81	5076.37	27	5570.71	2621.83	28	4256.11	1905.53	27	4433.33	1658.81	33
Immature Heterophils %	3.37	12.54	27	0.21	0.96	28	0.00	0.00	27	0.21	0.74	33
Imm. Heterophils/µl	504.44	1908.62	27	20.71	90.47	28	0.00	0.00	27	20.30	72.91	33
Lymphocytes %	21.07	7.74	27	32.14	12.10	28	33.11	10.69	27	37.06	10.70	33
Lymphocytes/µl	2883.85	1381.71	27	3747.96	1514.08	28	3006.93	813.67	27	3112.12	703.45	33
Monocytes %	6.93	4.34	27	15.71	10.27	28	16.19	10.06	27	10.00	3.00	33

Monocytes/μl	994.93	709.12	27	1974.07	1545.44	28	1747.48	1690.19	27	882.42	373.49	33
Eosinophils %	1.15	1.56	27	0.71	1.08	28	1.93	3.12	27	1.70	1.61	33
Eosinophils/μl	158.19	222.68	27	80.79	114.85	28	191.44	315.39	27	154.55	172.59	33
Basophils %	2.15	4.10	27	4.61	8.49	28	4.67	6.56	27	1.45	1.89	33
Basophils/μl	306.19	586.60	27	570.75	1117.76	28	398.78	552.73	27	139.09	214.12	33

Biochemistry

Biochemistry panel results are presented in Table 2.4. Calcium levels increased throughout rehabilitation ($p < 0.001$), while chloride and potassium increased from admission to mid-rehab and then decreased again by recovery, but stayed above admission levels (both $p < 0.001$). These three parameters, as well as the Ca:P ratio, were significantly lower in admission than free-ranging turtles (all $p < 0.001$). At recovery, calcium levels were significantly lower ($p = 0.001$), and phosphorus levels were significantly higher ($p < 0.01$) than in free-ranging turtles.

Uric acid and BUN were elevated for turtles at admission compared to wild turtles (both $p < 0.001$). Both decreased throughout rehabilitation. BUN in recovery animals was not significantly different than in free-ranging animals, and uric acid was significantly lower in recovery animals ($p < 0.01$). The glucose levels of admission turtles were not significantly different than in free-ranging turtles, however levels increased during rehabilitation and were significantly higher at recovery than in free-ranging turtles ($p < 0.01$).

Aspartate aminotransferase (AST) and CK were significantly elevated at admission compared to free-ranging turtles ($p < 0.001$ and $p < 0.05$, respectively). Both decreased throughout rehabilitation, and at recovery, CK values were not significantly different from those in free-ranging animals, however AST was still elevated ($p < 0.01$).

Table 2.4. Plasma chemistry parameters for green sea turtles (*Chelonia mydas*) in rehabilitation (admission, mid-rehabilitation, and recovery) at the Georgia Sea Turtle Center and free-ranging turtles from St. Lucie County Florida at time of capture.

Chemistry Panel	Admission			Mid-Rehab			Recovery			Free-Ranging		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
AST (U/L)	424.68	327.71	28	313.50	181.85	28	256.29	109.94	28	191.03	63.29	34
CK (U/L)	8864.37	14642.48	27	1835.04	2869.14	28	1302.39	1307.06	28	1319.91	1531.89	34
Albumin (g/dL)	0.70	0.22	28	1.23	0.33	28	1.47	0.48	28	1.13	0.26	34
TP (g/dL)	2.28	0.56	28	3.38	0.93	28	3.91	0.93	28	3.28	0.82	34
Globulin (g/dL)	1.58	0.38	28	2.15	0.61	28	2.44	0.51	28	2.15	0.61	34
BUN (mg/dL)	57.00	41.97	28	49.18	39.73	28	27.21	21.83	28	29.62	28.24	34
Cholesterol (mg/dL)	89.86	86.21	28	161.86	62.19	28	167.00	47.17	28	133.79	57.57	34
Glucose (mg/dL)	68.68	38.17	28	111.07	23.99	28	108.36	22.39	28	93.50	19.81	34
Calcium (mg/dL)	4.81	1.01	28	6.07	0.98	28	5.93	1.15	28	6.81	1.30	34

Phosphorus (mg/dL)	8.16	2.02	28	9.54	2.58	28	9.23	2.47	28	7.86	1.95	34
Ca:P Ratio	0.63	0.23	28	0.67	0.19	28	0.75	0.60	28	0.95	0.43	34
Chloride (mEq/L)	112.04	5.95	28	120.22	3.94	27	115.07	6.21	28	117.97	5.11	34
Potassium (mEq/L)	3.65	0.64	28	4.69	0.53	27	4.68	0.52	28	4.57	0.65	34
Sodium (mEq/L)	154.93	4.82	28	155.15	3.13	26	155.36	4.54	28	157.71	5.88	34
Uric Acid (mg/dL)	2.11	1.27	28	0.78	0.27	28	0.75	0.31	28	1.06	0.48	34
Triglycerides (mg/dL)	41.93	83.97	28	273.43	176.48	28	171.82	132.65	28	51.76	39.50	34

Electrophoresis

Total protein, pre-albumin, and albumin were significantly lower at admission compared to free-ranging turtles (all $p < 0.001$). All increased throughout rehabilitation, and averages were higher at recovery than in free-ranging animals, though the relationship was not significant.

Levels of all globulin fractions, except alpha-1 globulins, were significantly lower at admission compared to free-ranging turtles (all $p < 0.001$). Levels increased throughout rehabilitation, and at recovery, levels of alpha-1 and alpha-2 globulins were significantly higher compared to free-ranging turtles ($p < 0.001$ and $p < 0.01$, respectively) (Table 2.5).

Table 2.5. Protein electrophoresis parameters for green sea turtles (*Chelonia mydas*) in rehabilitation (admission, mid-rehabilitation, and recovery) at the Georgia Sea Turtle Center and free-ranging turtles from St. Lucie County Florida at time of capture. Results are presented in g/dL.

Protein Electrophoresis	Admission			Mid-Rehab			Recovery			Free-Ranging		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Total Protein	1.81	0.74	28	3.22	0.92	28	3.62	1.01	28	3.13	0.82	32
A/G Ratio	0.40	0.09	28	0.50	0.09	28	0.53	0.10	28	0.52	0.14	32
Pre-albumin	0.06	0.04	28	0.14	0.09	28	0.16	0.09	28	0.14	0.08	32
Albumin	0.45	0.21	28	0.92	0.29	28	1.08	0.33	28	0.92	0.31	32
Alpha 1 Globulins	0.13	0.08	28	0.26	0.12	28	0.30	0.16	28	0.17	0.10	32

Alpha 2 Globulins	0.25	0.10	28	0.46	0.17	28	0.57	0.20	28	0.41	0.12	32
Beta Globulins	0.34	0.20	28	0.57	0.21	28	0.65	0.23	28	0.52	0.19	32
Gamma Globulins	0.58	0.23	28	0.87	0.24	28	0.88	0.27	28	0.96	0.30	32

Lipids

Cholesterol levels at admission were significantly lower compared to free-ranging turtles
($p < 0.01$). Cholesterol and triglycerides increased during rehabilitation, both to levels
significantly higher than free-ranging turtles ($p < 0.05$ and $p < 0.001$, respectively). Cholesterol
increased throughout rehabilitation, while triglycerides peaked at mid-rehabilitation, and then
declined between mid-rehabilitation and recovery (Table 2.6).

Table 2.6. Lipid panel parameters for green sea turtles (*Chelonia mydas*) in rehabilitation
(admission, mid-rehabilitation, and recovery) at the Georgia Sea Turtle Center and free-ranging
turtles from St. Lucie County Florida at time of capture. Results are presented in mg/dL

Lipid Panel	Admission			Mid-Rehab			Recovery			Free-Ranging		
	Mean	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**N**
Cholesterol	93.14	84.15	28	144.11	45.31	28	161.43	51.63	28	126.60	53.52	31
Triglycerides	41.03	65.73	28	134.29	76.32	28	109.64	79.41	28	46.59	37.23	31
HDL	14.18	13.69	28	53.14	31.89	28	69.86	34.39	28	27.16	23.33	31

VLDL	17.42	16.32	12	26.86	15.26	28	21.82	15.84	28	9.83	7.31	29
LDL	109.00	86.16	10	64.11	30.09	28	69.71	26.85	28	92.27	28.43	26

Vitamin D Panel

Vitamin D, PTH, and iCa levels were all significantly lower in admission animals compared to free-ranging animals (all $p < 0.001$). Vitamin D and PTH did not change significantly throughout rehabilitation, and remained significantly lower in recovery than free-ranging animals. Ionized calcium did increase throughout rehabilitation, and was not significantly different in recovery and free-ranging turtles (Table 2.7).

Vitamins A and E

Retinol was significantly lower in admission compared to free-ranging turtles ($p < 0.001$). Levels increased throughout rehabilitation, and were significantly higher in recovery than in free-ranging animals ($p < 0.001$). However, α-tocopherol levels at admission were not significantly different from values in free-ranging turtles, yet levels increased throughout rehabilitation, and recovery animals had significantly higher levels than free-ranging turtles ($p < 0.001$) (Table 2.8).

Table 2.7. Vitamin D panel parameters for green sea turtles (*Chelonia mydas*) in rehabilitation (admission, mid-rehabilitation, and recovery) at the Georgia Sea Turtle Center and free-ranging turtles from St. Lucie County Florida at time of capture. Units are included in the table.

Vitamin D Panel	Admission			Mid-Rehabilitation			Recovery			Free-Ranging		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
25-Hydroxyvitamin D (nmol/L)	20.21	12.16	28	20.18	6.23	28	23.56	14.95	27	46.55	19.64	33
PTH (pmol/L)	0.48	0.28	28	0.55	0.38	28	0.51	0.34	28	1.12	0.70	33
iCa (mmol/L)	0.80	0.20	28	0.98	0.16	28	0.95	0.10	27	1.01	0.23	33

Table 2.8. Vitamin A (as aldehyde, palmitate, and retinol), vitamin E (as α-tocopherol) and carotenoid (lutein and zeaxanthin) levels in green sea turtles (*Chelonia mydas*) in rehabilitation (admission, mid-rehabilitation, and recovery) at the Georgia Sea Turtle Center and free-ranging turtles from St. Lucie County Florida at time of capture. Results are presented in μg/mL.

Vitamins	Admission			Mid-Rehabilitation			Recovery			Free-Ranging		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Aldehyde	8.90	13.38	28	10.98	21.31	28	8.25	9.64	28	16.75	57.74	34
Palmitate	21.47	30.16	28	14.04	20.74	28	18.02	43.14	28	24.62	56.97	34
Retinol	0.88	0.57	28	2.36	0.79	28	2.61	0.88	28	1.67	0.86	34
α-Tocopherol	0.75	0.74	28	2.02	1.63	28	1.91	1.10	28	0.74	0.41	34
Lutein	0.31	0.35	28	0.66	0.40	28	1.13	0.90	28	0.61	0.60	34
Zeaxanthin	0.35	0.40	28	0.45	0.21	28	0.62	0.41	28	0.59	0.31	34

Trace Minerals

Trace mineral panel results are presented in Table 2.9. Trace minerals that changed significantly over time in rehabilitation included copper and magnesium. Copper increased from admission to mid-rehabilitation, and then decreased again by recovery, but remained above admission levels ($p = 0.001$). Magnesium decreased significantly throughout rehabilitation ($p < 0.001$). Iron, selenium, and zinc did not change significantly throughout rehabilitation.

Iron and magnesium were the only significant variables when comparing admission and free-ranging samples. Magnesium levels of admission turtles were significantly lower than levels of free-ranging turtles ($p < 0.01$). In contrast, iron levels were significantly higher in admission compared to free-ranging turtles ($p = 0.001$).

Magnesium was the only significant variable when comparing recovery and free-ranging animals. Levels at recovery were again significantly lower than in free-ranging turtles ($p < 0.001$). Iron levels decreased throughout rehabilitation and were not significantly different in recovery and free-ranging turtles.

Table 2.9. Trace Mineral panel parameters for green sea turtles (*Chelonia mydas*) in rehabilitation (admission, mid-rehabilitation, and recovery) at the Georgia Sea Turtle Center and free-ranging turtles from St. Lucie County Florida at time of capture. Results are presented in ppm.

Trace Minerals	Admission			Mid-Rehab			Recovery			Free-Ranging		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Calcium	53.67	11.93	28	70.28	8.11	28	70.23	10.62	28	76.86	14.46	33
Copper	0.64	0.21	28	0.80	0.23	28	0.68	0.18	28	0.67	0.23	33
Iron	2.29	2.19	28	2.03	1.79	28	1.42	0.71	28	1.20	1.31	33
Magnesium	72.64	16.69	28	51.49	14.22	28	48.00	12.36	28	82.07	15.00	33
Phosphorus	119.68	37.04	28	201.50	41.32	28	193.53	51.06	28	132.73	30.80	33
Potassium	152.55	38.21	28	197.54	30.06	28	201.57	26.97	28	194.27	29.77	33
Selenium	0.16	0.09	27	0.16	0.05	28	0.16	0.03	28	0.22	0.13	33
Sodium	3465.63	402.43	16	3620.59	235.89	17	3612.63	272.21	19	3581.82	213.59	33
Zinc	1.01	0.51	28	1.05	0.24	28	1.16	0.35	28	1.19	0.44	33

DISCUSSION

The hematologic and plasma biochemical values of free-ranging turtles in this study were similar to values found in other free-ranging green sea turtle populations in the western Atlantic allowing us to use these animals as true representatives of wild turtles (i.e. Bolten and Bjorndal 1992, Anderson et al. 2011, Osborne et al. 2010). While other studies have looked at the effects of FP and cold-stunning on these parameters (i.e. Anderson et al. 2011, Aguirre and Balazs 2000), only one other study has focused on differences of rehabilitating and free-ranging green sea turtles (Stringer et al. 2010). However, this study focused solely on vitamin D, PTH, and iCa levels. Our study is the first to combine a diverse array of plasma health parameters to assess the effect of diet on health and recovery in rehabilitating green sea turtles. Significant differences at the recovery timepoint in rehabilitation compared to free-ranging turtles may represent areas where the nutritional needs of these animals were not being met.

Several significant differences were found in rehabilitation and free-ranging turtles, and many of these differences are likely attributable to the nutrition offered during rehabilitation. Some parameters demonstrate the positive aspects of nutrition in rehabilitation. For example, protein electrophoresis revealed that total protein levels at admission were far below the mean found in free-ranging animals, but levels gradually increased throughout rehabilitation to above that of free-ranging animals. Albumin and globulin protein fractions followed the same trend. This likely reflects improved overall nutrition, and the comparatively consistent food source in rehabilitation. Uric acid levels are often used as an indicator of renal compromise in reptiles, but can also be elevated due to dehydration. Levels in admission animals were likely elevated due to dehydration, as renal insufficiency was not diagnosed in any animal in this study, and levels steadily decreased throughout rehabilitation as animals were rehydrated.

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Another positive relationship elucidating the poor nutritional status of rehabilitation turtles was the potassium levels. Admission turtles had significantly lower potassium levels, likely due to anorexia and debilitation. Mid-rehabilitation and recovery animals had similar levels to free-ranging turtles. Similarly, stranded loggerhead turtles have been shown to have lower potassium levels than foraging loggerheads. The suggested cause of hypokalemia in stranded turtles was decreased food intake and potential diarrhea (Deem et al. 2009).

Levels of vitamin A (retinol) and vitamin E (α-tocopherol) also increased significantly throughout rehabilitation. Only one other study has assessed vitamin A and E levels in juvenile green sea turtles, and the authors found significant differences among study sites in Florida, which were attributed to differences in intake due to food availability (Frutchey 2004). Across sites, vitamin A (measured as retinol) concentrations averaged 0.61 ± 0.01 µg/ml (mean $\pm$ 1 SE) and vitamin E (measured as α-tocopherol) concentrations averaged 3.87 ± 0.13. These levels of vitamin A were lower than those found in all timepoints in our study, while the levels of vitamin E were higher than those found in our study. Elevations in vitamin A in rehabilitation animals may be due to daily multivitamin supplementation and fish intake early in our study. The multivitamin contains 310 IU/500mg, and vitamin analyses of the herring and mackerel fed to green sea turtles at the GSTC showed levels in these fish were quite high (i.e. mean 4830 IU/lb and 2957 IU/lb, respectively). The multivitamin also contains vitamin E (16 IU/500mg). Vitamin E levels fluctuated widely in the Frutchey (2004) study, and thus averages may not be directly comparable.

Other parameters call attention to the inadequacies of the rehabilitation diet. For example, Ca:P ratios were significantly lower in rehabilitation animals compared to free-ranging animals. This has been documented in a previous study of rehabilitating green sea turtles fed squid and

other seafood that has an inverted Ca:P ratio (Stringer et al. 2010). Hypocalcemia and hyperphosphatemia are common findings in rehabilitating turtles, and calcium supplementation may be added to attempt to normalize this ratio (Page-Karjian et al. 2014). Calcium supplementation was offered throughout rehabilitation, and plasma levels increased, however phosphorus levels also increased, and thus the ratio remained low in comparison to free-ranging turtles. As vitamin D is an important calcium-regulating hormone, calcium levels should be considered in concert with this vitamin. Vitamin D was low in all stages of rehabilitation compared to free-ranging turtles, even though the vitamin supplement contained 15 IU/500mg. This is likely because rehabilitating turtles in this study were maintained indoors and not afforded full-spectrum lighting. In the future, animals may be rotated to outside tanks to ensure adequate sunlight exposure for vitamin D production.

Cholesterol and triglyceride levels were also elevated at mid-rehabilitation and recovery timepoints compared to levels in free-ranging turtles. This is likely due to the seafood-based diet consumed early in rehabilitation. Triglycerides did decrease from mid-rehabilitation to recovery as the animals transitioned to a vegetable-based diet, however the levels remained elevated above those found in free-ranging animals. Circulating triglycerides are affected by the concentration of fat in the diet, and likely remained elevated in rehabilitation turtles compared to the free-ranging turtles due to the consumption of high-fat seafood. Levels of AST also improved throughout rehabilitation, but remained elevated compared to free-ranging turtles. Common causes of increased AST include hepatic disease or muscle injury, although high activity of this enzyme in many tissues makes its specificity low (Thrall et al. 2006).

Magnesium levels decreased significantly throughout rehabilitation, and by the recovery timepoint values were almost down to half that of free-ranging turtles. As magnesium is typically

found in high levels in aquatic and terrestrial vegetation, this may be an important nutrient that turtles are not getting when eating a primarily seafood-based diet. This nutrient is not included in typical biochemistry panels, and therefore may be missed by clinicians.

Interestingly, packed cell volume (PCV) did not change significantly throughout rehabilitation. This may indicate that PCV in green sea turtles is not correlated with their level of debilitation as it is in other species (e.g. loggerheads in Deem et al. (2009)).

Overall, we found many parameters in rehabilitating green sea turtles that are significantly different than in wild turtles. Some of the abnormal health parameters improved as individuals were transitioned from the seafood-based diet fed at admission to the primarily vegetable-based diet fed at recovery, while others never returned to values similar to wild turtles. This may indicate that feeding commercially-available vegetables (e.g. lettuce) may not be equivalent to the seagrass and algae that green sea turtles eat in the wild. This was the case found in Florida manatees (*Trichechus manatus latirostris*) fed romaine lettuce in rehabilitation as a substitute for the seagrass they normally consume in the wild (Siegal-Willott et al. 2010).

The information in this study highlights the need to develop a proper diet for green sea turtles in rehabilitation and in more permanent captive situations. With this information, we hope to facilitate the development of palatable, nutritionally complete, herbivorous gel diets that can be manipulated as needed through additions of a of fat, protein, and fiber. This is expected to enhance the recovery of injured and ill green sea turtles and to help maintain the nutritional health of animals held long term in captivity.

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CHAPTER 3

STABLE ISOTOPE AND FATTY ACID ANALYSIS OF HEALTHY AND

REHABILITATING ATLANTIC GREEN SEA TURTLES (*CHELONIA MYDAS*)

INTRODUCTION

The green sea turtle (*Chelonia mydas*) is listed as endangered or threatened throughout its range, both in the United States and other parts of the world (NOAA 2016). Green sea turtles are unique among the sea turtles in their foraging ecology. Hatchlings and young juveniles living in pelagic environments are thought to be primarily omnivorous to carnivorous, feeding on a range of food items including crustaceans, jellyfish, and ctenophores (Arthur et al. 2008). In Atlantic populations, once green sea turtles reach a size of 20 to 25 cm in straight carapace length, they leave pelagic habitats, enter benthic feeding areas and shift to a primarily herbivorous diet of seagrass and algae (Bjorndal and Bolten 1988).

Understanding this unique foraging ecology is critical to green sea turtle conservation for various reasons, including habitat protection. However, this is especially true when considering the implications for feeding injured and ill sea turtles that are often rescued and brought into rehabilitation facilities where they are cared for until they are deemed healthy enough for release. Not including mass stranding events (e.g. oil spill, cold stun), roughly 500 sea turtles are admitted yearly to the 16 sea turtle rehabilitation hospitals in Georgia and Florida (Florida Fish and Wildlife Conservation Commission, unpublished data). Proper nutrition during rehabilitation plays a critical role in the health and eventual release of an individual, however, the natural foraging ecology of green sea turtles makes prescribing a diet for them in rehabilitation settings

especially challenging. Facilities often offer a variety of seafood and vegetables, but injured and ill green sea turtles presenting for rehabilitation often initially prefer seafood, even when their size suggests they should have switched to a fully herbivorous diet. Individuals tend to become overweight when they are fed primarily seafood-based diets long term, and elevations in aspartate aminotransferase (AST) and significant calcium/phosphorous (Ca:P) imbalances occur. For this reason, individuals are typically shifted to a more natural, plant-based diet as soon as their body condition normalizes. However, there remains little scientific evidence surrounding the most appropriate diets to feed green sea turtles in rehabilitation. One relevant study on Florida manatees (*Trichechus manatus latirostris*) compared the proximate analysis of seagrass and algae eaten by free-ranging animals to the lettuce eaten by captive animals (Siegal-Willott et al. 2010). The authors found that these were not equivalent forages, and made suggestions to modify the rehabilitation diet to more closely resemble that of free-ranging animals. Similarly, this research seeks to utilize a more evidence-based analysis of the nutritional needs of sea turtles to improve conservation efforts in rehabilitation.

Due to the difficulty in studying the foraging ecology of marine species, studies often rely on analyses of stomach contents from necropsied animals. However, inference from this information is fraught with problems. Animals that are available for necropsies may have been ill and may not have consumed natural diets. In addition, stomach content analyses provides information about recent foods consumed, not the average diet consumed over time (Käkelä et al. 2007). In contrast, biochemical tracer studies utilizing stable isotope analysis (SIA) and fatty acid analysis (FAA) are commonly used to study foraging ecology because a consumer incorporates the "signature" of its food source in predictable ways (Guest et al. 2010). In stable isotope studies, nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$) primarily acts as a "trophometer," allowing researchers

to estimate the trophic level of individuals. Isotopic fractionation of nitrogen can vary depending on the tissue examined, and in sea turtles, average $\delta^{15}N$ enrichment from prey to predator in blood plasma is approximately 2.92‰, and in epidermis is approximately 2.80‰. Carbon ($^{13}C/^{12}C$, $\delta^{13}C$) enrichment through fractionation is minimal, however $\delta^{13}C$ can reveal feeding location (e.g. terrestrial versus marine or inshore versus offshore). Marine systems tend to be more enriched in ^{13}C relative to terrestrial biomes, and inshore food webs tend to be more enriched than offshore (Arthur et al. 2008, Hobson 1999, Rau et al. 1992). Similar to SIA, FAA uses the levels and proportions of fatty acids (FA) of consumers to elucidate foraging ecology. Fatty acids from diet items are incorporated into body tissues of consumers, and these "signatures" can provide information about spatial and temporal variations in diet among both individuals and populations (see Budge et al. 2006 for review). In monogastric animals, these FAs are incorporated with little modification, however in ruminants, intestinal bacteria may alter FA composition. As green sea turtles rely on bacterial fermentation in the hindgut, FA composition may be altered similarly to ruminants (Seaborn et al. 2005).

Both SIA and FAA may provide insight into diet over different temporal scales depending on the turnover rate for the particular tissue of interest. For SIA, fixed tissues like keratinous hair and feathers are metabolically inert after synthesis and therefore reflect the location or diet of an animal when that tissue was synthesized; metabolically active tissues reflect integration ranging from a few days in the case of blood plasma, to several weeks or months in the case of muscle and skin (Hobson and Wassenaar 2008). Similarly, in FAA, adipose tissue may provide a record of diet over several weeks to months, while blood may represent only a few hours (Budge et al. 2006).

Because SIA provides information about the trophic position and foraging location of an animal while FAA provides more specific information about potential prey items, combining the two techniques lends more resolving power to the study (Guest et al. 2010). While both SIA and FAA have been independently utilized to better understand the foraging ecology of green sea turtles, these techniques have not been combined to date for this species. Stable isotope analysis in green sea turtles has been used to evaluate trophic level (e.g. Lemons et al. 2011, Burkholder et al. 2011) and to more precisely estimate the timing of the shift in green sea turtles from a carnivorous to herbivorous diet (e.g. Reich et al. 2007, Arthur et al. 2008). Several studies show that the shift in feeding preference may be asynchronous with the arrival to neritic habitats in some areas, and that animal matter may be an important food resource for some populations of neritic green sea turtles. These areas include Argentina (González Carman et al. 2013), Australia (Burkholder 2011), California (Lemons et al. 2011), NW Africa (Cardona et al. 2009), and the Mediterranean (Cardona et al. 2010, Godley et al. 1998). Fewer studies have utilized FAA in green sea turtles. Joseph et al. (1985) showed that fatty acid composition in green sea turtles varies with dietary fatty acids consumed. The authors looked at rendered oils from wild green sea turtles from Panama and Somalia, and fat depots from wild turtles in Hawaii and the Caribbean, and found that the most common fatty acids were lauric acid (12:0), myristic acid (14:0), palmitic acid (16:0), and oleic acid (18:1ω9) (Joseph et al. 1985). Seaborn et al. (2005) used FAA to discern differences in pelagic versus benthic individuals. In particular, 22:6n-3, 7-methyl-7-hexadecaenoic acid (7M7H), t16:1n-10, 15:0, and 17:0 were associated with recent recruits (and therefore considered pelagic in origin), while 12:0 and 14:0 were associated with long-term benthic residents.

The objectives of this study were to utilize SIA and FAA as part of a larger study to understand the impact of diet on health and recovery of green sea turtles undergoing rehabilitation. Specifically, objectives were to evaluate the stable isotopes of skin and plasma and the fatty acids present in plasma from rehabilitating green sea turtles at the Georgia Sea Turtle Center (GSTC) compared to healthy, free-ranging turtles from St. Lucie County, Florida to evaluate how health and recovery of these turtles is impacted by the rehabilitation diet. Food items fed to green sea turtles at the GSTC were also analyzed for SIA and FAA. This research will aid in the successful rehabilitation and release of injured and ill green sea turtles admitted to rehabilitation facilities. As green sea turtles maintained in captivity more permanently (e.g. in aquaria) face similar issues (Erlacher-Reid et al. 2013), this research has major potential to aid decisions for what to feed these animals as well.

METHODS

Turtles

Samples from rehabilitating green sea turtles were collected from turtles presenting to the GSTC from January 2014 – May 2016. The GSTC is located on Jekyll Island, Georgia, USA which is a barrier island in Glynn County (31°N, 81°W). Rehabilitation animals primarily came from Georgia and Florida, however, three individuals from Massachusetts were also included. These animals were admitted to the GSTC after a cold-stun event in early 2016.

Standard morphometrics (mass and straight carapace length) were measured for each turtle at all sample collection times. This information was used to calculate a Body Condition Index (BCI) according to the following equation (Bjorndal et al. 2000):

$$BCI = [mass (kg)/straight\ carapace\ length\ (cm)^3] * 10,000$$

A subjective body condition score (BCS) on a scale of 1 – 5 was also recorded.

Samples from free-ranging green sea turtles were collected by the In-Water Research Group, Inc. out of Juniper, Florida from March 2015 – February 2016. The In-Water Group captures an average of 175 free-ranging green sea turtles annually at the St. Lucie power plant on Hutchinson Island, FL. About 50% of these are juveniles in the same size class as those brought into rehabilitation at the GSTC (see Appendix D for data). Turtles coming from the this area of Florida are thought to feed on algae rather than seagrass, a diet which is identical to the turtles received for rehabilitation by the GSTC (Allen Foley, personal communication, 1-15-15). However, there is recent evidence that they may eat both seagrass and algae (Gorham et al. 2016).

Rehabilitation Diets

Food items fed in rehabilitation included romaine lettuce and leafy lettuce (*Lactuca sativa*), cucumber (*Cucumis sativus*), green bell pepper (*Capsicum annuum*), mackerel (*Scomber scombrus*), herring (*Clupea harengus*), shrimp (*Penaeus* spp.), squid (*Loligo opalescens*), and a custom gel diet consisting of vegetables, seafood, and vitamins (recipe in Appendix B). In order to understand the impact of the typical rehabilitation diet on health, these diet items were not altered for this study. Instead, three timepoints relevant to the diet consumed by the turtles were defined: admission, mid-rehabilitation, and recovery. At admission, individuals consumed a mixture of all items. Animals were gradually transitioned throughout rehabilitation to a predominately vegetable-based diet. We defined mid-rehabilitation as the point when an individual was consuming 25% vegetation for two weeks, and recovery as the point when an individual was consuming 75% vegetation for two weeks.

<u>Stable Isotope Sample Collection and Preparation</u>

In general, we followed the methods of Reich et al. (2008), however we made modifications as needed. Brief descriptions of the methods for skin, plasma, and food item sample preparations are provided below.

Skin

A total of 36 skin biopsy samples were collected from green sea turtles presenting for rehabilitation (i.e. the admission timepoint), and 36 skin samples were collected from green sea turtles captured at the St. Lucie power plant. Five rehabilitation turtles sampled at admission were sampled again at the recovery timepoint to analyze the shift in isotopic composition over time.

Lidocaine HCL was mixed 50:50 with sodium bicarbonate for local anesthesia at the biopsy site. A skin sample was then aseptically collected using a sterile 6 mm biopsy punch from the dorsal surface of the shoulder area. Samples were stored in cryovials and immediately refrigerated, then transferred to a -80 °C freezer within 24 hours.

Prior to analysis, all samples were dried for a minimum of 24 hours at -40 °C. Samples were then finely diced with a scalpel blade and approximately 1 mg was weighed into sterilized tin capsules. Because the initial weight of sample needed was unknown, the first 10 samples varied more in weight (1.8 – 5.3 mg).

Epidermis was not separated from dermal tissue for an initial set of 25 samples from rehabilitation turtles. As this may influence comparison to studies which separate epidermis, all subsequent samples were run in duplicate with both whole, intact samples and epidermis alone represented. An analysis of epidermis compared to dermis samples was completed on all samples collected, regardless of being from rehabilitating or wild turtles.

Fat was extracted from a subset of whole skin samples (N=10). Fat extraction was completed by the Analytical Chemistry Laboratory at the University of Georgia using petroleum ether as a solvent. An analysis of extracted compared to non-extracted samples was completed on a subset of wild turtle samples.

Plasma

Blood was collected for SIA from 35 green sea turtles at admission to rehabilitation and 39 wild green sea turtles. Blood was collected from the dorsal cervical sinus using a sodium heparinized syringe and centrifuged to separate plasma. Plasma was stored in cryovials and immediately refrigerated; samples were transferred to a -80 °C freezer within four hours. Prior to analysis, all samples were dried for a minimum of 12 hours at -40 °C, homogenized with a micro spatula, and between 0.7 and 1.3 mg was weighed into sterilized tin capsules.

Food Items

All food items fed to green sea turtles at the GSTC (i.e. romaine lettuce, leafy lettuce, cucumber, green bell pepper, mackerel, herring, shrimp, squid, and gel) were sampled for SIA two times throughout the study (February and September 2015). At the beginning of rehabilitation, fish is deboned and squid are debeaked prior to being fed to the turtles; this is done until turtles are defecating and eating well, then bones and beaks are gradually added back in. Hence, for SIA all seafood items were analyzed both whole and deboned/debeaked. Prior to analysis, all food item samples were dried for at least 48 hours at -40 °C. Food items were then ground and homogenized using a mill grinder, and subsamples between 1.0 and 3.9 mg were weighed into sterilized tin capsules.

Stable Isotope Analysis

All skin, plasma, and food item samples were analyzed by a continuous-flow, isotope-ratio mass spectrometer in the Analytical Chemistry Laboratory at the University of Georgia, Athens, Georgia. A Thermo Finnigan Delta V Isotope Ratio Mass Spectrometer (Bremen, Germany) coupled to a Carlo Erba NA1500 CHN Analyzer (Milan, Italy) via a Thermo Finnigan Conflo III Interface (Bremen, Germany) was used for this analysis. Ratios of stable isotopes were expressed in the following conventional delta (δ) notation in parts per thousand (‰):

$$\delta^{13}C \text{ or } \delta^{15}N = (R_{sample} / R_{standard} - 1) \times 1000$$

where R_{sample} and $R_{standard}$ are the corresponding ratios of heavy to light isotopes ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) in the sample and standard. PeeDee Belemnite (PDB) was used as the carbon isotope standard, and atmospheric nitrogen was used as the nitrogen isotope standard. Samples of standard materials with known isotope ratios were inserted every six or seven samples for calibration and to compensate for any drift over time.

Fatty Acid Sample Collection and Preparation

Plasma

Blood for FAA was collected and stored in the same manner as that for SIA. Samples were collected from wild turtles (N=34) at a single timepoint, and from rehabilitation animals (N=27) at three timepoints: admission, mid-rehab, and recovery to reflect the different diets consumed. Blood was collected from the dorsal cervical sinus and centrifuged within 5 minutes to separate plasma. Plasma was collected and stored at -80 °C until analysis.

Food Items

All food items fed to green sea turtles at the GSTC were sampled for FAA three times throughout the study (February, June, and September 2015). As with SIA, seafood was sampled

whole and deboned/debeaked to accurately represent how they were fed to the animals during rehabilitation.

<u>Fatty Acid Analysis</u>

Plasma samples for FAA were shipped to Nestle Purina Analytical Laboratory (St. Louis, MO, USA) and analyzed using the Folch method to extract fatty acid methyl esters from plasma (Folch et al. 1957). Briefly, the fatty acids are esterified with methanolic sulfuric acid, taken up in heptane, and injected on a gas chromatograph with a flame ionization detector. Fatty acids are identified by comparison to external standards.

<u>Statistical Analysis</u>

Statistical analyses were performed using R 3.2.3 computer software (R Foundation for Statistical Computing, Vienna, Austria). For all analyses, a p-value of < 0.05 was considered statistically significant.

Stable Isotopes

Paired t-tests or Wilcoxon signed rank tests were utilized to determine if significant differences existed in several paired sets of $\delta^{13}C$ and $\delta^{15}N$ data. Paired t-tests were utilized to compare values from whole skin and epidermis samples, while Wilcoxon signed rank tests were used to compare sample sets with small sample sizes, including lipid extracted and non-extracted samples, and samples collected at admission and release.

Independent samples t-tests were used to determine if significant differences existed in $\delta^{13}C$ and $\delta^{15}N$ data from wild and rehabilitation turtles. In order to provide consistency with the first 25 samples for which epidermis was not separated, this analysis was completed using the whole samples. A generalized linear model was used to model $\delta^{13}C$ and $\delta^{15}N$ data while

exploring the influence of potential effects including capture location, straight carapace length (SCL), body condition index (BCI), and month of capture (See Appendix D for data).

Fatty Acids

Fatty acid data were analyzed using a combination of principle components analysis (PCA), multivariate and univariate analysis of variance (MANOVA and ANOVA) and classification trees using the rpart package in R (Therneau et al. 2015). For PCA and MANOVA, only FAs with at least one sample >1% were included. When FA data were less than the limit of detection (LOD), a value of LOD/sqrt(2) was used. Principle components analysis was used to identify the principle FAs that explained the most variance among wild and rehabilitation turtles. These FA data were then transformed prior to MANOVA, because proportional FA data are not multivariate normal (Budge et al. 2006). Data were transformed according to the equation from Budge et al. (2006), $x_{\text{trans}} = \ln (x_i/c_r)$, where x_{trans} is the transformed FA data, x_i is a given FA expressed as percent of total FA, and c_r is the percentage of a specific FA (18:0 was used as suggested by Budge et al. (2006)). Principle FAs identified in the PCA were included as dependent variables in the MANOVA. These included 16:1n7, 18:1n7C, 20:5n3, 18:1n9T, 16:0, 20:1n9, 20:4n6, 18:1n9C, and 22:6n3. The independent variables were timepoint and size class. Timepoints were analyzed three different ways, including a comparison of all three rehabilitation timepoints, admission compared to wild, and recovery compared to wild. Four size classes were determined for rehabilitation turtles based on SCL in cm: <30, 30.1 – 35, 35.1 – 40, 40.1 – 45. An additional two size classes, 45.1 – 50 and >50.1 were included for wild turtles. Follow-up ANOVAs for individual FA comparisons were done using a Bonferroni correction for number of tests (i.e. 0.05/N, where N is the number of dependent variables; Tabachnick and Fidell 2013).

The rpart package in R is based on the trademarked program and book Classification and Regression Trees (CART) by Breiman et al. (1984). For a detailed overview of how CART works, see Smith et al. (1997). Essentially, this package organizes a dataset into a series of dichotomous groups based on the FAs with the greatest deviances (Budge et al. 2006). First, an algorithm is used to find a FA that best splits the entire dataset into two groups. This process then continues with each subgroup (called intermediate nodes), until no improvement can be made (i.e., a terminal node is reached). This continuous dichotomous branching results in a structure called a tree. Terminal nodes can be conservative using this algorithm, and thus the tree can be complex. For this reason, a second stage is often performed where the original tree is "pruned" using cross-validation to minimize the number of terminal nodes without increasing the misclassification rate (MR) above a set level. (Therneau et al. 2015). The MR can be interpreted similar to a p-value, where a MR less than or equal to 5% is considered statistically significant (Samuel and Worthy 2004).

For this research, rpart was used to examine how effectively individual turtles could be classified into sampling timepoint (i.e. admission, mid-rehabilitation, recovery, or wild) based on differences in FA composition. All FA >1% were used to create classification trees.

RESULTS

<u>Stable Isotopes</u>

Skin and Plasma

There was no significant difference in $\delta^{13}C$ or $\delta^{15}N$ in whole skin and samples with just epidermis ($t_{49} = -0.64$, $p = 0.53$ and $t_{49} = -0.83$, $p = 0.41$ for $\delta^{13}C$ and $\delta^{15}N$, respectively; Table 3.1). Therefore, the final analysis of samples taken from rehabilitation compared to free-ranging turtles was completed only on whole skin samples.

Table 3.1. Stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ in whole skin (N=50) and epidermis (N=50) samples from green sea turtles (*Chelonia mydas*).

Treatment	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max
Whole	-15.4 ± 2.0	-19.2	-6.3	9.2 ± 1.2	5.1	10.6
Epidermis	-15.6 ± 1.9	-19.0	-6.7	9.1 ± 1.2	5.2	10.9

No significant differences in either $\delta^{13}C$ or $\delta^{15}N$ were found in lipid-extracted and non-extracted samples (V = 12, p = 0.13 and V = 30, p = 0.85 for $\delta^{13}C$ and $\delta^{15}N$, respectively; Table 3.2). Therefore, the final analysis of samples taken from rehabilitation compared to free-ranging turtles was completed only on non-extracted samples.

Table 3.2. Stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ in lipid-extracted (N=10) and non-extracted (N=10) skin samples from green sea turtles (*Chelonia mydas*).

Treatment	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max
Extracted	-16.0 ± 0.3	-16.3	-15.3	9.3 ± 0.9	8.1	10.9
Non-extracted	-15.8 ± 0.5	-16.4	-15.0	9.3 ± 0.8	8.2	10.4

There was also no significant differences in either $\delta^{13}C$ or $\delta^{15}N$ of skin samples taken at admission and at recovery (V = 0, p = 0.06 and V = 14, p = 0.13 for $\delta^{13}C$ and $\delta^{15}N$, respectively; Table 3.3).

Table 3.3. Stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ in skin samples from rehabilitating green sea turtles (*Chelonia mydas*; N=5) at admission compared to recovery.

Treatment	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)		
	Mean ± SD	Min	Max	Mean ± SD	Min	Max
Admission	-15.7 ± 0.8	-16.6	-14.5	8.8 ± 1.2	7.5	10.2
Recovery	-18.0 ± 1.5	-20.5	-16.9	10.0 ± 1.8	7.5	11.9

Skin stable isotope samples from rehabilitation turtles at admission and free-ranging turtles were not significantly different in $\delta^{13}C$ ($t_{45.39}$ = -1.88, p = 0.07) or $\delta^{15}N$ ($t_{69.78}$ = 0.84, p= 0.41; Fig. 3.1, 3.2). However, plasma stable isotopes samples from rehabilitation turtles were significantly depleted in $\delta^{13}C$ ($t_{58.02}$ = -2.58, p = 0.01) and enriched in $\delta^{15}N$ ($t_{64.54}$ = 4.16, p = <0.001) compared to free-ranging turtles (Figs. 3.1 and 3.3). Generalized linear models of plasma stable isotope data included the effects of capture location, SCL, BCI, and month of capture, but most were not significant. The only significant variable was capture location ($F_{3,53}$ = 3.04, p < 0.05); regardless of whether samples were collected from rehabilitation or free-ranging

animals, turtles captured in Massachusetts and Georgia had enriched $\delta^{15}N$ values compared with

animals captured in Florida.
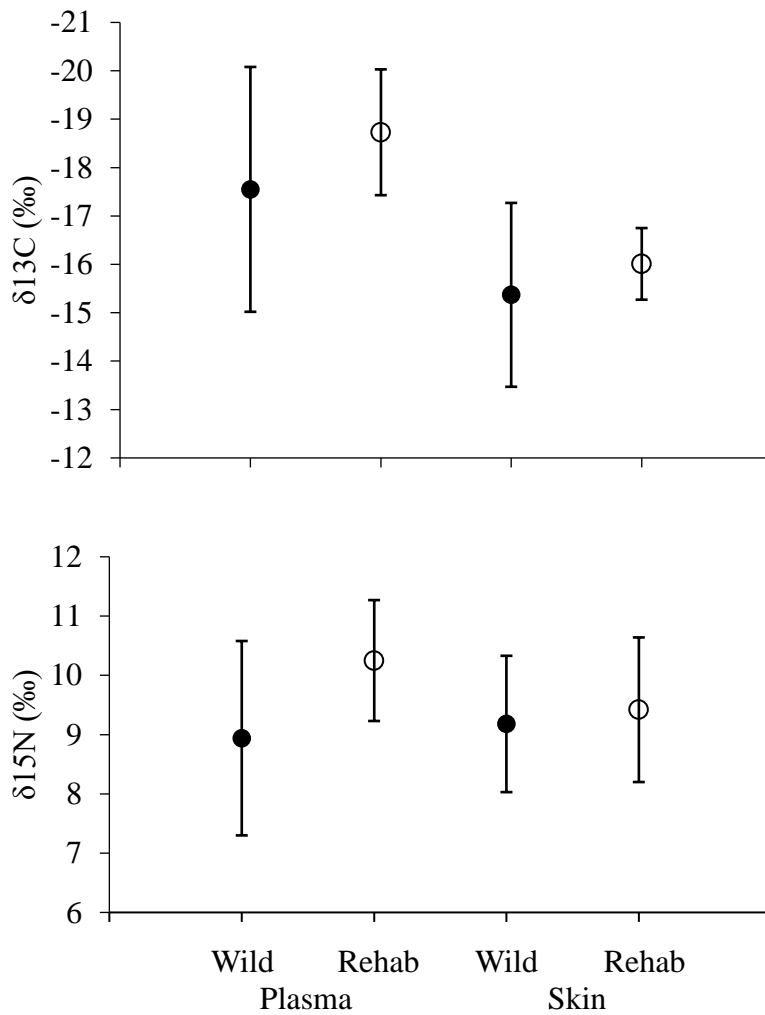
Figure 3.1. Stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ in plasma of rehabilitation (N=35) and wild

(N=39) turtles, and whole skin of rehabilitation (N=36) and wild (N=36) turtles.
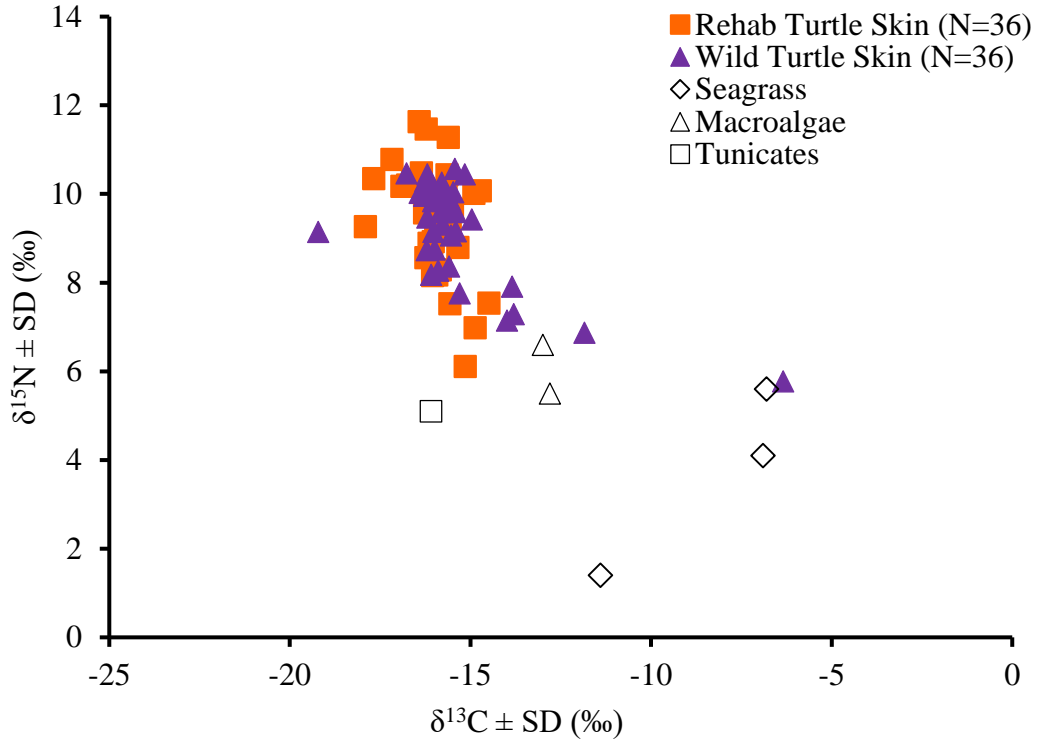
Figure 3.2. Stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ of whole skin of rehabilitation (N=36) and wild (N=36) green sea turtles (*Chelonia mydas*). Closed symbols represent individual turtles; open symbols represent previously published wild dietary food items taken from St. Joseph's Bay, FL (Williams et al. 2014).
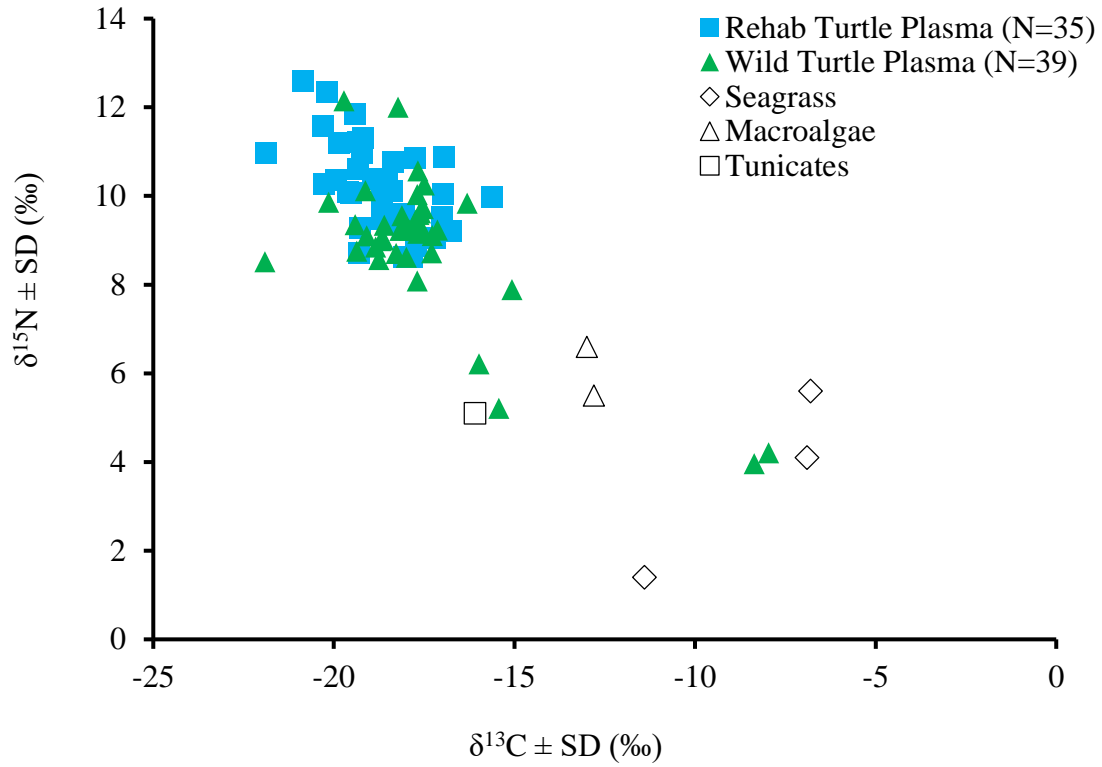
Figure 3.3. Stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ in plasma of rehabilitation (N=35) and wild (N=39) green sea turtles (*Chelonia mydas*). Closed symbols represent individual turtles; open symbols represent previously published wild dietary food items taken from St. Joseph's Bay, FL (Williams et al. 2014).

Food Items

For food items fed during rehabilitation, stable isotopes for vegetables grouped together and fish species grouped together. The gel diet and shrimp also grouped together. Within seafood items, whole and deboned/debeaked versions of the same items grouped together (Fig. 3.4).
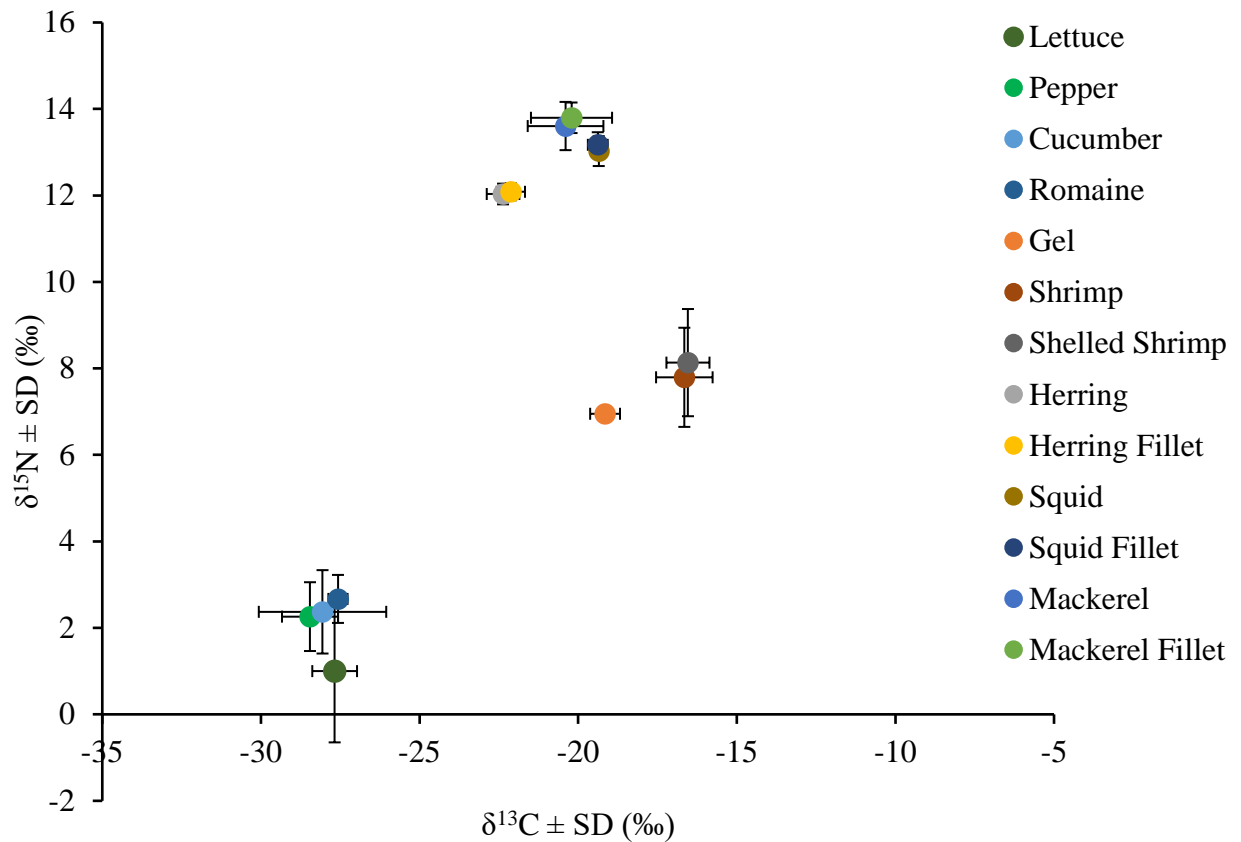
Figure 3.4. Stable isotope ratios of δ^{13}C and δ^{15}N in food items fed to green sea turtles (*Chelonia mydas*) at the GSTC.

Vegetables fed during rehabilitation were more depleted in carbon, and fish were more enriched in nitrogen, compared to potential wild food items. While stable isotopes of skin samples taken at recovery did not change significantly from those taken at admission, they did generally become more depleted in carbon and more enriched in nitrogen as the animals were fed the rehabilitation diet (Fig. 3.5).
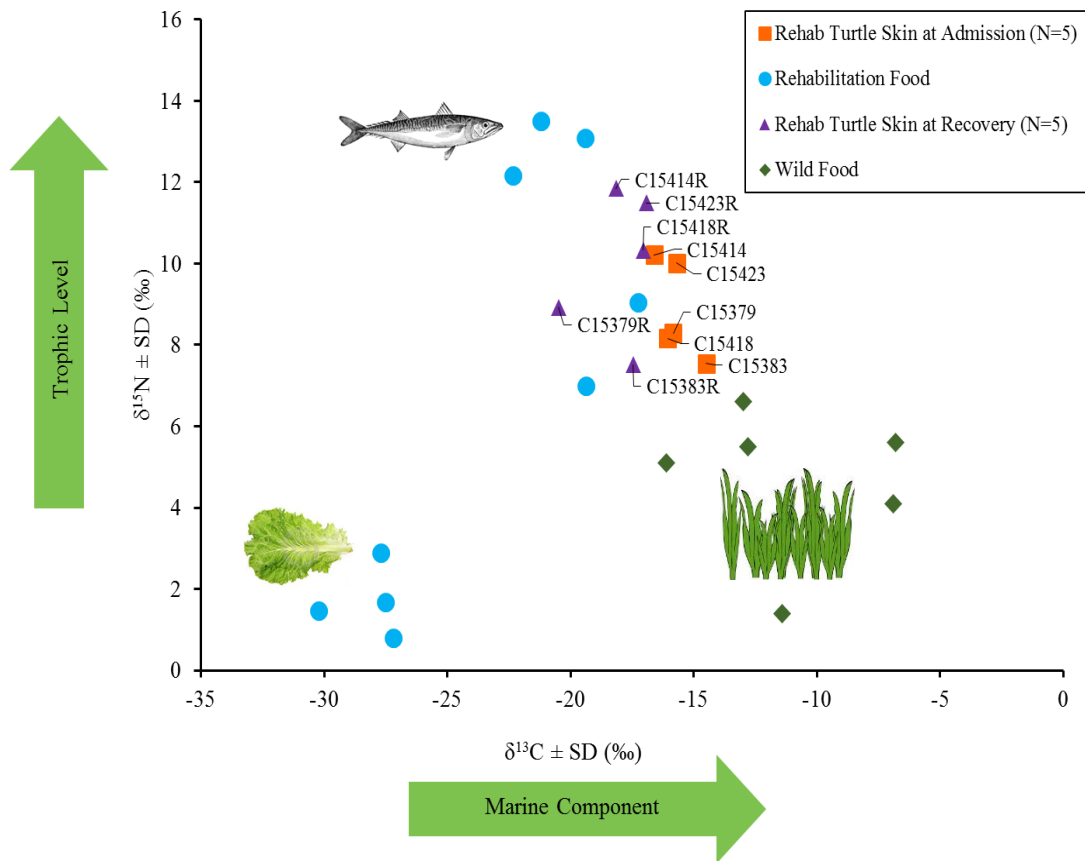
Figure 3.5. Stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ in food items fed to green sea turtles (*Chelonia mydas*) at the GSTC and previously published wild dietary food items taken from St. Joseph's Bay, FL (Williams et al. 2014) compared to individual turtles at admission (N=5) and recovery (N=5).

<u>Fatty Acids</u>

Plasma

The most abundant FA in plasma of wild green sea turtles was 18:1n9C followed by 16:0,

18:0, and 20:4n6 (Table 3.4). Fatty acid signatures of rehabilitation individuals sampled at

admission most closely resembled those of wild individuals. The most abundant FA in the

plasma of animals at admission to rehabilitation was 16:0 followed by 18:1n9C, 18:0, and 20:4n6

(Table 3.4). At mid-rehabilitation and recovery, 16:0 and 18:1n9C still dominated, however

22:6n3 was the third most prevalent FA (Table 3.4).

Table 3.4. Fatty acid composition (% of total) in the plasma of rehabilitating and wild green sea turtles (*Chelonia mydas*). Plasma was collected from rehabilitating green sea turtles at three timepoints in rehabilitation (i.e. admission, mid-rehabilitation, and recovery). The fatty acids that exceeded on average 1% in at least one timepoint are listed. Fatty acids are grouped by saturated (SAT), monounsaturated (MUFA), and polyunsaturated (PUFA).

Fatty Acid	Admission (N=28)				Mid-Rehabilitation (N=28)				Recovery (N=27)				Wild (N=34)			
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
SAT																
12:0	2.00	1.73	0.14	7.17	1.15	0.74	0.47	4.11	1.47	0.55	0.71	2.62	2.88	1.84	0.42	7.98
14:0	3.82	1.66	1.28	8.73	3.99	0.99	2.57	7.62	3.88	0.70	2.53	5.34	3.30	1.05	0.64	5.20
16:0	19.55	7.65	12.10	41.30	16.14	3.65	13.00	29.80	15.71	1.86	12.90	19.10	15.64	3.39	8.13	24.40
18:0	10.89	7.88	4.05	39.10	5.52	2.84	3.36	18.80	6.31	2.04	3.21	10.90	8.51	1.93	4.68	14.50
MUFA																
16:1n7	2.62	1.32	0.34	5.12	4.26	1.48	0.22	6.53	3.83	1.35	1.33	6.37	2.08	0.88	0.71	4.03

18:1n7C	2.39	0.69	0.91	3.68	2.23	0.61	0.45	3.33	2.21	0.48	1.47	3.56	2.24	0.65	0.92	4.00
18:1n9T	2.46	3.14	0.10	11.00	1.31	2.82	0.11	11.30	0.95	1.26	0.12	4.36	1.43	2.11	0.11	8.70
18:1n9C	16.16	7.00	1.40	29.90	11.74	2.97	3.15	17.70	13.60	3.29	8.47	20.60	19.10	3.66	10.50	25.50
20:1n9	1.81	1.96	0.10	5.72	4.38	1.68	1.23	7.90	3.25	1.68	0.59	6.73	0.31	0.20	0.10	0.85
22:1n11	2.09	2.04	0.10	5.35	3.45	2.12	0.32	9.62	2.12	1.70	0.14	6.74	0.20	0.16	0.10	0.43
24:1n9	1.18	0.92	0.18	3.43	0.93	0.42	0.19	1.78	1.04	0.48	0.46	2.62	1.09	0.43	0.39	2.42
PUFA																
18:2n6	2.61	2.19	0.30	8.79	3.94	2.96	0.14	11.80	6.38	4.43	0.91	15.60	2.15	2.70	0.32	14.90
18:3n3	1.10	0.73	0.23	2.71	3.67	2.93	0.11	13.90	4.38	2.49	0.39	9.08	0.46	0.77	0.10	4.18
20:4n6	8.14	4.93	0.88	18.10	2.17	0.80	0.42	3.98	2.09	0.76	0.76	4.30	8.48	3.14	3.34	17.30
20:5n3	2.91	1.99	0.14	7.58	6.09	1.92	0.26	8.44	5.63	1.62	3.15	8.81	2.00	0.84	0.39	4.33
22:5n3	1.29	0.76	0.23	3.13	1.22	0.40	0.16	2.08	1.36	0.68	0.41	3.66	1.64	0.96	0.16	3.70
22:5n6	1.04	1.11	0.19	4.11	0.44	0.17	0.19	0.96	0.32	0.11	0.14	0.54	0.74	0.58	0.18	2.36
22:6n3	5.26	3.41	0.86	13.60	9.79	3.30	0.43	14.40	8.42	3.43	3.85	14.20	5.23	2.29	1.78	9.72

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In the PCA of only rehabilitation turtles, the first axis explained 31% of the variance of the FA proportions of turtles at each timepoint, and the second axis explained an additional 20% (Table 3.5). In the PCA of rehabilitation and wild turtles combined, the first axis explained 29% of the variance of the FA proportions, and the second axis explained an additional 21% (Table 3.5). Plots of PCA data show the differences in the principle FA of different groups (i.e. admission, mid-rehabilitation, recovery, and wild; Fig. 3.6).

Table 3.5. Eigenvalues, cumulative proportion of variance explained, and principle component loadings for principle component analysis of fatty acids (>1%) in rehabilitation only and in rehabilitation and wild green sea turtles (*Chelonia mydas*).

	Rehab Only PCA		Rehab and Wild PCA	
	PC1	PC2	PC1	PC2
Eigenvalue	5.64	3.60	5.17	3.84
Cumulative proportion	0.31	0.51	0.29	0.50
	Loadings		Loadings	
	PC1	PC2	PC1	PC2
12:0	0.11	-0.11	0.14	0.03
14:0	0.02	-0.27	-0.04	-0.22
16:0	0.31	-0.10	0.22	-0.25
16:1n7	-0.36	-0.16	-0.39	-0.04

18:0	0.33	-0.02	0.30	-0.14
18:1n7C	-0.34	0.19	-0.25	0.30
18:1n9C	-0.17	0.37	-0.01	0.40
18:1n9T	0.31	-0.13	0.26	-0.27
18:2n6	-0.13	0.16	-0.14	0.03
18:3n3	-0.20	0.01	-0.25	-0.07
20:1n9	-0.17	-0.42	-0.29	-0.32
20:4n6	0.01	0.41	0.14	0.40
20:5n3	-0.36	-0.21	-0.40	-0.10
22:1n11	-0.17	-0.29	-0.26	-0.21
22:5n3	-0.22	0.09	-0.10	0.24
22:5n6	-0.05	0.28	0.01	0.28
22:6n3	-0.31	-0.21	-0.35	-0.04
24:1n9	-0.18	0.26	-0.12	0.30
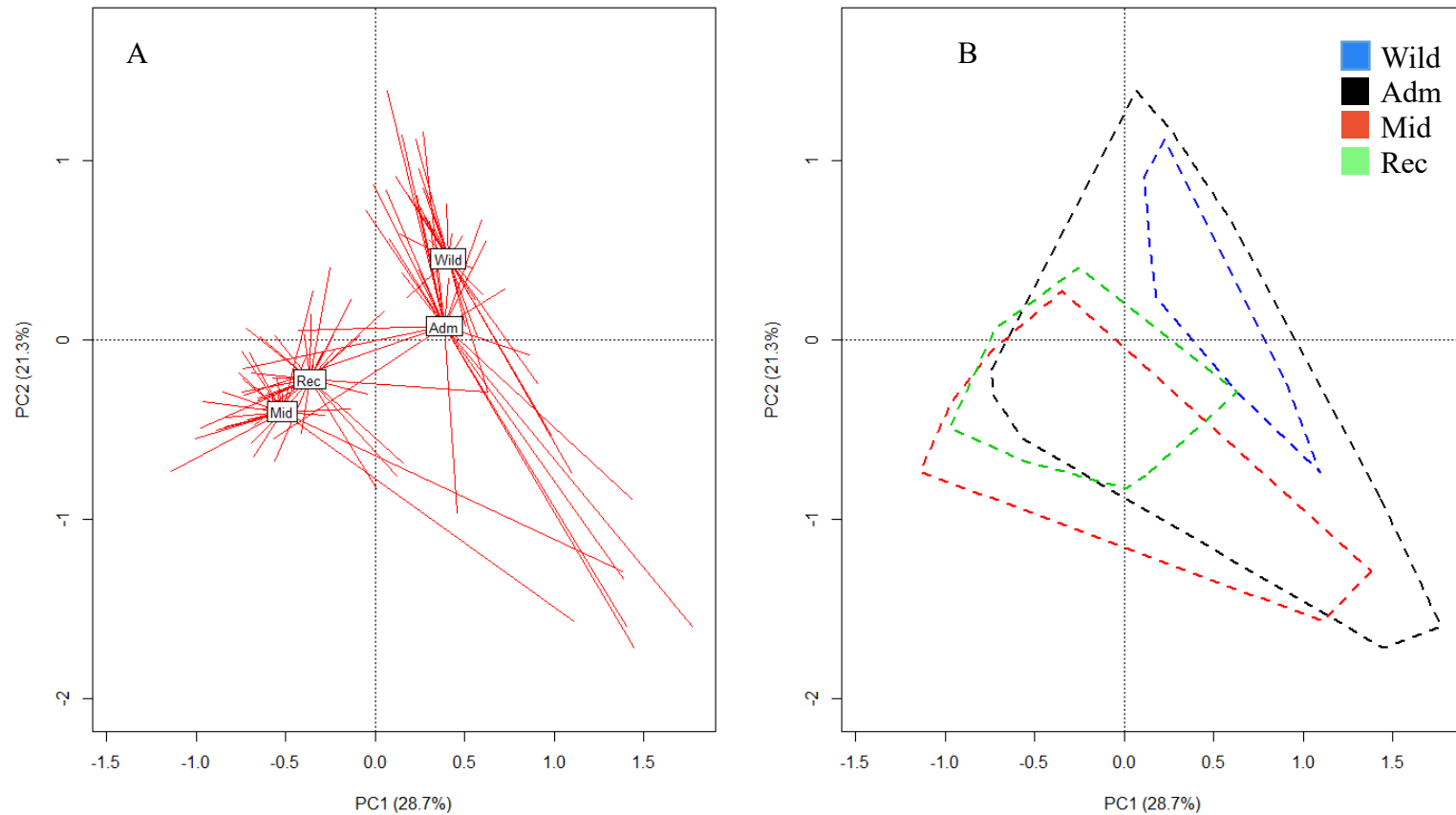
Figure 3.6. Principle components analysis of fatty acids (>1%) in rehabilitation (i.e. admission, mid-rehabilitation, and recovery) and wild green sea turtles (*Chelonia mydas*). Panel A shows lines drawn from the mean for that group to indivuals within the group. Panel B shows the hulls srrounding individuals within each group.

In the MANOVA comparing the three timepoints in rehabilitation, there was a statistically significant difference on the combined dependent variables ($F_{2,72} = 5.39$, p <0.001). In the ANOVAs of individual FAs, 16:0 and 18:1n7C decreased significantly over time in rehabilitation while 16:1n7, 20:1n9, 20:5n3, and 22:6n3 increased significantly over time. Fatty acids that did not vary significantly over the timepoints included 18:1n9T, 18:1n9C, and 20:4n6 (Fig. 3.7) (ANOVA with Bonferroni correction for number of tests, i.e., p = 0.006). Size class was significant in the MANOVA ($F_{3,72} = 1.70$, p <0.05), however, in the ANOVAs of individual FAs, size class was not significant. The interaction term was also not significant (p = 0.36).

When comparing admission and wild turtles, there was a statistically significant difference on the combined dependent variables ($F_{1,52} = 2.80$, $p < 0.05$). Size class was significant overall ($F_{5, 52} = 1.43$, $p < 0.05$), but the interaction term was not significant (p = 0.40). In the ANOVAs of individual FAs, no FA was statistically significant between admission and wild turtles.

When comparing recovery and wild turtles, there was a statistically significant difference on the combined dependent variables ($F_{1,52} = 44.01$, $p < 0.001$). Size class was not significant overall ($F_{5, 52} = 0.80$, p = 0.81). In the ANOVAs of individual FAs, all FA varied significantly over the timepoints except 18:1n9T and 18:1n9C. Fatty acid levels that were lower in recovery animals compared to wild included 18:1n7C and 20:4n6. Fatty acid levels that were lower in wild animals compared to recovery included 16:0, 16:1n7, 20:1n9, 20:5n3, and 22:6n3.
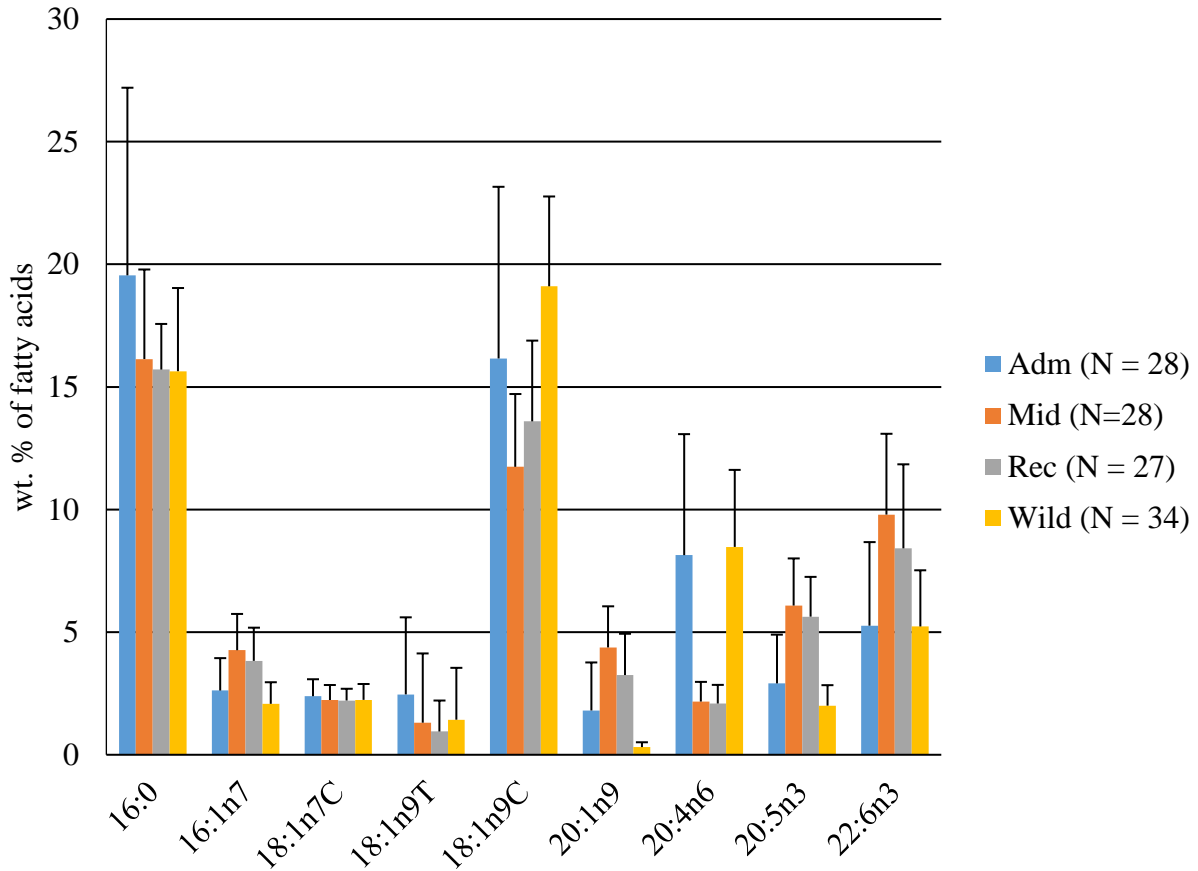
Figure 3.7. Mean (± SD) of the most common fatty acids in the plasma of rehabilitating and wild green sea turtles (*Chelonia mydas*). Rehabilitating animals were sampled at admission, mid-rehabilitation, and recovery, following changes in diet from primarily seafood to primarily vegetables.

Classification trees were fit using the rpart package in R. Classification of the plasma samples into the timepoints (i.e. admission, mid-rehabilitation, recovery, and wild) by the initial model required seven fatty acids, resulting in eight terminal nodes (Fig. 3.8). Nodes were

determined based on changes in deviance, and fatty acid 20:1n9 was chosen by the tree algorithm

as the first node. This original tree misclassified 26 of the 118 samples (MR = 26/118; p = 0.22)

		Predicted			
		Adm	Mid	Rec	Wild
Actual	Adm	21	0	0	7
	Mid	2	23	3	0
	Rec	2	6	16	4
	Wild	2	0	0	32
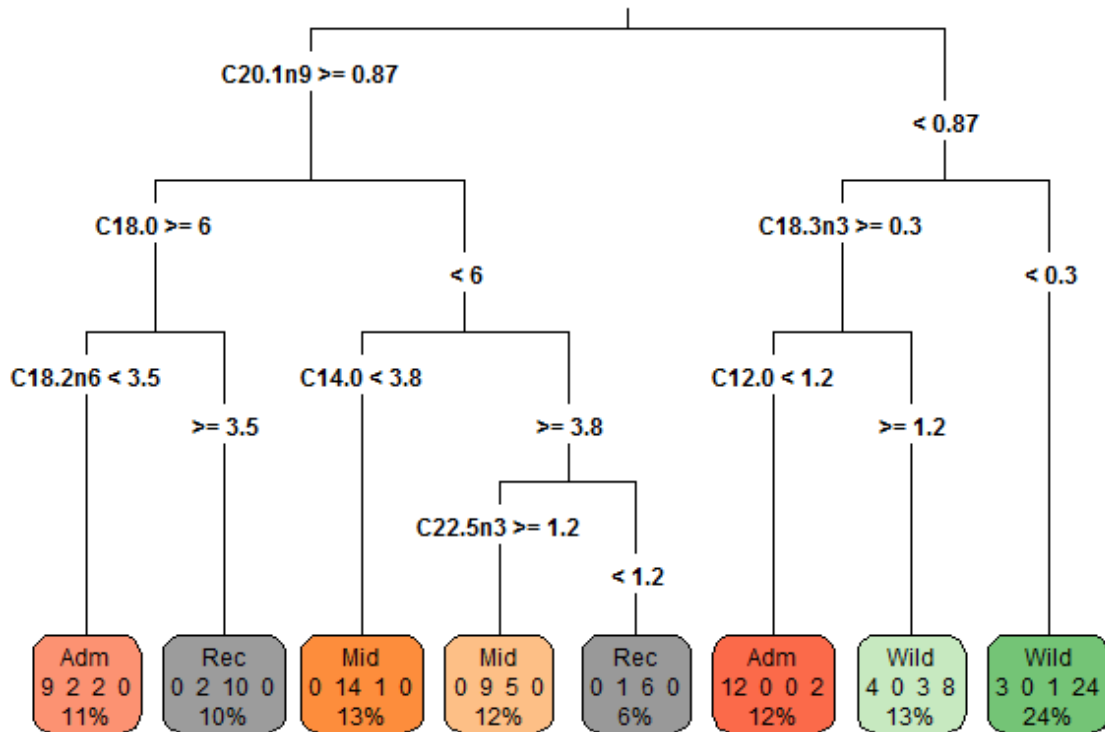
Figure 3.8. Classification tree of fatty acid data in rehabilitating (i.e. at admission, mid-rehab,

and recovery) and wild green sea turtles (*Chelonia mydas*). Each node is labelled with the FA

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and the cutoff that was chosen by the rpart package in R. Entries under the terminal nodes refer

to the number of turtles placed in each of the four categories (admission, mid-rehab, recovery,

and wild). The % within the terminal node refers to the % of the total animals placed in that

category. The matrix included at the top left of the figure displays the number of animals in each

category compared with the predicted number in rpart.

A pruned tree was also created by selecting the complexity parameter associated with the

minimum error (Fig. 3.9). This tree misclassified 35 of the 118 samples. (MR = 35/118; p =

0.30).

		Predicted			
		Adm	Mid	Rec	Wild
Actual	Adm	25	0	0	3
	Mid	2	24	2	0
	Rec	5	12	10	1
	Wild	10	0	0	24

C20.1n9 >= 0.87   < 0.87

C18.0 >= 6   < 6   C18.3n3 >= 0.3   < 0.3

C18.2n6 < 3.5   >= 3.5

Adm	Rec	Mid	Adm	Wild
9 2 2 0	0 2 10 0	0 24 12 0	16 0 3 10	3 0 1 24
11%	10%	31%	25%	24%
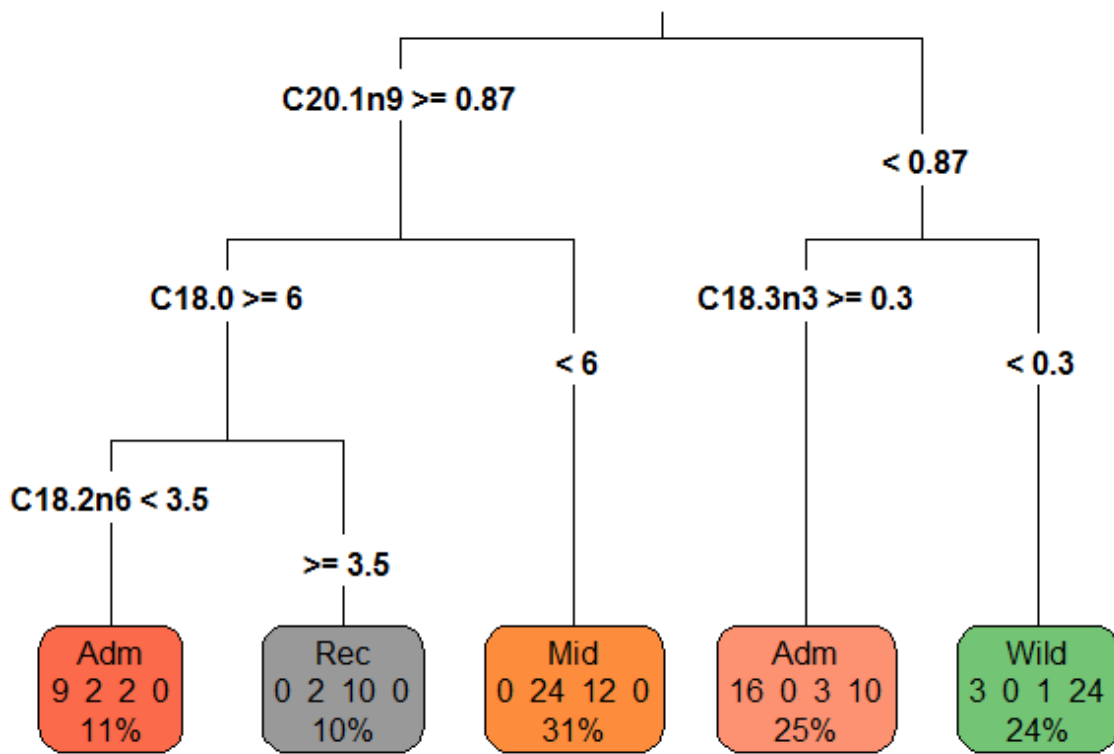
Figure 3.9. Pruned classification tree of fatty acid data in rehabilitating (i.e. at admission, mid-rehab, and recovery) and wild green sea turtles (Chelonia mydas). Each node is labelled with the FA and the cutoff that was chosen by the rpart package in R. Entries under the terminal nodes refer to the number of turtles placed in each of the four categories (admission, mid-rehab,

recovery, and wild). The % within the terminal node refers to the % of the total animals placed in that category. The matrix included at the top left of the figure displays the number of animals in each category compared with the predicted number in rpart.

Food Items

The most abundant FA in food fed to green sea turtles at the GSTC varied substantially by type (Tables 3.6 and 3.7). Within the gel diet and the vegetables, the most prevalent FAs were 16:0 and two polyunsaturated FA (PUFA), 18:2n6 and 18:3n3 (Table 3.6). Within the seafood, 16:0 was also prevalent, however levels of monounsaturated FA (MUFA) were generally higher than those in vegetables and the most common PUFA were 20:5n3 and 22:6n3. Levels of FA also varied substantially within different types of seafood. For example, the most abundant FAs in herring, 20:1n9 and 22:1n11, were <1% and <4%, respectively, of the FA proportion in mackerel, squid, and shrimp (Table 3.7).

Table 3.6. Fatty acid composition (% of total) of the gel and vegetable items fed to green sea turtles, *Chelonia mydas*, at the Georgia Sea Turtle Center. Fatty acids are grouped by saturated (SAT), monounsaturated (MUFA), and polyunsaturated (PUFA). The fatty acids that exceeded on average 1% in at least one food item are listed. Values that were below the limit of detection of 0.100% are listed as BDL. Values for the gel are presented as the mean and standard deviation of three samples collected over the duration of the study; only one sample was taken for vegetables, so that value is presented.

Fatty Acids	Gel					
	Mean	SD	Lettuce	Romaine	Cucumber	Pepper
SAT						
12:0	0.16	0.04	0.62	BDL	BDL	1.04
14:0	5.76	0.14	0.70	1.04	2.00	1.45
16:0	21.93	1.85	19.90	23.00	44.00	30.00
18:0	5.60	0.62	2.62	3.74	7.52	6.96
20:0	0.26	0.06	0.46	0.55	0.81	1.02
22:0	0.19	0.07	0.87	0.93	1.71	1.29
MUFA						
16:1n7	7.12	0.28	0.33	0.46	BDL	0.49
18:1n7C	2.60	0.20	0.57	0.68	BDL	0.56
18:1n9C	14.07	1.17	2.28	4.15	3.96	5.43

20:1n9	2.87	2.25	BDL	BDL	BDL	BDL
22:1n11	2.99	3.37	BDL	BDL	BDL	BDL
PUFA						
18:2n6	10.08	0.91	20.70	18.60	17.50	31.50
18:3n3	2.03	0.26	45.10	41.20	17.20	17.90
18:4n3	1.17	0.20	BDL	BDL	BDL	BDL
20:4n6	1.08	0.12	BDL	BDL	BDL	BDL
20:5n3	5.82	0.56	1.22	1.22	1.88	0.86
22:5n3	1.00	0.04	0.84	1.02	1.00	0.47
22:6n3	6.07	1.03	BDL	BDL	BDL	BDL

Table 3.7. Fatty acid composition (% of total) of the seafood items fed to green sea turtles, *Chelonia mydas*, at the Georgia Sea Turtle Center. Fatty acids are grouped by saturated (SAT), monounsaturated (MUFA), and polyunsaturated (PUFA). Values presented are the mean and standard deviation of three samples collected over the duration of the study. The fatty acids that exceeded on average 1% in at least one food item are listed. Fatty acids of food items that had one or more samples that were below the limit of detection of 0.100% are listed as BDL.

Fatty Acids	Herring		Deboned Herring		Mackerel		Deboned Mackerel		Squid		Debeaked Squid		Shrimp		Shrimp no Shell	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
SAT																
14:0	5.22	1.14	5.33	1.51	3.59	0.09	3.36	0.09	2.91	0.26	2.87	0.47	1.48	0.10	1.48	0.05
15:0	0.42	0.17	0.37	0.18	0.98	0.34	1.04	0.35	0.51	0.07	0.52	0.06	1.18	0.05	1.13	0.06
16:0	10.94	2.02	10.32	3.43	16.53	0.64	16.83	1.88	25.07	0.29	24.83	0.31	17.57	1.10	17.67	0.97
17:0	0.33	0.17	0.27	0.14	1.07	0.24	1.11	0.23	0.73	0.04	0.77	0.08	2.36	0.10	2.24	0.21
18:0	1.12	0.26	1.08	0.32	7.71	0.27	8.46	0.23	5.09	0.54	5.13	0.49	11.33	0.35	11.20	0.56

MUFA

16:1n7	6.48	0.76	6.70	0.68	2.90	0.40	2.81	0.51	0.86	0.31	0.90	0.31	4.02	0.49	4.61	0.81
18:1n7C	2.13	0.36	2.07	0.51	3.22	0.35	3.34	0.14	1.89	0.30	1.88	0.22	3.40	0.06	3.27	0.22
18:1n9C	6.93	1.33	7.44	3.03	7.96	1.13	7.98	1.14	3.62	1.08	3.22	0.47	8.12	0.62	8.37	1.18
20:1n9	15.13	4.04	17.30	4.81	1.49	0.16	1.24	0.35	3.43	0.74	3.62	0.33	0.41	0.04	0.51	0.22
22:1n11	20.77	5.67	22.33	8.62	0.87	0.84	0.45	0.37	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
22:1n9	2.53	0.73	2.72	1.00	0.52	0.12	0.39	0.11	0.49	0.13	0.50	0.07	BDL	BDL	BDL	BDL

PUFA

18:2n6	1.14	0.50	1.03	0.61	1.49	0.10	1.53	0.14	0.76	0.40	0.48	0.08	2.11	1.10	2.36	0.61
18:3n3	1.90	0.27	1.85	0.15	1.43	0.15	1.43	0.14	0.70	0.44	0.45	0.04	0.71	0.16	0.77	0.17
18:4n3	1.85	0.99	1.40	0.93	1.36	0.17	1.21	0.26	0.27	0.15	0.32	0.21	0.13	0.03	0.15	0.02
20:4n6	0.25	0.11	0.21	0.11	2.88	1.12	3.07	1.01	1.91	0.08	1.97	0.18	7.66	0.57	6.96	0.67
20:5n3	5.86	1.02	4.52	1.36	8.57	1.93	7.65	1.32	14.90	0.92	15.17	0.65	15.13	1.21	15.13	1.60
22:5n3	0.61	0.06	0.53	0.04	1.98	0.30	1.94	0.50	0.49	0.02	0.48	0.02	1.48	0.14	1.43	0.09
22:5n6	0.20	0.02	0.17	0.04	1.29	0.43	1.45	0.47	0.43	0.04	0.44	0.03	0.99	0.09	1.01	0.04
22:6n3	7.56	2.51	6.25	2.50	23.57	2.73	24.43	1.50	30.67	1.76	31.20	1.73	10.93	1.01	10.86	1.17

Discussion

We found no significant difference in SIA of whole skin and epidermis samples. Epidermis has traditionally been separated from dermal tissue before sample preparation, however these results suggest that this may be an unnecessary extra step, and could be eliminated. Another step in the process of sample preparation for SIA is lipid extraction. Fat is typically extracted prior to SIA because lipids are ^{13}C depleted compared to proteins, and not extracting may bias interpretation of results (DeNiro and Epstein 1978). This may be especially true in the whole skin samples used in this study, as they often included some subcutaneous fat. However, fat extraction yielded no significant difference in isotope values. Traditionally, it is not considered necessary to account for lipids in aquatic animal samples when the C:N ratio is <3.5, (Post et al. 2007). In this study, the C:N ratio of all skin samples ranged from 3.1 to 4.0. This narrow range is close to the recommended level for lipid extraction and this may be the reason that no significant difference in extracted and non-extracted samples was found. This evidence is supported by a study of 15 juvenile green sea turtles, in which fat extraction did not significantly alter δ^{13}C or δ^{15}N values in skin samples, leading the authors to suggest that this step is not required for SIA of skin in these animals (Bergamo et al. 2016).

Not performing lipid extraction on skin samples also allows direct comparison to SIA in plasma samples, for which lipids are often not extracted due to small sample size, as was the case in our study (Reich et al. 2008). Plasma samples appeared to be more depleted in δ^{13}C than skin samples in this study. This could be due to the shorter turnover time of plasma compared to skin. While skin of green sea turtles is thought to have a turnover time of several months, plasma reflects a shorter time of several weeks (Reich et al. 2008). These data were also not entirely the

same turtles (i.e. some turtles had plasma samples taken but no skin sample taken, and vice versa).

Stable isotopes in rehabilitation and wild skin samples were not significantly different in $\delta^{13}C$ or $\delta^{15}N$, however isotopes in rehabilitation and wild plasma samples were significantly different in both $\delta^{13}C$ and $\delta^{15}N$. This result was surprising; however, turnover time of these tissues may again explain the difference. Significant differences found in plasma stable isotopes between wild turtles and those admitted to rehabilitation are likely a result of short-term changes in diet. Elevated $\delta^{15}N$ in rehabilitation turtles could be explained by debilitation, as fasting can cause protein catabolism, leading to an increase in body ^{15}N values (Hobson and Wassenaar 2008). However, generalized linear models of stable isotope data indicated that BCI was not significant. This may be because BCI is too crude of an estimate of body condition, or it could indicate that animals do indeed forage at multiple trophic levels. The only significant effect found in the model was capture location, with animals captured in Massachusetts and Georgia reflecting more enriched $\delta^{15}N$ values than animals captured in Florida. Therefore, results may indicate that individuals at more northerly latitudes forage on a more carnivorous diet. No significant variables were found to help explain significant differences in $\delta^{13}C$ between rehabilitation and wild turtles, however, individuals with enriched $\delta^{13}C$ tended to be larger animals, and were primarily wild turtles from Florida. This could indicate a preference for seagrass over algae in these individuals, as recent research indicates that green sea turtles in this area forage on both algae and seagrass (Gorham et al. 2016). Seagrass is typically enriched in ^{13}C, and, in turn, seagrass consumers have an enriched carbon signature (Williams et al. 2014).

The most abundant FAs in plasma of wild green sea turtles was 18:1n9C followed by 16:0, 18:0, and 20:4n6. The most abundant FAs in the plasma of rehabilitating sea turtles were

also 16:0 and 18:1n9C, regardless of timepoint. However, levels of omega-3 FA, docosahexaenoic (22:6n3) and eicosapentaenoic (20:5n3), were higher in rehabilitation than wild animals. This likely reflected a larger amount of fish in the diet of rehabilitation animals than is typical of wild animals. This is supported by the high levels of these FAs in the seafood items fed in rehabilitation.

Principle components analysis showed that rehabilitation animals at admission varied much more than other groups, and actually encompassed all other groups (i.e. mid-rehabilitation, recovery, and wild). This could reflect a fasting condition or a propensity for debilitated animals to forage on a wider range of diet items. Mid-rehabilitation and recovery animals grouped closely together, and the wild group was separate, however recovery samples had shifted towards wild. This is intuitive, as mid-rehabilitation and recovery animals were being shifted to a more vegetable-based diet that is more similar to the wild diet than the seafood items fed after admission.

Using MANOVA and ANOVA to look at the principle FA, significant differences in FA signatures were found. In rehabilitation, all FA varied significantly over the three timepoints except 18:1n9T, 20:4n6, and 18:1n9C. These differences can be explained by the food items offered in rehabilitation. As discussed above, elevations in 20:1n9, 20:5n3, and 22:6n3 over time in rehabilitation likely reflect the high fish-based diet in these animals compared to what they consumed prior to admission. When comparing admission and wild turtles, no FA was statistically significant. This demonstrates that these two groups of animals were likely foraging on similar diets. When comparing recovery and wild turtles, however, all FA varied significantly over the timepoints except C18.1n9T and C18.1n9C, indicating that animals in rehabilitation consumed a diet not reflective of their wild counterparts. This was supported by the PCA

described earlier, and the changes throughout rehabilitation that were associated with seafood in the diet. Higher levels of arachidonic acid (20:4n6) in wild turtles should be monitored, as arachidonic acid plays an important role in mediating inflammatory reactions (Kuehl et al. 1980).

Comparisons of recovery animals to wild allowed conclusions to be made that rehabilitation diets are not matching the diets consumed by wild animals. This was particularly seen in the plasma FA data. Ideally, FA signatures in rehabilitation animals at recovery would match those of wild animals, and they did not. These results indicate that even when rehabilitation animals are fed 75% vegetables, these vegetables (i.e. lettuces, cucumber, and green pepper), are not equivalent to wild seagrass and algae. This has previously been shown by Siegal-Willott et al. (2010). This study did not evaluate FA, but utilized proximate analysis to compare the nutritional value of seagrass and algae eaten by free-ranging Florida manatees to that of romaine lettuce, which is typically fed to captive manatees. The study examined dry matter content, proximate nutrients (crude protein, ether-extracted crude fat, nonfiber carbohydrate, and ash), and digestible energy. Neutral-detergent fiber, acid-detergent fiber, and lignin were also compared. Results indicated that romaine lettuce and seagrasses and algae are not equivalent forages, and that captive manatees should be provided a diet higher in fiber and lower in fat, protein, digestible carbohydrates, and digestible energy to more closely mimic the diet of free-ranging manatees (Siegal-Willott et al. 2010). In the current study, as animals were transitioned to the higher proportion of vegetables (i.e. from mid-rehabilitation to recovery), a FA signature that more closely matched that of wild turtles emerged. This indicates that a vegetable-based diet more closely resembles that of wild turtles than the primarily seafood-based diet fed at admission and mid-rehabilitation.

Fatty acid and stable isotope analyses have not been combined in green sea turtles to the authors' knowledge. Thus far, the negative impacts of feeding high seafood-based diets to green sea turtles have been primarily anecdotal (e.g. obesity, elevations in AST, and Ca:P imbalances) or based on very specific nutritional parameters (i.e. vitamin D, parathyroid hormone, and ionized calcium levels (Stringer et al. 2010)). The combination of SIA and FAA can help elucidate the foraging strategies of wild turtles, and can allow comparison of diet signatures in wild and rehabilitating animals. This information can pinpoint areas where rehabilitation diets are lacking compared to wild food items and potentially indicate reasons for the pathologies described earlier. In turn, this information can be used to make dietary modifications and develop gelatin-based diets for rehabilitation centers that will enhance recovery and ensure optimal survival.

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CHAPTER 4

THE EFFECT OF DIET AND ANTIBIOTIC ADMINISTRATION ON THE

GASTROINTESTINAL MICROBIOME OF REHABILITATING GREEN SEA

TURTLES (*CHELONIA MYDAS*)

Introduction

Metagenomics is an emerging field that attempts to describe and quantify the genomes of entire communities of microbes in various tissues (e.g. skin, the gastrointestinal and respiratory tracts). It is estimated that microbial cells in the vertebrate gastrointestinal (GI) tract outnumber the cells that make up the host's body by a factor of at least 10 (Zoetendal et al. 2008). The composition of these microbes, collectively called the GI microbiota, has received particular attention because of its association with health. The GI-associated microbes produce vitamins and essential amino acids and influence fat and glucose utilization (Robinson et al. 2010). Disruptions to this composition is associated with problems such as obesity, inflammatory disease, diabetes, and cancer (Robinson et al. 2010). For example, a deficiency in *Faecalibacterium prausnitzii* in the phylum Firmicutes is associated with inflammatory bowel disease in people (i.e. Crohn's disease and ulcerative colitis; Swidsinski et al. 2008). The microbiota composition is also associated with diet. In humans, animal-based diets have been associated with increased abundances of bile-tolerant organisms (e.g. Alistapes, Bilophila, and Bacteroides) and decreased abundances of Firmicutes that break down plant polysaccharides

(David et al. 2014). Emerging research suggests that it may be possible to use our diet to manipulate our GI microbiota to improve health (Umu et al. 2013).

The majority of published microbiome studies (>90%) have focused on mammals, primarily humans. Within other taxa, the reptilian microbiome is the least studied (for review see Colston and Jackson 2016). Gastrointestinal bacterial metagenomes have been studied in American alligators (*Alligator mississippiensis*; Keenan et al. 2013), Burmese pythons (*Python molurus*; Costello et al. 2010), cottonmouths (*Agkistrodon piscivorus*; Colston et al. 2015), Galapagos marine iguanas (*Amblyrhynchus cristatus*) and land iguanas (*Conolophus subcristatus* and *C. pallidus*; Lankau et al. 2012 and Hong et al. 2015), and timber rattlesnakes (*Crotalus horridus*; McLaughlin et al. 2015). Only two studies focus on chelonians, including one on herbivorous gopher tortoises (*Gopherus polyphemus*) and one on carnivorous loggerhead sea turtles (*Caretta caretta*) (Yuan et al. 2015, Abdelrhman et al. 2016). The GI microbiome of the green sea turtle, *Chelonia mydas*, has not been investigated, and the unique physiology of this species presents a valuable opportunity to further the understanding of the chelonian microbiome.

Green sea turtles are unique among the sea turtles in that hatchlings and pelagic juveniles are thought to be primarily carnivorous, while coastal juveniles and adults are primarily herbivorous (Boyle and Limpus 2008). As an adaptation to their herbivorous diet, they have proportionally longer GI tracts than carnivorous sea turtle species (Wyneken and Witherington 2001). Their large intestine is approximately 2.5 times the length of the small intestine and the proximal colon is expanded into a functional cecum (Bjorndal 1979, Wyneken and Witherington 2001). Green sea turtles are similar to gopher tortoises in that they are monogastric hindgut fermenters, and rely on microbial flora in their hindgut to digest cellulose and hemicellulose.

This microbial fermentation produces volatile fatty acids (VFAs) such as acetic, butyric, and proprionic acids, which are an important energy source (Bjorndal 1979).

Understanding the GI microbiota and its role in the overall health of green sea turtles is important in providing the best care for animals in rehabilitation and permanent captive situations. This is particularly true in understanding how the diets consumed in captivity impact the GI bacterial composition. In addition, understanding how antibiotic treatment affects the GI microbiota is important. In humans, antibiotics consumed at therapeutic doses have been shown to disturb the gastrointestinal ecosystem, and may lead to overgrowth of pathogenic bacteria (Rafii et al. 2008). Sea turtles presenting to rehabilitation hospitals often require antibiotic treatment, and its effect on the GI microbiota composition has not previously been described.

The objective of this study was to evaluate the genotypic bacterial community composition in feces from green sea turtles in rehabilitation (N=19) at the Georgia Sea Turtle Center (GSTC) on Jekyll Island, GA. The GI microbial diversity and composition of rehabilitated sea turtles was expected to change over time in rehabilitation with changes in diet and with changes in antibiotic treatments.

METHODS

Turtles

Samples from 19 rehabilitating green sea turtles at the GSTC were collected from January 2014 – April 2016. Samples were collected at each of the three timepoints defined above. At each timepoint, turtles were weighed and physical exams were performed. A subjective body condition score (BCS) on a scale of 1 – 5 was recorded. Straight carapace length

(SCL) was also recorded. When both weight and SCL were available, body condition index

(BCI) was calculated according to the following formula from Bjorndal et al. (2000):

$$BCI = [mass\ (kg)/straight\ carapace\ length\ (cm)^3] * 10,000$$

<u>Rehabilitation Diets</u>

Food items fed to green sea turtles at the GSTC include romaine lettuce and leafy lettuce

(*Lactuca sativa*), cucumber (*Cucumis sativus*), green bell pepper (*Capsicum annuum*), mackerel

(*Scomber scombrus*), herring (*Clupea harengus*), shrimp (*Penaeus* spp.), squid (*Loligo*

opalescens), and a custom gelatin-based diet consisting of vegetables, seafood, and vitamins

(recipe in Appendix B). In addition, multivitamin (Mazuri® Vita-Zu® Sea Turtle Vitamin for

Fish-based diets, 500mg, catalog number 1815523-300, Mazuri, Richmond, IN 47374; recipe in

Appendix C) and calcium supplements (Calcium Carbonate 10 gr, 648mg, catalog number

00536-1024-10, Rugby, Livonia, MI 48152) were offered daily. In order to understand the

impact of the typical rehabilitation diet on health, three timepoints during rehabilitation were

defined according to the typical diet consumed. At admission, individuals consumed a mixture of

all items. Animals were gradually transitioned throughout rehabilitation to a predominately

vegetable-based diet. We defined mid-rehabilitation as the point when an individual was

consuming 25% vegetables for two weeks, and recovery as the point when an individual was

consuming 75% vegetables for two weeks.

<u>Antibiotics</u>

Antibiotic treatment history for each turtle at each timepoint was defined as one of three

levels: none, short term, or long term. None indicated that the individual had not received

antibiotics within two weeks prior to fecal collection. Short term was defined as an individual

having received less than three doses of an antibiotic, whereas long term was used in cases where

individuals had received more than three doses. Antibiotic administration was determined by clinical need based on the individual turtle's presentation and diagnosis, and dose frequency depended on the antibiotic prescription. A range of antibiotic types were administered to different turtles including: amikacin, ampicillin, ceftazidime, clavamox, danofloxacin, enrofloxacin, metronidazole, and oxytetracycline.

Sample Collection

Fecal samples (N=57) were collected from 19 turtles over three time periods. All samples were collected from individual turtles within 4 hours of defecation. Turtles were often housed in the same tanks as other turtles, but dividers were in place to prevent feces from moving between defined areas. A sterile swab was placed in the center part of the fecal sample so as to collect feces not exposed to salt water from the tank. The swab was then placed in a cryovial with 0.5mL of RNA*later*® and put into a refrigerator and then a -80°C freezer within 24 hours.

To test for the effects of saltwater exposure on fecal microbiota, a subset of feces (N=2) were collected and swabbed immediately after defecation, and kept in a container of salt water from the tank. Subsequent swabs were then taken after 1 min, 5 min, 15 min, 30 min, and 1 hour of exposure. One sample was additionally swabbed at 2 hours and 4 hours after defecation. Results from this time-series analysis indicated that bacterial community composition did not change significantly over time. Thus samples for the study were always collected within 4 hours of defecation, although attempts were made to collect the samples as soon as possible.

DNA Extraction, PCR, and Library Quantification and Pooling

Samples were randomized prior to extraction using "research randomizer" (www.randomizer.org/). DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen Ltd., West Sussex, England) following the manufacturer's instructions. Each sample was vortexed and

20µL of feces in RNA*later*® was added to each kit sample tube. Six extraction rounds were completed with samples, and each included a reagent control. A single water control was also completed as a control for the Taq polymerase. After extraction, fluorometric quantitation was used to confirm presence of DNA (Qubit™ 3.0 Fluorometer, ThermoFisher Scientific). DNA extracts were stored at -80°C until further analysis.

The remaining steps broadly follow the Illumina 16S protocol (16S Metagenomic Sequencing Library Preparation; URL: http://www.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). First, a 35µL amplicon PCR reaction was completed. Positive (*Salmonella enteritis*) and negative (water) controls were included for all PCR reactions. PCR was performed using the following program: 3 min of initial denaturation at 95°C, followed by 30 cycles of denaturation (30 sec at 95°C), annealing (30 sec at 55°C) and extension (30 sec at 72°C), with a final extension at 72°C for 5 min. The size of the PCR products was verified by gel electrophoresis.

Amplicon PCR products were purified according to the Illumina 16S protocol. Following purification, a 50µL index PCR reaction was completed using the library primers from the Nextera XT DNA Library Preparation Kit (Illumina). All samples, reagent controls, and the water control were indexed. PCR was performed using the following program: 3 min of initial denaturation at 95°C, followed by 8 cycles of denaturation (30 sec at 95°C), annealing (30 sec at 55°C) and extension (30 sec at 72°C), with a final extension at 72°C for 5 min. Index PCR products were purified according to the Illumina 16S protocol. The presence and size of the PCR products was verified by gel electrophoresis, and fluorometric quantitation was used to quantify the concentration of each library (Qubit™).

After all samples were extracted and indexed, a pure isolate of a bacteria (*Mycobacterium chelonae*) was extracted and indexed as a positive control to act as a sequence control for the MiSeq run.

The methodology used for library pooling deviated from the Illumina 16S protocol. Rather than diluting each sample to a constant concentration and then adding 5µL of each, the library concentration was used to calculate the amount of each sample needed to add 20ng/µL of DNA. This amount of each sample and the positive control were pooled. The amount of each extraction control added was equal to the highest amount of any sample added, 2.8µL. This gave a total pooled volume of 107.6µL. Quantitative PCR was used to determine the final concentration of the pooled libraries (19.1nM). The sample was then diluted to 4nM using 10 mM Tris pH 8.5.

Remaining steps for library denaturing and MiSeq sample loading were completed according to the Illumina 16S protocol. Briefly, 5µL of the pooled library was combined with 5µL of 0.2 N NaOH for denaturation. A hybridization buffer (HT1) was used to dilute the denatured library to 20 pM. A PhiX control was prepared in the same manner as the DNA sample. Both the denatured library and PhiX control were then diluted to 4pM. Finally, the 4pM PhiX control (30 µL) and the 4pM denatured library (570 µL) were combined and loaded onto the MiSeq v3 reagent cartridge and put onto the MiSeq.

Data Analysis

Pre-processing of the raw sequence data was performed using the QIIME (Caporaso et al. 2010) pre-processing application within Illumina basespace (https://basespace.illumina.com). Operational Taxonomic Units (OTUs) were assigned based on at least 97% sequence similarity against the Greengenes reference database (DeSantis et al. 2006). For downstream analysis,

sequences assigned as Chloroplast, Mitochondria and Unassigned were removed. Sequences were rarefied to an even depth of 800 sequences per sample to account for unequal sequencing depth across samples. For the two samples that were swabbed multiple times to study the effect of time spent in salt water on bacterial composition, sequences were rarefied to an even depth of 22,600 sequences per sample. Rarefaction curves showing alpha diversity indices (Chao1, Shannon and Observed OTUs) and beta-diversity analysis using principal coordinate analysis (PCoA) plots and unweighted Unifrac (Lozupone et al. 2011) distance metrics were generated within QIIME 1.9.

Analysis of Similarity (ANOSIM) within the PRIMER 6 software package (PRIMER-E Ltd., Luton, UK) was used to analyze significant differences in microbial communities across samples. R-values of the ANOSIM test range from -1 to +1 and are an estimate of the effect size. R values closer to zero indicate that the samples are similar to each other, and values closer to 1 indicate that the samples are dissimilar.

For summary statistics in regards to bacterial taxa and alpha-diversity measures, the data was tested for normality using the Shapiro-Wilk test (JMP Pro 11, SAS software Inc. Cary, NC, USA). Most of the datasets did not meet the assumptions of normality, hence Friedman's test within (Prism v .5.0, GraphPad Software Inc.) was used. Benjamini & Hochberg's False Discovery Rate (FDR) was used to adjust the resulting p-values for multiple comparisons, and an adjusted $p<0.05$ was considered statistically significant.

Additionally, linear discriminant analysis (LDA) effect size (LEfSe) algorithm (Segata et al. 2011) was used to elucidate the bacterial taxa with significant differential relative abundances associated with timepoints. The threshold was set at 3.5 to identify the differentially abundant

taxa. LEfSe was used online in the Galaxy workflow framework

(https://huttenhower.sph.harvard.edu/galaxy/).

RESULTS

<u>Turtles</u>

Table 4.1 shows the physical exam findings for each turtle at each timepoint. The average size for individuals enrolled in this study was a SCL of 29.7 cm (range 23.1 to 37.0 cm). The average number of days that a turtle was in the rehabilitation center prior to feces being collected for the admission sample was 12 days (range 0 to 68 days), for the mid-rehabilitation sample it was 52 days (range 24 to 114 days), and for the recovery sample it was 104 days (range 49 to 231 days). Of the 19 rehabilitating turtles included in this study, 10 were admitted from Florida, seven from Georgia, and two from Massachusetts.

Table 4.1. Physical exam parameters for green sea turtles (*Chelonia mydas*) at three timepoints in rehabilitation (admission, mid-rehabilitation, and recovery) at the Georgia Sea Turtle Center. Parameters include weight (kg), straight carapace length (SCL; cm), body condition index (BCI $= (\text{mass}/\text{SCL}^3) * 10,000 \text{ kg/cm}^3$) and body condition score (BCS) on a scale of $1 - 5$. Values recorded as NA were not recorded or could not be calculated.

GSTC ID	Timepoint	Date Collected	Days in Rehabilitation Before Sampling	SCL	BCI	BCS	Capture Location	Current Antibiotic Treatment
C14001	Admission	01/10/14	4	30.2	1.1	2.00	Florida	short term
C14001	Mid-rehab	04/02/14	86	31.5	1.4	3.00	Florida	long term
C14001	Recovery	05/06/14	120	33.1	1.4	3.50	Florida	long term
C14002	Admission	01/11/14	3	25.3	1.1	2.25	Florida	short term
C14002	Mid-rehab	02/21/14	44	26.7	1.1	2.50	Florida	long term
C14002	Recovery	03/11/14	62	27.2	1.3	3.50	Florida	none
C14003	Admission	01/13/14	4	34.5	1.3	2.50	Florida	short term
C14003	Mid-rehab	02/26/14	48	34.4	1.4	3.50	Florida	none
C14003	Recovery	05/19/14	130	36.2	1.3	3.75	Florida	none
C14004	Admission	01/15/14	5	33.8	1.0	1.75	Georgia	short term
C14004	Mid-rehab	02/21/14	42	34.5	1.1	3.00	Georgia	none
C14004	Recovery	03/09/14	58	35.1	1.2	3.75	Georgia	none
C14008	Admission	02/02/14	6	37.0	1.2	2.00	Georgia	short term
C14008	Mid-rehab	02/23/14	27	37.4	1.3	3.50	Georgia	long term

C14008	Recovery	03/31/14	63	38.1	1.3	3.50	Georgia	long term
C14009	Admission	01/30/14	2	31.4	1.1	2.25	Georgia	short term
C14009	Mid-rehab	02/21/14	24	31.5	1.0	2.50	Georgia	long term
C14009	Recovery	04/01/14	63	32.5	1.2	3.50	Georgia	none
C14010	Admission	01/31/14	1	27.0	1.1	1.50	Florida	none
C14010	Mid-rehab	02/28/14	29	27.0	1.0	2.00	Florida	long term
C14010	Recovery	03/21/14	50	28.3	1.1	2.75	Florida	long term
C14011	Admission	02/03/14	3	32.5	1.2	2.50	Georgia	short term
C14011	Mid-rehab	02/26/14	26	32.7	1.0	2.50	Georgia	long term
C14011	Recovery	03/21/14	49	34.0	1.2	3.00	Georgia	long term
C14012	Admission	02/08/14	1	33.2	1.1	2.25	Georgia	short term
C14012	Mid-rehab	03/04/14	25	33.2	1.1	2.25	Georgia	long term
C14012	Recovery	05/04/14	86	34.8	1.3	3.75	Georgia	none
C14027	Admission	04/03/14	0	NA	NA	1.75	Florida	short term
C14027	Mid-rehab	05/18/14	45	NA	NA	2.50	Florida	long term
C14027	Recovery	08/21/14	140	28.1	1.4	3.50	Florida	none
C14389	Admission	10/06/14	1	26.5	1.2	2.00	Florida	none
C14389	Mid-rehab	11/10/14	36	NA	NA	3.50	Florida	none
C14389	Recovery	05/12/15	219	31.0	1.2	3.75	Florida	none
C14396	Admission	10/08/14	0	25.6	1.2	3.00	Florida	none
C14396	Mid-rehab	12/29/14	82	NA	NA	4.00	Florida	long term
C14396	Recovery	05/27/15	231	29.3	1.4	4.00	Florida	none
C14405	Admission	11/28/14	8	27.2	1.3	3.00	Georgia	none

C14405	Mid-rehab	03/14/15	114	NA	NA	3.50	Georgia	short term
C14405	Recovery	05/22/15	183	30.5	1.3	3.75	Georgia	long term
C15030	Admission	05/28/15	68	30.4	1.1	2.00	Florida	long term
C15030	Mid-rehab	06/15/15	86	NA	NA	2.50	Florida	long term
C15030	Recovery	07/21/15	122	33.4	1.3	3.50	Florida	none
C15051	Admission	05/03/15	20	30.2	1.3	2.50	Florida	long term
C15051	Mid-rehab	05/12/15	29	NA	NA	2.50	Florida	long term
C15051	Recovery	06/04/15	52	30.9	1.2	3.00	Florida	none
C15414	Admission	01/16/16	35	23.1	1.3	3.00	Mass.	long term
C15414	Mid-rehab	02/20/16	70	NA	NA	3.00	Mass.	none
C15414	Recovery	03/10/16	89	27.0	1.4	3.50	Mass.	none
C15418	Admission	12/12/15	0	23.6	1.2	2.50	Mass.	short term
C15418	Mid-rehab	02/08/16	58	NA	NA	NA	Mass.	none
C15418	Recovery	03/10/16	89	25.5	1.3	3.00	Mass.	none
C16004	Admission	02/03/16	53	32.8	1.3	2.25	Georgia	long term
C16004	Mid-rehab	03/01/16	40	NA	NA	2.50	Georgia	long term
C16004	Recovery	04/09/16	79	34.3	1.3	3.50	Georgia	long term
C16005	Admission	01/31/16	6	30.5	1.2	2.25	Florida	short term
C16005	Mid-rehab	04/16/16	82	NA	NA	3.50	Florida	long term
C16005	Recovery	04/28/16	94	35.2	1.2	3.50	Florida	none

Alpha diversity measures, as described by species richness, Chao 1, and Shannon diversity index, were not significantly different across timepoints. However, in a principle coordinate analysis (PCoA) of unweighted Unifrac distances, there was a significant difference in microbial community composition among timepoints (Fig. 4.1; R = 0.225, p < 0.001). Composition at admission was significantly different from mid-rehabilitation (R = 0.309, p < 0.001) and from recovery (R = 0. 397, p < 0.001). Compositions at mid-rehabilitation and recovery were not significantly different.

Figure 4.1. Principle Coordinate Analysis (PCoA) of unweighted Unifrac distances of 16S rRNA genes for turtles in each timepoint. Each shape represents a fecal sample, and timepoints (i.e. admission, mid-rehabilitation, and recovery) are color-coded.

Overall, the dominant bacteria across the three timepoints were of the phyla Firmicutes and Bacteroidetes, followed by Proteobacteria (Appendix E). The composition of the two dominant phyla changed depending on timepoint; samples taken at admission were primarily Firmicutes (55.0%) with less Bacteroidetes (11.1%) and samples taken at recovery were primarily Bacteroidetes (45.3%) with less Firmicutes (32.5%). The primary classes found in admission animals were Clostridia (55%), Bacteroidia (11.1%) and Verrucomicrobiae (1.8%), while those in recovery animals were Bacteroidia (45.2%), Clostridia (29.4%), Gammaproteobacteria (6.4%) and Verrucomicrobiae (5.0%).

Using LEfSe to compare individual bacterial groups based on LDA effect size, several bacterial taxa were identified as significantly different among timepoints (Appendices E and F). Turtles at admission had significantly more of the order Clostridiales, and the families Clostridiaceae, Ruminococcaceae, and Mogibacteriaceae. Turtles at mid-rehabilitation had significantly more of the orders Bacteroidales, Enterobacteriales and Verrucomicrobiales and the families Enterobacteriaceae, Erysipelotrichaceae, and Porphyromonadaceae. At recovery, turtles had significantly more of the families Porphyromonadaceae, and Erysipelotrichaceae.

Antibiotics

Overall, 22 samples were from turtles not on antibiotics within the two weeks prior to sampling, 12 from turtles that had been on antibiotics short term, and 23 from turtles that had been on antibiotics long term. In a principle coordinate analysis (PCoA) of unweighted Unifrac distances, there was a significant difference in microbial community composition among groups (Fig. 4.2; $R = 0.237$, $p < 0.001$). Significant differences were found between all groups, i.e. none and short term ($R = 0.351$, $p < 0.001$), none and long term ($R = 0.104$, $p < 0.01$), and short term and long term ($R = 0.407$, $p < 0.001$).
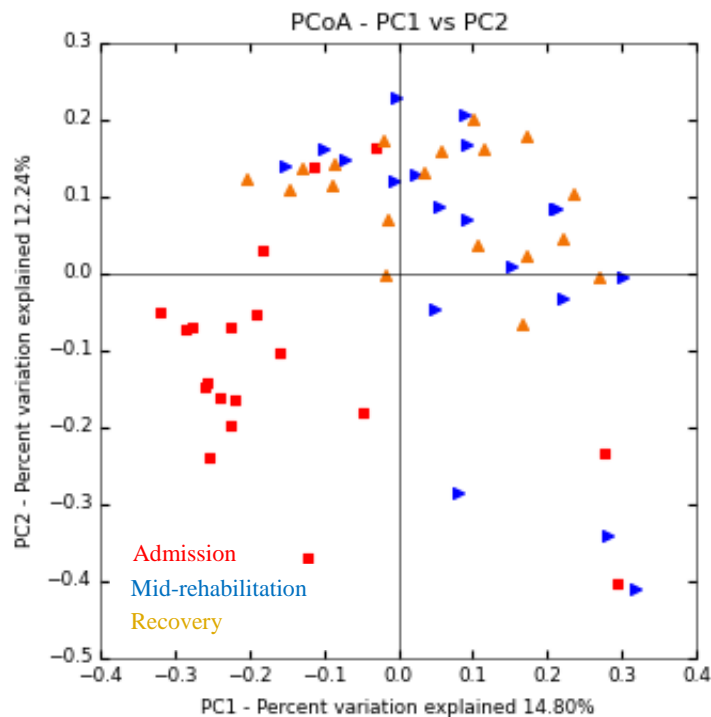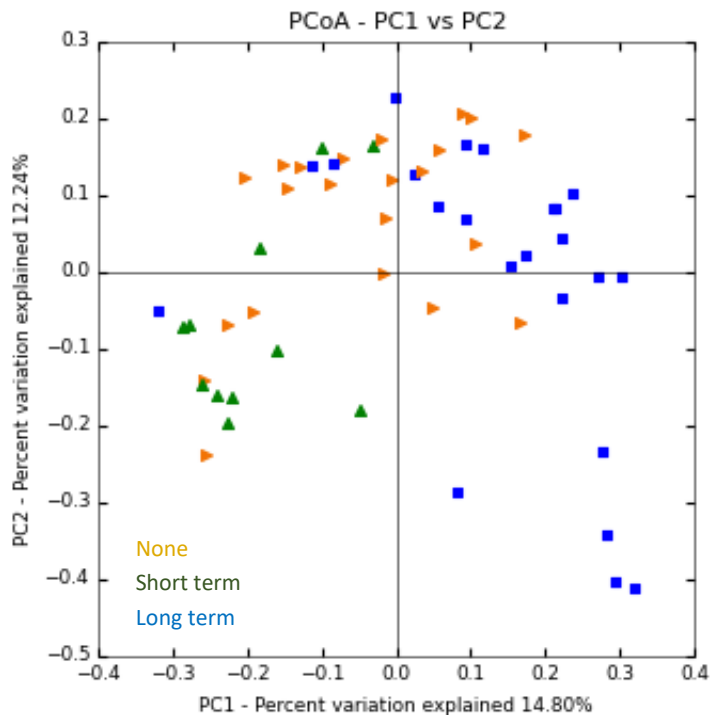
Figure 4.2. Principle Coordinate Analysis (PCoA) of unweighted Unifrac distances of 16S rRNA genes for turtles in different antibiotic groups. Each shape represents a fecal sample, and groups (i.e. none, short term, and long term antibiotic exposure) are color-coded.

DISCUSSION

This was the first study of the GI microbiome in green sea turtles. The dominant bacteria phyla found in our study, Firmicutes and Bacteroidetes, were first described as the core GI microbiota in humans (Rajilić-Stojanović et al. 2009). Costello et al. (2010) found that the GI microflora of fasting Burmese pythons is also dominated by these two bacterial phyla. This core appears to be conserved across many vertebrate taxa regardless of diet (Ley et al. 2008b). In a survey of the GI microbes in mammalian species, Ley et al. (2008a) found that the predominate bacteria were of the phyla Firmicutes (65.7%), Bacteroidetes (16.3%), Proteobacteria (8.8%),

Actinobacteria (4.7%), and Verrucomicrobia (2.2%). In this survey, Ley et al. (2008a) found that phylogeny plays a role in microbial community composition; in particular, conspecifics are more similar to each other than to those of other species. They also found that diet and gut physiology are significant predictors of microbiota composition. Carnivores, herbivores, and omnivores harbor distinct microbial communities. Herbivores harbor the most phyla, omnivores an intermediate number, and carnivores the least. Within herbivores, foregut and hindgut fermenters can be differentiated based on their GI microbiota (Ley et al. 2008a).

Based on the findings of Ley et al. (2008a), green sea turtles should have similar GI microbiome compositions to other sea turtles, but also to other hindgut fermenters. Only one other study has been completed in sea turtles, and that was in the carnivorous loggerhead species (Abdelrhman et al. 2016). This study compared fecal and colorectal sections. Fecal samples were taken from three living animals admitted to a rehabilitation hospital, while colorectal samples were collected from five recently dead individuals. In these animals, fecal bacterial community composition was primarily the phyla Firmicutes (66%), Proteobacteria (23%) and Bacteroidetes (6.2%). The Firmicutes were primarily composed of the class Clostridia (63%). The colorectal sections were composed of the same phyla, but in different proportions: Firmicutes (87%), Proteobacteria (4.2%), and Bacteroidetes (3.4%). In these samples, the Firmicutes were primarily represented by the classes Clostridia (43%) and Bacilli (42.5%). Extrapolation of these results to the current study is limited because of small sample sizes and the carnivorous nature of these turtles; however, the dominant bacterial phyla were conserved across the two studies (i.e. Firmicutes, Bacteroidetes, and Proteobacteria).

The closest comparison in the literature for green sea turtles within gut physiology is a study on terrestrial hindgut fermenting gopher tortoises (Yuan et al. 2015). In this study, fresh

feces was collected from a population of tortoises captured in south-central Florida. The core microbiome in this species was dominated by Firmicutes (36.0%) and Bacteroidetes (36.5%), and Proteobacteria were not well-represented. Other minor phyla included Euryarchaeota, candidate phylum Termite Group 3, Spirochaetes, Tenericutes, and Verrucomicrobia. The Firmicutes were primarily composed of the class Clostridia.

Our findings offer a look into how the microbiota of a hindgut fermenting sea turtle changes with diet. Green turtles in our study had the same dominant phyla as both loggerheads and gopher tortoises, although the representation was different and depended on timepoint in rehabilitation. In a study of mice that were switched from a low-fat, plant-based diet to a high-fat/high-sugar diet, the microbiota composition shifted within a single day (Turnbaugh et al. 2009). In our study, animals sampled at admission had been in rehabilitation an average of 12 days, so their microbiota composition was expected to reflect the mixture of seafood and vegetables fed during this time. The composition in these animals was Firmicutes > Bacteroidetes > Proteobacteria. At mid-rehabilitation and recovery, when turtles were eating rehabilitation diets that were 25% and 75% vegetables, respectively, the composition shifted to Bacteroidetes > Firmicutes > Proteobacteria. Therefore, at admission, the phyla in green sea turtles are more similar to loggerheads, while at recovery, the phyla are more similar to gopher tortoises. This is intuitive in that at admission these animals have been eating seafood (i.e. a similar diet to loggerheads), while at recovery these animals have been eating primarily vegetables (i.e. a diet similar to gopher tortoises).

Microbial community composition at the class level was also significantly different depending on timepoint. Based on LEfSe analysis, turtles at admission had significantly more of the class Clostridia, while turtles at mid-rehabilitation and recovery had significantly more

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Bacteroidia and Erysipelotrichi. This was similar for fecal samples from gopher tortoises, in which the phylum Firmicutes was primarily made up of the class Clostridia with some Erysipelotrichi, and the phylum Bacteroidetes was primarily the class Bacteroidia. Thus, as green sea turtles were transitioned to a primarily herbivorous diet, their microbiota shifted to be more similar to a physiologically similar species.

Antibiotic administration also had an effect on fecal bacterial composition. Unfortunately it is difficult to separate the effect of diet (i.e. timepoint) and antibiotics, however both were independently significant.

The importance of the GI microbiota to health is only recently being investigated and appreciated by clinicians. Little attention has been paid to these communities in non-mammalian species, and it likely often goes unconsidered in clinical treatment plans for the animals. It is important for clinicians to consider the potential impacts that diet and antibiotic treatment can have on the GI microbiota.

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CHAPTER 5

CONSERVATION EDUCATION CENTERS AS BOUNDARY ORGANIZATIONS FOR VETERINARY OUTREACH

INTRODUCTION

There are seven species of sea turtle worldwide. Six species can be found in the United States, and all six are either threatened or endangered (NOAA 2016). Because of this, when sea turtles are found stranded, injured or diseased, they are often rescued and brought into rehabilitation care facilities. Wildlife rehabilitation is defined as "the treatment and temporary care of injured, diseased, and displaced indigenous animals, and the subsequent release of healthy animals to appropriate habitats in the wild" (Miller 2012, page ix). Rehabilitation of individual animals has been criticized for its lack of effect at the population level, its potential to interfere with natural selection, and its potential to increase disease transmission between individuals; however, many argue that humans have a duty to protect and improve the welfare of animals, especially because so many are harmed as a result of human activity (Sleeman 2008).

Rehabilitation centers, especially those with a public education initiative, play a critical role in conservation (Ballantyne et al. 2007, Feck and Hamann 2013). Environmental education (EE) programs offered at similar facilities foster responsible environmental behavior, effect long-term changes of attitudes toward conservation and nature, and provide basic ecological knowledge (Ballantyne and Packer 2005, Bogner 1998). Environmental educators engage the public and can help foster enthusiasm towards conservation (Athman and Monroe 2001). In this

way, effective EE programs empower learners to address environmental issues and instill in learners a sense of conservation stewardship (Athman and Monroe 2001).

Rehabilitation centers with effective EE programs work at the interface of science and the general public. Educators at these facilities are tasked with translating scientific information for the public in a way that is easily digestible and yet fosters an appreciation and an initiative for future conservation action. We propose that rehabilitation centers, and other organizations with EE programs, can act as boundary organizations by serving as mediators between science and the public. Boundary organization theory defines boundary organizations as those that work at the interface of two different social worlds (Guston 1999). Traditionally, boundary organization theory has been applied to the theoretical boundary between politics and science (Guston 2001). In 2001, *Science, Technology, & Human Values* published a special issue on "Boundary Organizations in Environmental Policy and Science." Topics ranged from climate change (Agrawala et al. 2001, Miller 2001) to air quality and public health (Keating 2001) to agriculture (Cash 2001).

While more recent applications of boundary organization theory still include environmental organizations (e.g. Cutts et al. 2011), the scope has broadened to include other entities. For example, Kelly (2003) and Leinhos (2005) applied boundary organization theory to bioethics committees working at the interface of science and the politics of ethics in stem cell research. Additionally, Parker and Crona (2012) extended this theory to include university-based organizations. Although boundary organization theory has been extended to include non-environmental entities, it has rarely been applied to organizations working at boundaries other than that of politics and science. To our knowledge, the only study to do this described the Florida Cooperative Extension Service (FCES) as a boundary organization between science and

the public because of the Extension's role as an intermediary between a research consortium producing climate data and farmers using the data (Breuer et al. 2010). This study used pre-post survey data to measure the evolution of the FCES as an effective boundary organization.

We propose the boundary organization concept can be applied more often and in more diverse situations. More specifically, we propose the concept can be expanded to include conservation and rehabilitation entities that work at the interface of science and the general public. Hence, we present the Georgia Sea Turtle Center (GSTC) as a case study. The GSTC is a sea turtle rehabilitation hospital on Jekyll Island, GA. Each year, approximately 130,000 people visit the Center. Once inside, visitors have the opportunity to walk through a gallery of sea turtle exhibits with information on conservation. Visitors may look through the treatment window and see the veterinary team treating patients and may walk through the Rehabilitation Pavilion to view the holding tanks with all current patients. In addition to these activities, the GSTC offers various educational programs to increase visitor awareness of conservation efforts.

The objective of this study was to determine whether the GSTC may act as a boundary organization for conservation. We designed and implemented a survey instrument to test this theory. The survey was completed by visitors to the GSTC who did and did not participate in a Behind-the-Scenes (BTS) program. All GSTC visitors can view veterinary treatments through a window and explore the gallery of sea turtle exhibits, but visitors who participate in the BTS program are guided on an hour-long tour of the "behind-the-scenes" operations of the hospital, meet and discuss turtle care with the veterinarian, and view treatments up-close. We hypothesized that visitors who completed a BTS tour would have more knowledge of sea turtles, more positive attitudes towards sea turtles and sea turtle rehabilitation, and more conservation ethics in general than people who only visited the gallery. We also hypothesized that BTS

participants would be more likely to donate money or volunteer their time for sea turtle rehabilitation than people who only visited the gallery.

METHODS

Survey Methodology

A survey was developed to measure the effect of conservation programming at the GSTC on visitor attitudes and perceptions toward sea turtle conservation, and to also examine how conservation entities may serve as boundary organizations. A draft survey was loosely created based on existing questions and scales in current literature (i.e. Larson et al. 2011). However, some additional new questions were created to specifically measure visitor's attitudes and perceptions towards sea turtles and their rehabilitation. A pilot study (N=47) of the draft survey was performed in summer 2014 (see Appendix G for pilot survey).

For visitors who did not participate in the BTS program, every third person over the age of 18 encountered in the exhibit gallery was approached. Visitors were approached after they spent time at the various exhibits on sea turtle conservation and viewed treatments through the window. Individuals were asked if they would be willing to participate in a graduate student research project aimed at better understanding the attitudes and perceptions of GSTC visitors towards sea turtle conservation. Participants were offered an incentive of 10% off in the GSTC gift shop for completing the in-person, self-administered survey. All visitors over the age of 18 who completed a BTS program were invited to participate in the study at the conclusion of their program, and the same incentive was offered.

Questions from the pilot survey were adjusted based on reliability and validity analyses. Some items were simply clarified by modifying the language, while others were moved to different sections or removed entirely based on results from Cronbach's alphas and principal

components analysis (PCA). Some questions were also added in order to look further into

visitors' attitude towards rehabilitation of sea turtles. The resulting survey (N=217) was

implemented in summer 2015 (see Appendix H for final survey; Institutional Review Board

approval ID number STUDY00001134). The final survey instrument took approximately ten

minutes to complete and consisted of seven sections (Table 5.1).

Table 5.1. Sections and items for final survey instrument.

Section	Items	Type of Question
Your experience today	6	checked/unchecked box
	4	1 = never, 5 = very often
Your experience with and	4	1 = never, 5 = very often
knowledge of sea turtles		
	7	1 = strongly disagree, 5 = strongly agree
	15	true/false/unsure
Your attitudes and behaviors in	5	1 = strongly disagree, 5 = strongly agree
relation to sea turtles		
	11	1 = strongly disagree, 5 = strongly agree
Environmental ethics and attitudes	10	1 = never, 5 = very often
Sociodemographic characteristics	7	checked/unchecked box or fill-in-the-blank
Willingness to pay	2	yes/no
Optional follow-up	1	fill-in-the-blank

An optional question at the end of the survey invited participants to include an email for an online follow-up (or delayed posttest) survey in four months. This survey took five minutes to complete (using Qualtrics, 2015) and questions were duplicated from the initial survey (see Appendix I for delayed posttest survey). A survey link was emailed to everyone who had provided an email contact. The link remained open for 14 days. One week after the original email, a reminder was sent to complete the survey. Two days before the survey was closed, a final reminder email was sent.

Data Analysis

Likert-type questions within each section were grouped into subsections or constructs. Resultant constructs included "perceived knowledge of sea turtles," "ecosystem importance of sea turtles," "importance of sea turtle rehabilitation," and "general environmental ethics." Reliability and validity analyses were conducted on all Likert-type scales in SPSS (IBM SPSS Statistics 23) using Cronbach's alpha and PCA with direct oblimin rotation. Kaiser-Meyer-Olkin (KMO) Measure of Sampling Adequacy and Bartlett's Test of Sphericity were used to confirm that factor analysis was appropriate. Catell's scree test (Cattell 1966) was used to extract factors; all eigenvalues above the "elbow" were retained as factors. This elbow was at an eigenvalue of approximately one (the highest value not retained was 1.17). Scree test results were confirmed with Horn's parallel analysis (Horn 1965). A mean score was calculated for each scale, and independent samples t-tests were utilized to explore differences in sample populations that did and did not complete a BTS tour. Chi square tests for independence (with Yate's Continuity Correction) were utilized to understand differences in willingness to donate and volunteer time towards sea turtle conservation among groups who did and did not participate in a BTS tour.

RESULTS

Response Rates and Respondents

A total of 217 surveys were completed with a response rate of 88.6%. Of these, 111 participated in a BTS tour, and 106 did not. A total of 86 people provided email addresses for the follow-up or delayed posttest survey. Of these, 38 completed the survey (24 BTS and 14 non-BTS), giving a response rate of 44.2%. Demographics of the participants for both surveys were reflective of the demographic of typical Jekyll Island visitors (Table 5.2).

Table 5.2. Demographics of study participants for the original survey (N=217) and the follow-up (delayed posttest) survey (N=38).

Variable	Original Survey		Follow-up Survey	
	Count	Percent	Count	Percent
Gender				
Female	153	71.2	28	73.7
Male	62	28.8	8	21.1
Age				
18-24	33	15.3	8	21.1
15-34	42	19.5	5	13.1
35-44	62	28.8	11	29.0
45-54	46	21.4	10	26.3
55-64	24	11.2	3	7.9
65-74	8	3.7	1	2.6

Ethnicity

Caucasian	196	91.2	33	86.8
African American	5	2.3	0	0
American Indian	2	0.9	0	0
Asian/Pacific islander	1	0.5	0	0
Hispanic	5	2.3	1	2.6

Education

High school not completed	2	0.9	1	2.6
High school completed or GED	14	6.5	0	0
Some college or technical school	47	21.8	9	23.7
College degree or higher	153	70.8	28	73.7

Income

≤$25K	16	7.5	2	5.3
$25,001 - $50K	16	7.5	4	10.5
$50,001 - $75K	45	21.0	6	15.8
$75,001 - $100K	29	13.6	5	13.2
>$100K	73	34.1	10	26.3

Scales

Cronbach's alpha values indicated that each scale had adequate internal consistency (Table 5.3). Values for KMO and Bartlett's Test of Sphericity indicated that scales were adequate for factor analysis (Table 5.4). Factor analysis confirmed that each of the sections had single factor solutions (Table 5.5).

Table 5.3. Survey constructs and associated Cronbach's alpha values for reliability analysis.

Scale	Items	Cron. α	Mean	Var.	SD
Perceived knowledge of sea turtles	7	0.90	22.94	78.44	8.86
Ecosystem importance of sea turtles	5	0.96	23.88	13.66	3.70
Importance of sea turtle rehabilitation	11	0.91	49.14	40.91	6.40
General environmental ethics	8	0.75	28.31	30.11	5.49

Table 5.4. KMO and Bartlett's test results.

Scale	Items	KMO	X^2	df	sig
Perceived knowledge of sea turtles	7	0.86	989.21	21	<0.001
Ecosystem importance of sea turtles	5	0.85	1510.51	10	<0.001
Importance of sea turtle rehabilitation	11	0.89	1604.19	55	<0.001
General environmental ethics	8	0.72	381.72	28	<0.001

Table 5.5. Eigenvalues and percent variance explained for each construct.

Scale	Items	Factors	Eigenvalues	% of Variance
Perceived knowledge of sea turtles	7	1	4.54	64.8
Ecosystem importance of sea turtles	5	1	4.39	87.9
Importance of sea turtle rehabilitation	11	1	6.27	57.0
General environmental ethics	8	1	2.91	36.4

Descriptive Statistics

The general attitude of all visitors, regardless of whether they participated in the BTS program, was in favor of sea turtle rehabilitation, as implied by the high mean scores for all questions in that section. These overall scores remained high in the four-month follow-up survey, indicating that these positive values were retained over time (Table 5.6).

Table 5.6. Mean scores among GSTC visitors for items (N=11) in the "Importance of Sea Turtle Rehabilitation" scale. Mean scores for the original survey (N=217) and the follow-up survey (N=38) are provided. Items are arranged in the order they appeared in the survey.

Item	Original Survey		Follow-up Survey	
	Mean	SD	Mean	SD
I believe sea turtles should have similar rights to those of humans.	3.38	1.15	3.55	1.16
I believe sea turtles should be protected.	4.75	0.69	4.68	0.74
I want to learn ways to help protect sea turtles.	4.33	0.94	4.29	0.87
I believe stricter laws are needed to protect sea turtles.	4.22	0.94	4.34	0.88
I am interested in learning more about sea turtles.	4.23	0.96	4.37	0.85
I believe rehabilitating sea turtles is important.	4.67	0.69	4.66	0.75
I believe releasing healthy sea turtles back to the ocean is important.	4.82	0.58	4.66	0.75
I believe sea turtles are easily hurt by humans.	4.54	0.77	4.55	0.80
I believe humans should help repair or rehabilitate injured sea turtles.	4.67	0.69	4.50	0.83
I believe it is important for humans to visit places like the Georgia Sea Turtle Center.	4.7	0.66	4.55	0.80
I believe sea turtle rehabilitation centers help people learn more about sea turtles.	4.82	0.53	4.66	0.75

Emphasizing this overall positive attitude towards sea turtle rehabilitation, 94.0% of those surveyed with the original survey instrument agreed or strongly agreed that rehabilitating sea turtles was important, and 92.0% agreed or strongly agreed that humans should help rehabilitate injured sea turtles.

Comparing BTS Participants and Non-BTS Participants

Independent samples t-tests on scale means revealed there was a significant difference for visitors who did or did not participate in the BTS program for two of the collapsed scales (total knowledge and total rehabilitation importance; Table 5.7). Overall, individuals who completed a BTS tour perceived they had more knowledge of sea turtles and were more likely to agree that rehabilitation is important.

Table 5.7. Independent samples t-test results for non-BTS participants compared to BTS participants.

	Non-BTS			BTS					
Scale	**Mean**	**SD**	**n**	**Mean**	**SD**	**n**	**t**	**df**	**Sig.**
Total Knowledge	2.83	0.98	102	3.31	1.09	108	3.306	208	0.001
Total Ecosystem Importance	4.81	0.61	106	4.74	0.85	110	-0.653	214	0.514

Total Rehabilitation Importance	4.31	0.66	106	4.62	0.44	110	4.11	183	<0.001
Total Ethics	3.49	0.70	105	3.58	0.67	110	0.95	213	0.343

Chi-square analysis indicated a significant difference between groups who did and did not participate in a BTS tour and their willingness to donate or volunteer time towards sea turtle conservation. Participants who completed a BTS tour were significantly more likely to be willing to donate (χ^2 (1, n = 212) = 12.76, p = <0.001, *phi* = 0.26) and volunteer their time (χ^2 (1, n = 212) = 10.73, p = 0.001, *phi* = 0.24).

DISCUSSION

Boundary organization theory is primarily limited to organizations playing a role at the boundary of science and politics. To our knowledge, only one other study has proposed that this theory could be extended to include organizations that work at the boundary between science and the public, and this study was restricted to a specific organization that translates climate data for farmers (Breuer et al. 2010).

Rehabilitation centers may also act as boundary organizations by translating the science of sea turtle rehabilitation and conservation for the public. As a case study, we created a survey to measure the effect of conservation programming on visitors' attitudes and perceptions of sea turtle rehabilitation and conservation at the GSTC, a sea turtle rehabilitation hospital on Jekyll Island, GA. Visitors to the GSTC are exposed to exhibits about sea turtle conservation, can view treatments of sea turtles through a window, and can view current rehabilitation turtles in their holding tanks. Visitors that participate in a BTS tour are led on an hour-long behind-the-scenes

tour of the hospital, meet the veterinarian, and view treatments up close. When comparing BTS and non-BTS participants, we found that BTS participants perceived they had more knowledge of sea turtles and were more likely to agree sea turtle rehabilitation is important. Participants who completed a BTS tour were also significantly more likely to be willing to donate and volunteer their time for sea turtle conservation.

Another study looked at the educational role of sea turtle rehabilitation centers in Australia and found that all visitors to these centers were willing to make lifestyle changes to protect sea turtles, and many were willing to donate annually to sea turtle conservation (Feck and Hamann 2013). However, results may be confounded by the fact that all study participants were visitors to a sea turtle hospital, and therefore the sample population may be biased towards individuals who are already interested in sea turtle rehabilitation and conservation. However, we compared individuals who did and did not complete a BTS tour, and results showed that even within a population of people interested in sea turtle rehabilitation, differences in attitudes and perceptions of rehabilitation exist.

A potential limitation of this study is that admission to the center is $7 for adults, and participation in the BTS tour is an additional $15. Therefore comparison of individuals who do and do not participate in a BTS tour may be confounded by income. However, there was no significant difference in income level for these two groups. Future studies could include a control group of members of the general public who do not visit the rehabilitation facility. This approach would help control for income as well as any bias of prior interest in sea turtle rehabilitation and conservation.

Boundary organization theory should be expanded to include conservation education organizations that translate scientific information for the public. The survey created in this study

was confirmed to be valid and reliable and could be used by various conservation entities to measure their ability to act as boundary organizations, including rehabilitation centers, zoos, aquaria, museums, and other similar facilities worldwide. If these facilities can use the theory of a boundary organization as a framework, they may be empowered to create a stronger conservation-education initiative.

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CHAPTER 6

CONCLUSION

In this dissertation, my main objective was to integrate the science of veterinary medicine and sea turtle rehabilitation with environmental education to reinforce the conservation message offered by the Georgia Sea Turtle Center (GSTC) on Jekyll Island, GA. I presented a literature review for this research in Chapter 1. For the veterinary medicine portion, I focused on three aspects of green sea turtle (*Chelonia mydas*; GST) nutrition in order to understand how nutrition in rehabilitating sea turtles affects overall health. These parameters made up three chapters of this dissertation, as follows: blood clinical parameters (Chapter 2), skin and plasma stable isotopes and plasma fatty acid analysis (Chapter 3), and fecal metagenomics (Chapter 4). For the environmental education portion, I focused on using in-person surveys to understand how people perceive sea turtle conservation, and I used boundary organization theory to describe how conservation centers like the GSTC bridge the gap between science and the general public (Chapter 5).

In Chapter 2, I had two major objectives. The first objective was to compare clinical pathology and plasma nutritional parameters for GSTs in rehabilitation at the GSTC over three time periods: admission, mid-rehabilitation, and release. The second objective was to compare clinical pathology and plasma nutritional parameters from each timepoint in rehabilitation to the same parameters in wild GSTs at a single timepoint. This was the first study to combine such a diverse array of plasma health parameters to assess the effect of diet on health and recovery in rehabilitating GSTs. One of the most significant findings in this chapter was that vitamin D was

low in all stages of rehabilitation compared to free-ranging turtles. This was likely because rehabilitating turtles in this study were maintained indoors without full-spectrum lighting. One individual turtle was rotated to an outdoor tank, and the plasma vitamin D levels in that individual increased dramatically. In the future, animals may be rotated to outside tanks more often to ensure adequate sunlight exposure for vitamin D production.

In Chapter 3, I focused on skin and plasma stable isotopes and plasma fatty acid analysis. Two major findings of the stable isotope study were technique-based. There was no significant difference in stable isotope analysis of whole skin and epidermis samples, and no significant difference in fat-extracted and non-extracted samples. These results suggest that epidermis and dermal tissue may not need to be separated before sample preparation, and fat may not need to be extracted. These modifications would make sample preparation easier for future researchers. The major finding of the stable isotope analysis was that rehabilitation and wild skin samples were not significantly different in δ^{13}C or δ^{15}N, however isotopes in rehabilitation and wild plasma samples were significantly different in both δ^{13}C and δ^{15}N. The different findings for skin and plasma could be due to differing turnover time of these tissues. Significant differences found in plasma stable isotopes were likely a result of short term changes in diet, and could be explained by debilitation or could indicate that these animals forage at multiple trophic levels. In the plasma fatty acid analysis, admission and wild turtles were not significantly different, however recovery and wild turtles were. This demonstrates that admission and wild turtles were likely foraging on similar diets, but that animals in rehabilitation consumed a diet not reflective of their wild counterparts.

In Chapter 4, I analyzed gastrointestinal (GI) microbial diversity and composition of rehabilitated sea turtles. This was the first study of the GI microbiome in green sea turtles. There

was one major objective, to evaluate the bacterial community composition in feces from GSTs in rehabilitation over three time periods: admission, mid-rehab, and recovery. Once samples were taken, a confounding factor of antibiotic administration was realized, and analysis was repeated to evaluate the bacterial community composition in response to antibiotics. Both diet and antibiotic use had a significant impact on GI microbial flora in this study. However, the dominant bacteria phyla found, Firmicutes and Bacteroidetes, were the same ones that have been noted as "core" phlya across many animal taxa (Ley et al. 2008).

In Chapter 5, I focused on the environmental education portion of my research. The objective was to develop a survey to measure the knowledge, attitudes, and behaviors of GSTC visitors towards sea turtle conservation. Another concept these surveys measured was whether rehabilitation centers may act as boundary organizations for conservation by translating scientific research in a way the general public can enjoy and get excited about. To explore this idea, I compared two populations of visitors to the GSTC: those who did and did not participate in a behind-the-scenes program. Results from this research indicated rehabilitation centers may indeed act as boundary organizations. Behind-the-scenes participants perceived they had more knowledge of sea turtles and were more likely to agree sea turtle rehabilitation is important. Participants who completed a BTS tour were also significantly more likely to be willing to donate and volunteer their time for sea turtle conservation. To my knowledge, the concept of a boundary organization has never been applied to a rehabilitation center. If these facilities can use the theory of a boundary organization as a framework, they may be empowered to create a stronger conservation-education initiative.

Interdisciplinary research is that which brings together multiple disciplinary perspectives to gain insight into complex problems (Hirsch et al. 2013). In my dissertation, I approached sea

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turtle conservation from multiple perspectives, primarily those of conservation medicine and environmental education. I could, in the natural sciences, have looked strictly at the sea turtle patients that come into the GSTC for rehabilitation, and made recommendations about how to improve the rehabilitation of these animals based on bloodwork, stable isotope, and metagenomic analyses. However, rehabilitation is just one piece of the conservation puzzle. People are another piece. Whether talking about policy, values, public education, or any of the many other lenses one could use to view sea turtle conservation, it is important to realize that rehabilitation and veterinary care is just one angle. Thus, the major goal of my PhD research was to bring together the disciplines of veterinary medicine and social science in order to approach sea turtle conservation from a more holistic perspective. I have always been interested in how the general public understands veterinary medicine, especially conservation medicine, defined as a "transdisciplinary approach to study the relationships among the health states of humans, animals, and ecosystems to ensure the conservation of all" (Deem 2014). Chapter 5 was an opportunity for me to branch out of my disciplinary silo and bring in the social dimension of sea turtle conservation.

The research discussed in Chapter 5 was also an opportunity for me to delve into the social science of sea turtle conservation because of my experience working as part of the GSTC education department for a summer. As an Education Volunteer, I learned how to interpret scientific programs for the public that visits the GSTC. I learned how to lead the BTS tour groups, how to give a program about the "Sea Turtles of the Georgia Coast," and how to discuss treatments going on in the window to the public that was watching. All of these opportunities gave me anecdotal experience with the general public's attitudes and perceptions of sea turtle conservation. It was truly rewarding to discuss a sea turtle patient's case and how the

veterinarian, Dr. Terry Norton, would plan to treat it. The concept of medical treatment for animals is something that most people have only experienced with their dog or cat; but when visiting the turtle hospital, visitors get to see and understand that physical exams, diagnostics, and state-of-the-art medical care are also needed and applied to an endangered species. The kind of understanding gained when someone watches a shell wound caused by a boat strike being treated in a similar way to what would be performed in a human emergency room, or having the veterinarian explain how to assess pain in a turtle, is invaluable. Experiencing these revelations with the GSTC visitors was an experience I would not have had without volunteering with the education department that summer.

Unfortunately, I was unable to complete one of my original objectives for delving into the social science of sea turtle conservation. This was to understand the effect of the GSTC release programs on attendees' perceptions, attitudes, and behaviors towards sea turtles and conservation. Releases are events centered around the release of successfully rehabilitated sea turtles back to the ocean. These events are well attended, often gaining the attention hundreds of onlookers. Some of these onlookers plan in advance to come to the event; they may have been recent visitors to the GSTC or participants in the adopt-a-sea-turtle program. The adoption program allows visitors to symbolically adopt a GSTC patient and receive periodic updates on their care and an invitation to attend the release. For the analysis of program attendees' attitudes and perceptions, I planned to compare the individuals who came to event on purpose to those who happened to be at the beach and see something going on. I did develop a survey to address this objective, and began implementation. The survey instrument was based on the same instrument I used to evaluate the behind-the-scenes program (Chapter 5), and thus was already

validated. Unfortunately, I was unable to attend enough releases during my PhD tenure to achieve enough surveys for analysis. However, this research could be completed at a later date.

While I was not able to complete my objective for evaluating attitudes and perceptions of release program attendees, I was able to add a different style of social science to my research. I created a promotional video about the adopt-a-sea-turtle program that the GSTC can be displayed in their gift shop or museum gallery. To create this video, recorded interviews with GSTC staff members as well as participants in the adopt-a-sea-turtle program. The staff members discussed what the program is and how to become an adoptive parent, while the participants described their experiences as adoptive families. I recorded two young girls, ages 5 and 7, who had adopted multiple turtles and were very involved in summer camp and other activities at the GSTC. I also contacted a kindergarten teacher who adopts sea turtles for her class every year, and a lawyer who adopts sea turtles for her clients. The teacher was able to send photos of her students with their adoption certificate for me to add to the video. The stories of all of these individual's experiences were a valuable addition to the video, as well as a valuable experience for me to listen to. They helped me understand more deeply how people perceive sea turtle rehabilitation and conservation, the objective I set out to complete via pen-and-paper surveys in Chapter 5.

In this dissertation, I strove to integrate the natural and social sciences of sea turtle conservation. As discussed earlier, this research could have been completed by only looking at the veterinary medicine aspect of sea turtle rehabilitation, or only the environmental education aspect. I believe that the issue of endangered species conservation can be better addressed when viewed from multiple perspectives. Places like the GSTC, which open their doors to the public and allow visitors the opportunity to view and experience sea turtle conservation first hand, are

active participants in integrative research. Conservation initiatives could have a more holistic

approach if more conservation entities can act as boundary organizations in this way.

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APPENDIX A

RESULTS FOR ALL STATISTICAL ANALYSES PRESENTED IN CHAPTER 2

	Throughout Rehabilitation				Admission-Wild				Recovery-Wild			
	DF1	**DF2**	**F**	**P**	**DF1**	**DF2**	**F**	**P**	**DF1**	**DF2**	**F**	**P**
PCV	2	41	3.04	0.059	1	46	2.57	0.116	1	45	2.49	0.122
Hematology												
Combined	**2**	**40**	**14.84**	**<0.001**	**6**	**39**	**10.35**	**<0.001**	**6**	**38**	**3.33**	**0.010**
Independent (Bf = 0.008)												
Total WBC	**2**	**41**	**15.50**	**<0.001**	**1**	**64**	**24.46**	**<0.001**	1	63	1.78	0.187
Heterophils	**2**	**41**	**26.14**	**<0.001**	**1**	**64**	**19.73**	**<0.001**	1	63	0.02	0.877
Lymphocytes	2	41	3.85	0.029	1	64	0.61	0.439	1	63	0.20	0.655
Monocytes	**2**	**41**	**6.63**	**0.003**	1	64	0.73	0.397	**1**	**63**	**8.11**	**0.006**
Eosinophils	2	41	3.00	0.061	1	64	1.33	0.253	1	63	0.04	0.846

Basophils	2	41	3.66	0.035	1	64	0.08	0.777	1	63	4.91	0.030

Chemistry

Combined	**2**	**40**	**15.00**	**<0.001**	**6**	**41**	**11.54**	**<0.001**	**6**	**39**	**7.43**	**<0.001**

Independent (Bf = 0.008)

Calcium	**2**	**54**	**15.38**	**<0.001**	**1**	**66**	**48.09**	**<0.001**	**1**	**66**	**11.12**	**0.001**
Phosphorus	2	54	4.13	0.021	1	66	0.25	0.618	**1**	**66**	**8.18**	**0.006**
Ca/P Ratio	2	54	0.92	0.405	**1**	**66**	**16.03**	**<0.001**	1	66	6.83	0.011
Chloride	**2**	**53**	**16.57**	**<0.001**	**1**	**66**	**15.26**	**<0.001**	1	65	3.12	0.082
Potassium	**2**	**53**	**39.66**	**<0.001**	**1**	**66**	**33.46**	**<0.001**	1	65	1.03	0.315
Sodium	2	52	0.07	0.931	1	66	4.76	0.033	1	65	3.41	0.069
Combined	**2**	**42**	**9.78**	**<0.001**	**3**	**44**	**12.03**	**<0.001**	**3**	**43**	**10.73**	**<0.001**

Independent (Bf = 0.017)

BUN	**2**	**43**	**8.86**	**<0.001**	**1**	**52**	**26.28**	**<0.001**	1	66	0.02	0.896
Glucose	**2**	**43**	**18.82**	**<0.001**	1	52	5.72	0.020	**1**	**66**	**10.49**	**0.002**
Uric Acid	**2**	**43**	**43.03**	**<0.001**	**1**	**52**	**25.55**	**<0.001**	**1**	**66**	**9.70**	**0.003**
Combined	**2**	**41**	**6.28**	**0.004**	**2**	**44**	**28.33**	**<0.001**	**2**	**44**	**5.20**	**0.009**

Independent (Bf = 0.025)

	df	df	F	p	df	df	F	p	df	df	F	p
AST	2	54	5.65	0.006	1	52	32.88	<0.001	1	66	9.36	0.003
CK	2	53	17.89	<0.001	1	52	5.45	0.023	1	66	0.14	0.714

Electrophoresis

	df	df	F	p	df	df	F	p	df	df	F	p
Combined	2	42	57.87	<0.001	3	42	26.55	<0.001	3	41	1.43	0.247

Independent (Bf = 0.017)

	df	df	F	p	df	df	F	p	df	df	F	p
Total Protein	2	43	49.51	<0.001	1	52	38.80	<0.001	NA	NA	NA	NA
Pre-albumin	2	43	25.29	<0.001	1	52	35.31	<0.001	NA	NA	NA	NA
Albumin	2	43	69.27	<0.001	1	52	45.51	<0.001	NA	NA	NA	NA
Combined	2	42	57.87	<0.001	3	42	26.55	<0.001	4	40	13.45	<0.001

Independent (Bf = 0.013)

	df	df	F	p	df	df	F	p	df	df	F	p
Alpha 1 Globulins	2	43	34.27	<0.001	1	52	2.51	0.120	1	52	15.96	<0.001
Alpha 2 Globulins	2	43	47.08	<0.001	1	52	25.51	<0.001	1	52	10.68	0.002
Beta Globulins	2	43	27.37	<0.001	1	52	18.24	<0.001	1	52	5.28	0.026
Gamma Globulins	2	43	25.83	<0.001	1	52	28.69	<0.001	1	52	2.01	0.163

Lipids

	df1	df2	F	p	df1	df2	F	p	df1	df2	F	p
Combined	**2**	**42**	**22.58**	**<.0001**	**2**	**42**	**4.12**	**0.023**	**2**	**41**	**14.89**	**<.0001**
Independent (Bf = 0.025)												
Cholesterol	**2**	**43**	**22.26**	**<.0001**	**1**	**49**	**8.24**	**0.006**	**1**	**51**	**6.21**	**0.016**
Triglycerides	**2**	**43**	**28.02**	**<.0001**	1	49	0.07	0.789	**1**	**51**	**31.96**	**<.0001**

Vitamin D Panel

	df1	df2	F	p	df1	df2	F	p	df1	df2	F	p
Combined	2	41	1.56	0.221	**3**	**43**	**38.44**	**<0.001**	**3**	**41**	**32.92**	**<0.001**
Independent (Bf = 0.017)												
25 Hydroxyvitamin D	NA	NA	NA	NA	**1**	**59**	**45.57**	**<0.001**	**1**	**47**	**46.50**	**<0.001**
PTH	NA	NA	NA	NA	**1**	**59**	**35.98**	**<0.001**	**1**	**48**	**25.68**	**<0.001**
iCa	NA	NA	NA	NA	**1**	**59**	**15.91**	**<0.001**	1	47	3.66	0.062

Vitamin A and E Panel

	df1	df2	F	p	df1	df2	F	p	df1	df2	F	p
Combined	**2**	**42**	**24.03**	**<0.001**	**4**	**43**	**5.56**	**0.001**	**4**	**42**	**14.97**	**<0.001**
Independent (Bf = 0.013)												
α-Tocopherol	**2**	**54**	**23.60**	**<0.001**	1	60	0.84	0.363	**1**	**66**	**55.29**	**<0.001**

Aldehyde	2	54	0.01	0.989	1	60	0.00	0.958	1	66	0.21	0.648
Palmitate	2	54	1.72	0.188	1	60	0.02	0.885	1	66	0.69	0.408
Retinol	**2**	**54**	**68.26**	**<0.001**	**1**	**60**	**16.41**	**<0.001**	**1**	**66**	**26.50**	**<0.001**

Trace Minerals

Combined	**2**	**40**	**7.81**	**0.001**	**5**	**40**	**4.93**	**0.001**	**5**	**40**	**17.58**	**<0.001**

Independent (Bf = 0.010)

Copper	**2**	**42**	**7.83**	**0.001**	1	55	0.22	0.644	1	65	0.05	0.828
Iron	2	42	2.74	0.076	**1**	**55**	**11.88**	**0.001**	1	65	3.02	0.087
Magnesium	**2**	**42**	**27.72**	**<0.001**	**1**	**55**	**8.15**	**0.006**	**1**	**65**	**94.51**	**<0.001**
Selenium	2	41	0.80	0.456	1	54	5.91	0.018	1	65	4.06	0.048
Zinc	2	42	2.70	0.079	1	55	4.44	0.040	1	65	0.97	0.329

APPENDIX B

SEA TURTLE GELATIN DIET RECIPE

Ingredients	Amount (grams)
Trout chow sinking pellets	426
Mackerel fillet pieces	106
Herring fillet pieces	107
whole smelt pieces	71
squid (pens removed)	170
Shell on Shrimp	170
Broccoli/Bok Choy (fresh leaves)	142
carrots (chopped)	142
Pet-Cal vitamin supplements	8 tabs finely ground
Sea Tabs	15 tabs finely ground
unflavored gelatin	227
water to soak pellets	250 mL
water for gelatin	500 mL
extra	250 mL
STAY-C	234.12 grams

APPENDIX C

NUTRIENT COMPOSITION OF MAZURI® VITA-ZU® SEA TURTLE VITAMIN FOR

FISH-BASED DIETS, 500MG, CATALOG NUMBER 1815523-300

Ingredients	Amount
Thiamin (mg)	16
Riboflavin (mg)	1.2
Niacin (mg)	1.2
Panthothenic acid (mg)	1.2
Choline (mg)	1.6
Folic acid (μg)	40
Pyridoxine (mg)	1.2
Biotin (μg)	20
Vitamin C (mg)	23
Vitamin B_{12} (μg)	0.18
Vitamin A, IU	310
Vitamin D_3, IU	15
Vitamin E, IU	16
Vitamin K (menadione), (μg)	30
Beta-carotene (μg)	90
Calcium (mg)	1.2
Iron (mg)	4.8
Zinc (mg)	1.6
Manganese (mg)	4.2
Copper (μg)	80
Iodine (μg)	16
Selenium (μg)	0.0

STABLE ISOTOPE COMPOSITION (Δ^{13}C AND Δ^{15}N) OF PLASMA AND SKIN IN

REHABILITATING AND WILD GREEN SEA TURTLES (CHELONIA MYDAS)

INCLUDING POTENTIAL EFFECTS OF MONTH OF CAPTURE, CAPTURE

LOCATION, STRAIGHT CARAPACE LENGTH (SCL), AND BODY CONDITION

INDEX (BCI).

GSTC ID	Timepoint	Plasma		Skin		Month	Capture Location	SCL	BCI
		δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N				
C14001	Rehab	-16.96	10.89	-14.73	10.08	January	Florida	30.2	1.09
C14002	Rehab	NA	NA	-15.57	9.16	January	Florida	25.3	1.14
C14003	Rehab	-18.41	10.12	NA	NA	January	Florida	34.5	1.27
C14004	Rehab	-15.63	9.98	-14.91	10.01	January	Georgia	33.8	1.01
C14008	Rehab	NA	NA	-15.62	11.28	January	Georgia	37	1.23
C14009	Rehab	-18.35	9.36	NA	NA	January	Georgia	31.4	1.07
C14010	Rehab	-16.77	9.21	-15.57	7.53	January	Florida	27	1.07
C14011	Rehab	NA	NA	-16.9	10.18	January	Georgia	32.5	1.15
C14012	Rehab	-19.33	10.6	-17.18	10.78	February	Georgia	33.2	1.13
C14023	Rehab	-18.36	10.78	-15.96	9.35	March	Florida	33.6	0.98
C14027	Rehab	-20.28	10.28	-16.15	8.89	April	Florida	NA	NA
C14059	Rehab	-19.43	11.84	-16.23	11.47	May	Georgia	40.7	1.17
C14063	Rehab	-19.34	11.23	-16.42	11.63	May	Georgia	45.4	1.17
C14389	Rehab	-18.06	9.6	-15.94	8.18	October	Florida	26.5	1.21
C14396	Rehab	-17.73	8.88	-14.88	6.99	October	Florida	25.6	1.19
C14405	Rehab	-18.14	9.47	-15.5	9.65	November	Georgia	27.2	1.27
C15004	Rehab	-17.2	9.06	-16.74	10.2	January	Florida	24.7	1.23
C15005	Rehab	-18.68	9.48	-15.96	9.39	January	Florida	NA	NA
C15009	Rehab	-18.68	9.97	-15.15	6.11	February	Florida	28.4	1.2
C15010	Rehab	-16.99	10.04	-16.04	8.92	February	Georgia	28.5	1.4
C15026	Rehab	-19.3	8.71	-16.24	8.57	March	Florida	27.3	1.25
C15029	Rehab	-19.61	10.08	-16.28	9.58	March	Florida	41.3	1.24
C15030	Rehab	-19.54	10.07	-15.35	8.8	March	Florida	30.4	1.12
C15044	Rehab	-19.87	11.21	-17.68	10.35	April	Georgia	39.5	1.09
C15051	Rehab	NA	NA	-17.92	9.27	April	Florida	30.2	1.27
C15094	Rehab	-19.21	11.31	NA	NA	May	Georgia	63	1.5

C15100	Rehab	-18.54	10.35	-16.21	9.59	May	Florida	31.8	1.09
C15363	Rehab	-19.22	10.97	-16.6	10.28	August	Florida	26.4	1.06
C15379	Rehab	-18.05	8.63	-15.84	8.29	September	Florida	25.1	1.14
C15383	Rehab	NA	NA	-14.49	7.54	September	Florida	31	1.26
C15412	Rehab	-20.85	12.6	-16.36	10.48	December	Mass	29.7	1.24
C15413	Rehab	-20.31	11.58	-15.66	10.44	December	Mass	27.2	1.14
C15414	Rehab	-20.2	12.35	-16.61	10.22	December	Mass	23.1	1.3
C15418	Rehab	NA	NA	-16.06	8.17	December	Mass	23.6	1.22
C15423	Rehab	-17.77	10.85	-15.68	10.01	December	Florida	29.8	1.23
C16004	Rehab	-17.01	9.52	-15.98	9.6	January	Georgia	32.8	1.18
C16005	Rehab	-17.84	8.63	-16.14	9.8	January	Florida	30.5	1.23
C16009	Rehab	-18.92	10.39	NA	NA	February	Florida	28	1.32
C16010	Rehab	-19.28	9.29	-16.08	8.73	February	Florida	26.1	1.15
C16011	Rehab	-19.96	10.37	-15.78	9.44	February	Florida	26.7	1.26
L32757	Wild	-17.13	9.23	NA	NA	July	Florida	48.6	1.22
L4AOB	Wild	NA	NA	-15.43	10.57	July	Florida	48.6	1.22
L473052	Wild	-18.17	9.22	-16.03	9.84	January	Florida	46.6	1.34
L843473	Wild	-18.12	9.55	-16.21	8.73	October	Florida	25.5	1.21
L938198	Wild	-18.85	8.85	-15.3	7.76	December	Florida	27.3	1.23
L938249	Wild	-18.28	8.71	-15.59	8.36	November	Florida	27.7	1.22
LBBR110	Wild	-16.31	9.84	-15.16	10.44	February	Florida	52.4	1.15
LLA141	Wild	-18.1	9.27	-15.48	10.04	November	Florida	50.1	1.31
LLA378	Wild	-18.62	9.01	-16.41	10.02	July	Florida	48.7	1.37
LLH610	Wild	-20.15	9.86	-16.03	9.14	March	Florida	31.7	1.29
LLH617	Wild	-19.72	12.15	-16.77	10.47	March	Florida	31	1.31
LLH634	Wild	-19.09	9.1	-15.56	9.85	April	Florida	43	1.25
LLH794	Wild	-18.7	8.99	-15.78	9.59	July	Florida	28.8	1.3
LLH931	Wild	-17.67	9.16	-16.26	10.29	February	Florida	51.4	1.24
LLP203	Wild	-19.42	9.35	-15.99	8.75	September	Florida	29.6	1.12
LLP210	Wild	-17.55	9.22	-16.02	10.13	October	Florida	48.7	1.21
LLP214	Wild	-17.99	8.62	-15.5	9.68	October	Florida	46.2	1.23
LLP218	Wild	-17.69	8.08	-14.97	9.42	October	Florida	31.1	1.23
LLP229	Wild	-18.76	8.57	-15.89	8.27	October	Florida	29.7	1.11
LLP238	Wild	-19.37	8.75	-16.09	8.18	October	Florida	30.5	1.27
LLP248	Wild	-17.29	8.72	-15.54	9.06	October	Florida	46.7	1.36
LLP262	Wild	-8.37	3.96	-6.34	5.78	October	Florida	60	1.23
LLP272	Wild	-17.91	9.23	NA	NA	November	Florida	44.1	1.19
LLP303	Wild	-18.06	9.35	-15.41	9.59	December	Florida	40	1.3
LLP328	Wild	-7.96	4.21	NA	NA	January	Florida	43.2	1.24
LLP330	Wild	-17.61	9.62	-15.77	9.95	December	Florida	53.6	1.29
LLP350	Wild	-17.5	10.25	-16.19	10.45	December	Florida	48.2	1.2
LLP357	Wild	-19.13	10.12	NA	NA	January	Florida	55.6	1.27
LLP383	Wild	-17.74	9.45	-16.28	9.96	January	Florida	56.2	1.27
LLP390	Wild	-17.65	9.58	-15.77	9.31	January	Florida	32.9	1.18
LLP418	Wild	-17.67	10.57	-15.82	9.6	January	Florida	30.8	1.2

LLP423	Wild	-18.61	9.34	-16.19	9.47	January	Florida	40.9	1.23
LLP427	Wild	-17.27	9.11	-13.85	7.91	January	Florida	57.4	1.2
LLP429	Wild	-17.53	9.71	NA	NA	January	Florida	32.4	1.32
LLS231	Wild	-17.69	10.03	-15.8	10.25	February	Florida	47.4	1.18
LLS239	Wild	NA	NA	-15.78	10.07	February	Florida	34.1	1.24
LLS252	Wild	-18.22	12	-15.41	9.16	February	Florida	28.8	1.26
LLS259	Wild	-15.07	7.89	-11.85	6.87	February	Florida	44.3	1.43
LLS278	Wild	-15.43	5.21	-13.99	7.14	February	Florida	42.9	1.3
LLS282	Wild	-21.91	8.52	-19.22	9.14	February	Florida	41	1.13
LLS304	Wild	-15.98	6.22	-13.81	7.29	February	Florida	34.8	1.07

APPENDIX E

DIFFERENCES IN GASTROINTESTIONAL MICROBIAL COMPOSITION OF GREEN SEA TURTLES AT THREE TIMEPOINTS IN REHABILITATION

Taxa	Admission			Mid-rehabilitation			Recovery			p-value	Q Value
	Med	Min	Max	Med	Min	Max	Med	Min	Max		
Phylum											
p__Actinobacteria	0.125	0.000	2.125	0.250	0.000	1.875	0.250	0.000	2.000	0.104	0.174
p__Bacteroidetes	11.125[a]	0.000	48.625	37.625[b]	0.125	74.125	45.25[a,b]	12.375	66.250	0.016	0.039
p__Firmicutes	55[a]	0.125	94.875	25.625[b]	0.000	68.000	32.5[b]	8.375	63.250	0.008	0.038
p__Proteobacteria	4.750	0.000	99.500	7.500	0.250	99.875	6.500	0.125	73.750	0.505	0.505
p__Verrucomicrobia	0.625	0.000	56.750	9.500	0.000	45.375	6.375	0.000	34.750	0.200	0.250
Class											
c__Coriobacteriia	0.000	0.000	2.125	0.250	0.000	1.875	0.250	0.000	0.000	0.109	0.145
c__Bacteroidia	11.125[a]	0.000	48.625	37.625[a,b]	0.125	74.125	45.25[b]	12.375	2.000	0.016	0.031
c__Bacilli	0.000	0.000	0.250	0.125	0.000	10.250	0.000	0.000	0.000	0.052	0.083
c__Clostridia	55[a]	0.125	94.875	23.625[b]	0.000	62.750	29.375[a,b]	7.125	0.000	0.002	0.006
c__Erysipelotrichi	0[a]	0.000	0.625	0.5[b]	0.000	3.750	0.75[b]	0.000	1.500	<0.001	0.002
c__Deltaproteobacteria	0.000	0.000	13.375	0.375	0.000	1.625	0.500	0.000	0.125	0.774	0.774
c__Verrucomicrobiae	1.750	0.000	98.625	6.625	0.000	99.625	5.000	0.000	5.375	0.200	0.348
c__Gammaproteobacteria	0.625	0.000	56.750	9.500	0.000	45.375	6.375	0.000	0.000	0.232	0.266
Order											
o__Coriobacteriales	0.000	0.000	2.125	0.250	0.000	1.875	0.250	0.000	2.000	0.109	0.081
o__Bacteroidales	11.125[a]	0.000	48.625	37.625[b]	0.125	74.125	45.25[a,b]	12.375	66.250	0.016	0.016
o__Lactobacillales	0.000	0.000	0.250	0.125	0.000	10.250	0.000	0.000	1.375	0.008	0.01464*
o__Clostridiales	55[a]	0.125	94.875	23.625[b]	0.000	62.750	29.375[a,b]	7.125	62.875	0.002	0.005
o__Erysipelotrichales	0[a]	0.000	0.625	0.5[b]	0.000	3.750	0.75[a,b]	0.000	3.500	<0.001	0.001
o__Desulfovibrionales	0.000	0.000	13.375	0.375	0.000	1.625	0.500	0.000	5.375	0.774	0.516
o__Enterobacteriales	0[a]	0.000	91.375	6.5[b]	0.000	99.250	2.125[a,b]	0.000	73.500	0.032	0.028
o__Vibrionales	0.375[a]	0.000	2.750	0.125[a,b]	0.000	4.500	0[b]	0.000	16.750	0.012	0.015
o__Verrucomicrobiales	0.625[a]	0.000	56.750	9.5[a,b]	0.000	45.375	6.375[a]	0.000	34.750	0.012	0.015

Family

f__Coriobacteriaceae	0.000	0.000	2.125	0.250	0.000	1.875	0.250	0.000	2.000	0.109	0.145
f__Bacteroidaceae	10.625	0.000	48.000	29.500	0.125	72.000	31.000	6.500	64.250	0.331	0.378
f__Porphyromonadaceae	0.125[a]	0.000	20.625	7.125[b]	0.000	22.000	8.625[b]	0.000	27.000	<0.001	<0.001
f__Rikenellaceae	0.125	0.000	2.125	0.375	0.000	3.750	0.250	0.000	2.875	0.424	0.453
o__Clostridiales;f__	6.625	0.000	41.125	2.750	0.000	13.750	3.250	0.375	11.000	0.065	0.115
f__Clostridiaceae	5.125[a]	0.000	40.125	0.375[b]	0.000	59.875	0.5[a,b]	0.000	7.750	0.007	0.018
f__Eubacteriaceae	0.000	0.000	3.500	0.000	0.000	2.000	0.125	0.000	0.500	0.942	0.942
f__Lachnospiraceae	19.750	0.125	54.250	6.875	0.000	37.250	13.375	2.625	48.375	0.080	0.116
f__Peptostreptococcaceae	1.250	0.000	10.000	0.125	0.000	1.375	0.125	0.000	1.250	0.080	0.116
f__Ruminococcaceae	8.125[a]	0.000	37.250	3.125[b]	0.000	10.250	5.25[a,b]	0.500	14.750	0.022	0.044
f__[Mogibacteriaceae]	1.125[a]	0.000	15.250	0[b]	0.000	0.250	0[b]	0.000	0.375	<0.001	<0.001
f__Erysipelotrichaceae	0[a]	0.000	0.625	0.5[b]	0.000	3.750	0.75[a,b]	0.000	3.500	<0.001	<0.001
f__Desulfovibrionaceae	0.000	0.000	13.375	0.375	0.000	1.625	0.500	0.000	5.375	0.7736	0.82517
f__Enterobacteriaceae	0[a]	0.000	91.375	6.5[b]	0.000	99.250	2.125[a,b]	0.000	73.500	<0.001	<0.001
f__Vibrionaceae	0.125	0.000	2.750	0.000	0.000	2.875	0.000	0.000	16.625	0.012	0.026971*
f__Verrucomicrobiaceae	0.625	0.000	56.750	9.500	0.000	45.375	6.375	0.000	34.750	0.200	0.246

Genus

g__Eggerthella	0.000	0.000	2.125	0.125	0.000	1.875	0.250	0.000	2.000	0.078	0.153
g__Bacteroides	10.625	0.000	47.875	29.500	0.125	72.000	31.000	6.500	64.250	0.078	0.153
g__Parabacteroides	0.125[a]	0.000	20.625	7.125[b]	0.000	22.000	8.625[b]	0.000	27.000	<0.001	<0.001
f__Rikenellaceae;g__	0.000	0.000	2.125	0.250	0.000	3.750	0.250	0.000	2.625	0.259	0.341
o__Clostridiales;f__;g__	6.625	0.000	41.125	2.750	0.000	13.750	3.250	0.375	11.000	0.065	0.151
f__Clostridiaceae;g__	0.875	0.000	6.125	0.125	0.000	6.750	0.125	0.000	0.750	0.065	0.151
g__Clostridium	3.625[a]	0.000	32.500	0.125[b]	0.000	51.500	0.25[b]	0.000	7.000	0.002	0.011
g__SMB53	0.375[a]	0.000	26.875	0[b]	0.000	1.000	0[a,b]	0.000	1.250	0.000	0.003
g__Pseudoramibacter_Eubacterium	0.000	0.000	3.500	0.000	0.000	2.000	0.125	0.000	0.500	0.938	1.000
f__Lachnospiraceae;g__	9.875	0.000	19.875	4.875	0.000	36.000	11.000	2.125	45.000	0.268	0.341
g__Blautia	0[a]	0.000	0.875	0.125[b]	0.000	2.125	0.125[b]	0.000	1.375	0.006	0.032
g__Coprococcus	2.5[a]	0.000	51.750	0[b]	0.000	0.625	0.125[b]	0.000	1.500	0.001	0.006
g__Dorea	0.125	0.000	6.125	0.375	0.000	3.250	0.375	0.000	2.000	0.301	0.367
g__Epulopiscium	0.125	0.000	7.250	0.000	0.000	7.125	0.000	0.000	1.375	0.093	0.153
g__Robinsoniella	0.125	0.000	1.500	0.125	0.000	1.500	0.125	0.000	1.250	0.093	0.153
g__Roseburia	0.000	0.000	2.125	0.000	0.000	1.375	0.125	0.000	2.875	0.123	0.182
f__Peptostreptococcaceae;g__	0.250	0.000	4.500	0.000	0.000	1.000	0.125	0.000	0.750	0.123	0.182
g__Clostridium	0.375	0.000	8.250	0.000	0.000	0.375	0.000	0.000	1.250	0.028	0.078
f__Ruminococcaceae;g__	6.000	0.000	31.750	2.000	0.000	9.625	4.625	0.375	14.000	0.028	0.078
g__Oscillospira	0.500	0.000	1.875	0.500	0.000	2.000	0.500	0.000	1.625	0.550	0.616
g__Ruminococcus	0.375	0.000	12.500	0.000	0.000	4.250	0.000	0.000	0.875	0.550	0.616
f__[Mogibacteriaceae];g__	1[a]	0.000	15.250	0[b]	0.000	0.250	0[b]	0.000	0.375	<0.001	<0.001

f__Erysipelotrichaceae;g__	0[a]	0.000	0.375	0.125[a,b]	0.000	1.750	0.375[b]	0.000	3.250	<0.001	<0.001
g__Bilophila	0.000	0.000	13.375	0.375	0.000	1.625	0.500	0.000	5.375	0.086	0.153
f__Enterobacteriaceae;g__	0[a]	0.000	46.750	1.75[b]	0.000	56.125	2.125[a,b]	0.000	59.125	0.018	0.061
g__Citrobacter	0.000	0.000	1.375	0.125	0.000	3.625	0.000	0.000	1.500	0.012	0.048*
g__Vibrio	0.125	0.000	2.000	0.000	0.000	2.875	0.000	0.000	16.625	0.079	0.248
g__Akkermansia	0.625	0.000	56.750	9.500	0.000	45.375	6.375	0.000	34.750	0.200	0.280

*medians not sharing a common superscript are significantly different

*no significance after Dunn's post test

APPENDIX F

SIGNIFICANT DIFFERENCES IN BACTERIAL TAXA ON VARIOUS PHYLOGENETIC

LEVELS BASED ON LEFSE ANALYSIS IN GREEN SEA TURTLES AT THREE

TIMEPOINTS IN REHABILITATION
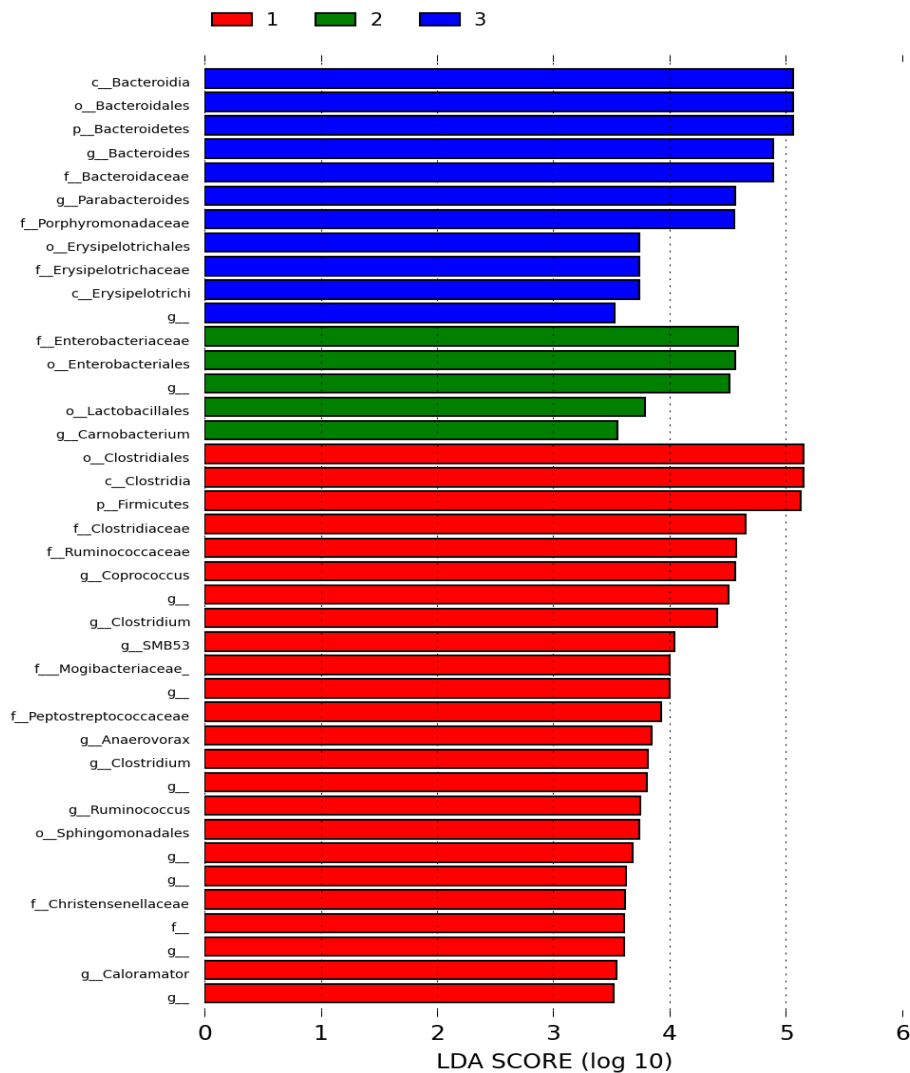
APPENDIX G

PILOT SURVEY INSTRUMENT FOR MEASURING ATTITUDES AND PERCEPTIONS

OF GSTC VISITORS

Section A: Your Experience Today

A1. What is your residence on Jekyll Island? *(Please check ONE box.)*

☐ Non-resident ☐ Seasonal resident ☐ Year-round resident

A2. Which of the following best describes your visitation to the Georgia Sea Turtle Center (GSTC)? *(Please check ONE box.)*

☐ Never visited ☐ Visitor (1 time) ☐ Visitor (2+ times) ☐ Annual Member

A3.
Did you speak with an educator during your visit today? *(Please check ONE box.)*

☐ Yes ☐ No ☐ No, but I have before

A4. Did you go into the operating room today? *(Please check ONE box.)*

☐ Yes ☐ No ☐ No, but I have before

A5. Did you see a live sea turtle at the GSTC today? *(Please check ONE box.)*

☐ Yes ☐ No ☐ No, but I have before

A6. Did you see a treatment today? *(Please check ONE box.)*

☐ Yes ☐ No ☐ No, but I have before

A7. Indicate how often you have done the following. **(Check ONE box per ROW.)**

	Never	**Seldom**	**Sometimes**	**Often**	**Very Often**
Attended a sea turtle release.	☐	☐	☐	☐	☐
Attended an educational program about sea turtles.	☐	☐	☐	☐	☐
Used your phone to look up information about sea turtles.	☐	☐	☐	☐	☐
Used your computer to look up information about sea turtles.	☐	☐	☐	☐	☐

Section B: Your Experience with and Knowledge of Sea Turtles

B1. Indicate how often you have done the following. **(C*heck ONE box per ROW.*)**

	Never	Seldom	Sometimes	Often	Very Often
Seen a sea turtle in captivity (before today).	☐	☐	☐	☐	☐
Seen a sea turtle in a rehabilitation center (before today).	☐	☐	☐	☐	☐
Seen a wild sea turtle on a beach.	☐	☐	☐	☐	☐
Seen a wild sea turtle in the ocean.	☐	☐	☐	☐	☐

B2. Please indicate the extent to which you strongly disagree to strongly agree with the following statements. **(*Circle ONE number per ROW.*)**

	Strongly Disagree		Neither Disagree or Agree		Strongly Agree
	1	2	3	4	5
I can recognize different types of sea turtles.	1	2	3	4	5
I know the names of some types of sea turtles.	1	2	3	4	5
I can identify a sea turtle as opposed to a terrapin.	1	2	3	4	5
I can identify a sea turtle as opposed to a land turtle.	1	2	3	4	5
I can identify a sea turtle as opposed to a tortoise.	1	2	3	4	5
I know about sea turtle behaviors.	1	2	3	4	5
I know a lot about sea turtles in general.	1	2	3	4	5
I find sea turtles interesting.	1	2	3	4	5
I am interested in learning more about sea turtles.	1	2	3	4	5

B3. Please indicate whether you believe the following statements are true or false, or if you are unsure. **(C*heck ONE box per ROW.*)**

	True	False	Unsure
Sea turtles have lungs.	☐	☐	☐
Sea turtles have teeth.	☐	☐	☐
Sea turtles are cold-blooded.	☐	☐	☐

Sea turtles crawl out of their shells when they need a bigger one.	☐	☐	☐
Sea turtles can retreat into their shells.	☐	☐	☐
Sea turtles are roughly the same size as land-dwelling turtles.	☐	☐	☐
Sea turtles are reptiles.	☐	☐	☐
Sea turtles have beaks.	☐	☐	☐
Sea turtles have gills.	☐	☐	☐

B4. Please indicate whether you believe the following statements are true or false, or if you are unsure. (**C*heck ONE box per ROW.*)

	True	False	Unsure
The GSTC uses a multivitamin supplement in sea turtle diets.	☐	☐	☐
The GSTC uses a gel diet with ground-up seafood, broccoli, carrots, and vitamins.	☐	☐	☐
The GSTC does not take blood from any of its patients.	☐	☐	☐
	True	False	Unsure
The GSTC uses honey to treat some types of wounds.	☐	☐	☐
The GSTC does not use anesthesia.	☐	☐	☐
The GSTC does not have a machine to take x-rays.	☐	☐	☐

Section C: Your Attitudes and Behaviors in Relation to Sea Turtles

C1. Please indicate the extent to which you strongly disagree to strongly agree with the following statements. (***Circle ONE number per ROW.***)

	Strongly Disagree		Neither Disagree or Agree		Strongly Agree
	1	2	3	4	5
Sea turtles are an important part of nature.	1	2	3	4	5
Sea turtles are important in preserving ecosystems.	1	2	3	4	5
Sea turtles should be protected on Jekyll Island.	1	2	3	4	5
Sea turtles are an important part of the scenic beauty of Jekyll Island.	1	2	3	4	5
Sea turtles are an important part of nature on Jekyll Island.	1	2	3	4	5

C2. Please indicate the extent to which you strongly disagree to strongly agree with the following statements. **(*Circle ONE number per ROW.*)**

	Strongly Disagree		Neither Disagree or Agree		Strongly Agree
	1	**2**	**3**	**4**	**5**
I believe human needs come before the needs of sea turtles.	1	2	3	4	5
I believe sea turtles should have similar rights to those of humans.	1	2	3	4	5
I feel a strong connection to sea turtles.	1	2	3	4	5
I believe sea turtles should be protected.	1	2	3	4	5
I want to learn ways to help protect sea turtles.	1	2	3	4	5
I believe stricter laws are needed to protect sea turtles.	1	2	3	4	5
I enjoy seeing sea turtles in the wild.	1	2	3	4	5
I believe rehabilitating sea turtles is important.	1	2	3	4	5
I believe releasing sea turtles back to the wild is important.	1	2	3	4	5

C3. Would you be willing to donate money to help protect sea turtles (we are only interested in your opinions, not in receiving donations at this time)? **(*Please check ONE box.*)**

☐ Yes. If yes, how much?   ☐ No

_____

C4. Would you be willing to volunteer your time to help protect sea turtles (again, we are only interested in your opinions, not in having you sign up as a volunteer at this time)? **(*Please check ONE box.*)**

☐ Yes. If yes, how many hours/week?   ☐ No

_____

Section D: Environmental Ethics and Attitudes

D1. Indicate how often you do the following. **(*Check ONE box per ROW.*)**

	Never	**Seldom**	**Sometimes**	**Often**	**Very Often**
Give some of my own money to help save wild plants or animals.	☐	☐	☐	☐	☐
Cook things I can grow or find outside in nature.	☐	☐	☐	☐	☐

Compost my food waste.	☐	☐	☐	☐	☐
Turn off the sink when washing or rinsing dishes to save water.	☐	☐	☐	☐	☐
Turn off the lights when I leave a room to save energy.	☐	☐	☐	☐	☐
Help to clean up parks and forests in my neighborhood.	☐	☐	☐	☐	☐
Tell my friends or my family about things they can do to help protect nature.	☐	☐	☐	☐	☐
Carpool.	☐	☐	☐	☐	☐
Use re-usable grocery bags.	☐	☐	☐	☐	☐
Recycle paper, plastic, or glass.	☐	☐	☐	☐	☐

Section E: Demographics

These questions will help us to ensure that the people we are surveying are representative of all GSTC visitors. **All answers will be kept strictly confidential.**

E1. What is your gender? ☐ Female   ☐ Male   ☐ Other/do not wish to respond

E2. What is your age? _____ years old

E3. Which of the following categories best describes your race/ethnicity? (*Check ALL that apply.*)

☐ African American   ☐ Caucasian

☐ American Indian   ☐ Hispanic, Latino, or Spanish

☐ Asian or Pacific Islander   ☐ Other/do not wish to respond

E4. What is the highest level of education you have completed? (*Please check ONE box.*)

☐ High school not completed   ☐ High school completed or GED   ☐ Some college or technical school   ☐ College degree or higher

E5. Please indicate your total household income range before taxes last year. (*Please check ONE box.*)

☐ $25,000 or less   ☐ $50,001 to $75,000   ☐ $100,001 or more

☐ $25,001 to $50,000   ☐ $75,001 to $100,000   ☐ Do not wish to respond

E6. Please provide the state and zip code of your primary residence:_____

Please use the space provided below for any additional comments.
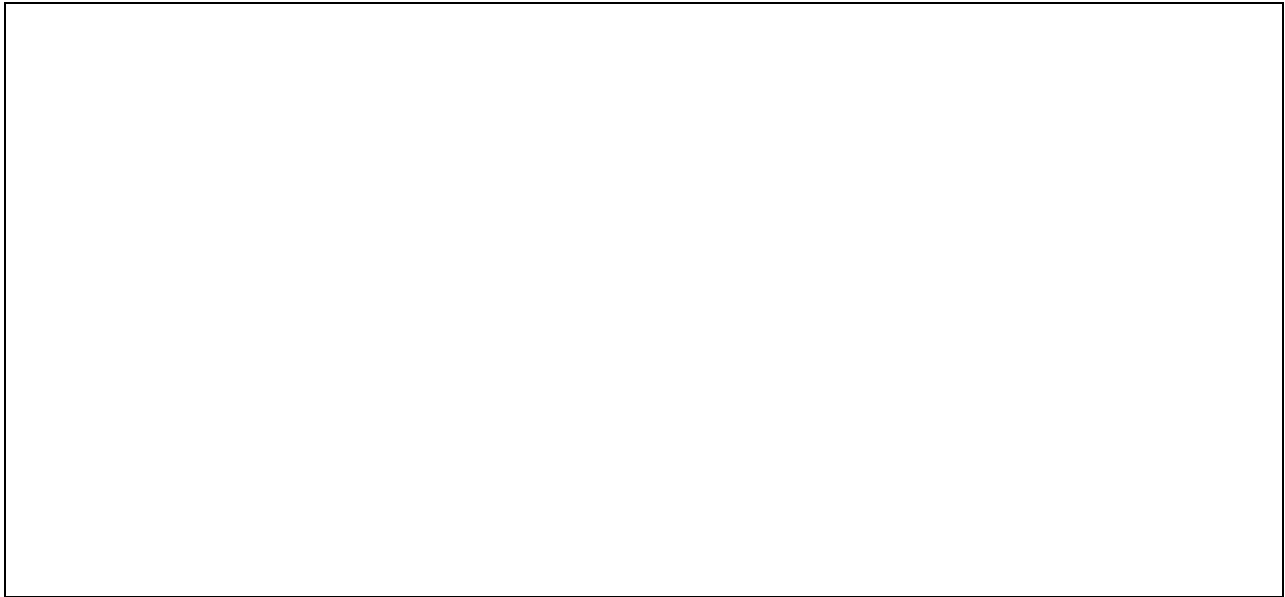
Thank you for completing this survey. If you have any additional questions, please contact:
Jennifer Bloodgood
Georgia Sea Turtle Center
214 Stable Road
Jekyll Island, GA 31527
912-635-4444
706-206-3254
jcbloodg@uga.edu

APPENDIX H

FINAL SURVEY INSTRUMENT FOR MEASURING ATTITUDES AND PERCEPTIONS

OF GSTC VISITORS

For GSTC Staff: **Date:**_____   **Time:**_____   **Survey Number:**_____   **Gallery**

Section A: Your Experience Today

A1. What is your residence on Jekyll Island? *(Please check ONE box.)*

☐ Non-resident   ☐ Seasonal resident   ☐ Year-round resident

A2. Which of the following best describes your visitation to the Georgia Sea Turtle Center (GSTC) before today? *(Please check ONE box.)*

☐ Never visited   ☐ Visitor (1 time)   ☐ Visitor (2+ times)   ☐ Annual Member

A3. Did you speak with an educator during your visit today? *(Please check ONE box.)*

☐ Yes   ☐ No   ☐ No, but I have before

A4. Did you go *into* the operating room today? *(Please check ONE box.)*

☐ Yes   ☐ No   ☐ No, but I have before

A5. Did you see a live sea turtle at the GSTC today? *(Please check ONE box.)*

☐ Yes   ☐ No   ☐ No, but I have before

A6. Did you see a treatment today? *(Please check ONE box.)*

☐ Yes   ☐ No   ☐ No, but I have before

A7. Indicate how often you have done the following. **(Check ONE box per ROW.)**

	Never	**Seldom**	**Sometimes**	**Often**	**Very Often**
Attended or seen a sea turtle release.	☐	☐	☐	☐	☐
Attended an educational or nature program about sea turtles.	☐	☐	☐	☐	☐
Used your phone to look up information about sea turtles.	☐	☐	☐	☐	☐
Used your computer to look up information about sea turtles.	☐	☐	☐	☐	☐

Section B: Your Experience with and Knowledge of Sea Turtles

B1. Indicate how often you have done the following. **(Check ONE box per ROW.)**

	Never	Seldom	Sometimes	Often	Very Often
Seen a sea turtle in an aquarium or nature center (before today).	☐	☐	☐	☐	☐
Seen a sea turtle in a rehabilitation center (before today).	☐	☐	☐	☐	☐
Seen a sea turtle on a beach.	☐	☐	☐	☐	☐
Seen a sea turtle in the ocean.	☐	☐	☐	☐	☐

B2. Please indicate the extent to which you strongly disagree to strongly agree with the following statements. **(Circle ONE number per ROW.)**

	Strongly Disagree		Neither Disagree or Agree		Strongly Agree
I can recognize different types of sea turtles.	1	2	3	4	5
I know the names of some types of sea turtles.	1	2	3	4	5
I can identify a sea turtle as opposed to a terrapin.	1	2	3	4	5
I can identify a sea turtle as opposed to a land turtle.	1	2	3	4	5
I can identify a sea turtle as opposed to a tortoise.	1	2	3	4	5
I know about sea turtle behaviors.	1	2	3	4	5
I know a lot about sea turtles in general.	1	2	3	4	5

B3. Please indicate whether you believe the following statements are true or false, or if you are unsure. **(Check ONE box per ROW.)**

	True	False	Unsure
Sea turtles have lungs.	☐	☐	☐
Sea turtles have teeth.	☐	☐	☐
Sea turtles are cold-blooded.	☐	☐	☐
Sea turtles crawl out of their shells when they need a bigger one.	☐	☐	☐
Sea turtles can retreat into their shells.	☐	☐	☐
Sea turtles are roughly the same size as land-dwelling turtles.	☐	☐	☐

	True	False	Unsure
Sea turtles are reptiles.	☐	☐	☐
Sea turtles have beaks.	☐	☐	☐
Sea turtles have gills.	☐	☐	☐

B4. Please indicate whether you believe the following statements are true or false, or if you are unsure. (**C***heck ONE box per ROW.*)

	True	False	Unsure
The GSTC uses a multivitamin supplement in sea turtle diets.	☐	☐	☐
The GSTC uses a gel diet with ground-up seafood, broccoli, carrots, and vitamins.	☐	☐	☐
The GSTC does not take blood from any of its patients.	☐	☐	☐
The GSTC uses honey to treat some types of wounds.	☐	☐	☐
The GSTC does not use anesthesia.	☐	☐	☐
The GSTC does not have a machine to take x-rays.	☐	☐	☐

Section C: Your Attitudes and Behaviors in Relation to Sea Turtles

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C1. Please indicate the extent to which you strongly disagree to strongly agree with the following statements. (*Circle ONE number per ROW.*)

	Strongly Disagree		Neither Disagree or Agree		Strongly Agree
Sea turtles are an important part of nature.	1	2	3	4	5
Sea turtles are important in preserving ecosystems.	1	2	3	4	5
Sea turtles should be protected on Jekyll Island.	1	2	3	4	5
Sea turtles are an important part of the scenic beauty of Jekyll Island.	1	2	3	4	5
Sea turtles are an important part of nature around Jekyll Island.	1	2	3	4	5

C2. Please indicate the extent to which you strongly disagree to strongly agree with the following statements. (*Circle ONE number per ROW.*)

	Strongly Disagree		Neither Disagree or Agree		Strongly Agree
I believe human needs come before the needs of sea turtles.	1	2	3	4	5
I believe sea turtles should have similar rights to those of humans.	1	2	3	4	5
I believe sea turtles should be protected.	1	2	3	4	5
I want to learn ways to help protect sea turtles.	1	2	3	4	5
I believe stricter laws are needed to protect sea turtles.	1	2	3	4	5
I am interested in learning more about sea turtles.	1	2	3	4	5
I believe rehabilitating sea turtles is important.	1	2	3	4	5
I believe releasing healthy sea turtles back to the ocean is important.	1	2	3	4	5
I believe sea turtles are easily hurt by humans.	1	2	3	4	5
I believe humans should help repair or rehabilitate injured sea turtles.	1	2	3	4	5
I believe it is important for humans to visit places like the Georgia Sea Turtle Center.	1	2	3	4	5
I believe sea turtle rehabilitation centers help people learn more about sea turtles.	1	2	3	4	5

Section D: Environmental Ethics and Attitudes

D1. Indicate how often you do the following. (**C*heck ONE box per ROW.***)

	Never	Seldom	Sometimes	Often	Very Often
Give some of my own money to help save wild plants or animals.	☐	☐	☐	☐	☐
Grow vegetables in my own garden.	☐	☐	☐	☐	☐
Compost my food waste.	☐	☐	☐	☐	☐
Turn off the sink when washing or rinsing dishes to save water.	☐	☐	☐	☐	☐
Turn off the lights when I leave a room to save energy.	☐	☐	☐	☐	☐
Help to clean up parks and forests in my neighborhood.	☐	☐	☐	☐	☐

Tell my friends or my family about things they can do to help protect nature.	☐	☐	☐	☐	☐
Carpool.	☐	☐	☐	☐	☐
Use re-usable grocery bags.	☐	☐	☐	☐	☐
Recycle paper, plastic, or glass.	☐	☐	☐	☐	☐

Section E: Demographics

These questions will help us to ensure that the people we are surveying are representative of all GSTC visitors. **All answers will be kept strictly confidential.**

E1. What is your gender?   ☐ Female   ☐ Male   ☐ Other/do not wish to respond

E2. What is your age? _____ years old

E3. Do you have children?   ☐ Yes   ☐ No

E4. Which of the following categories best describes your race/ethnicity? (*Check ALL that apply.*)

☐ African American   ☐ Caucasian

☐ American Indian   ☐ Hispanic, Latino, or Spanish

☐ Asian or Pacific Islander   ☐ Other/do not wish to respond

E5. What is the highest level of education you have completed? *(Please check ONE box.)*

☐ High school not completed   ☐ High school completed or GED   ☐ Some college or technical school   ☐ College degree or higher

E6. Please indicate your total household income range before taxes last year. *(Please check ONE box.)*

☐ $25,000 or less   ☐ $50,001 to $75,000   ☐ $100,001 or more

☐ $25,001 to $50,000   ☐ $75,001 to $100,000   ☐ Do not wish to respond

E7. Please provide the state and zip code of your primary residence: _____

Section F: Willingness to Pay

The following questions are for research purposes only—we are NOT soliciting your money or time.

F1. Would you be willing to donate money to help protect sea turtles (again, this is for research only, we are NOT soliciting donations)? *(Please check ONE box.)*

☐ Yes. If yes, how much? _____   ☐ No   ☐ Do not wish to respond

F2. Would you be willing to volunteer your time to help protect sea turtles (again, this is for research only, we are NOT soliciting your time)? *(Please check ONE box.)*

☐ Yes. If yes, how many hours/week? _____   ☐ No   ☐ Do not wish to respond

Section G: Optional Follow-Up

G1. If you would like to participate in a very brief, one time follow-up study via internet survey in 6 months, please supply your email address:

_____

Please use the space provided below for any additional comments.

Thank you for completing this survey. If you have any additional questions, please contact:

Jennifer Bloodgood
Georgia Sea Turtle Center
214 Stable Road
Jekyll Island, GA 31527
912-635-4444
706-206-3254
jcbloodg@uga.edu

APPENDIX I

DELAYED POSTTEST SURVEY INSTRUMENT FOR MEASURING ATTITUDES AND

PERCEPTIONS OF GSTC VISITORS

Thank you for your recent visit to The Georgia Sea Turtle Center (GSTC), and for participating in our survey! As you know, the GSTC rehabilitates several species of sea turtles, such as the green sea turtle pictured below. The Center is interested in following up on your opinions and attitudes towards the protection of sea turtles. Your input is important in helping us better understand how to educate people about protecting sea turtles so future generations may enjoy them.   This survey takes less than 10 minutes to complete. Questions refer to your summer 2015 visit to the GSTC. Please complete this survey only if you completed the original survey in summer 2015. We appreciate your time and effort in helping us complete this study. Your participation is voluntary, and your responses will be anonymous and confidential.

What is your residence on Jekyll Island?
- ⭕ Non-resident
- ⭕ Seasonal Resident
- ⭕ Year-round resident

Which of the following best describes your visitation to the Georgia Sea Turtle Center (GSTC)?
- ⭕ Visitor (1 time)
- ⭕ Visitor (2+ times)
- ⭕ Annual Member

Did you speak with an educator during your visit?
- ⭕ Yes
- ⭕ No
- ⭕ No, but I have before
- ⭕ I don't remember

Did you go into the operating room as part of the Behind-the-Scenes Tour?
- ⭕ Yes
- ⭕ No
- ⭕ No, but I have before

Did you see a live sea turtle at the GSTC?
- ⭕ Yes
- ⭕ No
- ⭕ No, but I have before
- ⭕ I don't remember

Did you see a veterinary treatment at the GSTC?
- ⭕ Yes
- ⭕ No
- ⭕ No, but I have before
- ⭕ I don't remember

Please indicate the extent to which you strongly disagree to strongly agree with the following statements. (Select ONE option per ROW.)

	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
I can recognize different types of sea turtles.	○	○	○	○	○
I know the names of some types of sea turtles.	○	○	○	○	○
I can identify a sea turtle as opposed to a terrapin.	○	○	○	○	○
I can identify a sea turtle as opposed to a land turtle.	○	○	○	○	○
I can identify a sea turtle as opposed to a tortoise.	○	○	○	○	○
I know about sea turtle behaviors.	○	○	○	○	○
I know a lot about sea turtles in general.	○	○	○	○	○

Please indicate whether you believe the following statements are true or false, or if you are unsure. (Select ONE option per ROW.)

	True	False	Unsure
Sea turtles have lungs.	○	○	○
Sea turtles have teeth.	○	○	○
Sea turtles are cold-blooded.	○	○	○
Sea turtles crawl out of their shells when they need a bigger one.	○	○	○
Sea turtles can retreat into their shells.	○	○	○
Sea turtles are roughly the same size as land-dwelling turtles.	○	○	○
Sea turtles are reptiles.	○	○	○
Sea turtles have beaks.	○	○	○
Sea turtles have gills.	○	○	○

Please indicate whether you believe the following statements are true or false, or if you are unsure. (Select ONE option per ROW.)

	True	False	Unsure
The GSTC uses a multivitamin supplement in sea turtle diets.	○	○	○
The GSTC uses a gel diet with ground-up seafood, broccoli, carrots, and vitamins.	○	○	○
The GSTC does not take blood from any of its patients.	○	○	○
The GSTC uses honey to treat some types of wounds.	○	○	○
The GSTC does not use anesthesia.	○	○	○
The GSTC does not have a machine to take x-rays.	○	○	○

Please indicate the extent to which you strongly disagree to strongly agree with the following statements. (Select ONE option per ROW.)

	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
Sea turtles are an important part of nature.	○	○	○	○	○
Sea turtles are important in preserving ecosystems.	○	○	○	○	○
Sea turtles should be protected on Jekyll Island.	○	○	○	○	○
Sea turtles are an important part of the scenic beauty of Jekyll Island.	○	○	○	○	○
Sea turtles are an important part of nature around Jekyll Island.	○	○	○	○	○

	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
I believe human needs come before the needs of sea turtles.	○	○	○	○	○
I believe sea turtles should have similar rights to those of humans.	○	○	○	○	○
I believe sea turtles should be protected.	○	○	○	○	○
I want to learn ways to help protect sea turtles.	○	○	○	○	○
I believe stricter laws are needed to protect sea turtles.	○	○	○	○	○
I am interested in learning more about sea turtles.	○	○	○	○	○
I believe rehabilitating sea turtles is important.	○	○	○	○	○
I believe releasing healthy sea turtles back to the ocean is important.	○	○	○	○	○

I believe sea turtles are easily hurt by humans.	○	○	○	○	○
I believe humans should help repair or rehabilitate injured sea turtles.	○	○	○	○	○
I believe it is important for humans to visit places like the Georgia Sea Turtle Center.	○	○	○	○	○
I believe sea turtle rehabilitation centers help people learn more about sea turtles.	○	○	○	○	○

The following questions are for research purposes only--we are NOT soliciting your money or time.

Have you donated money to help protect sea turtles since your summer 2015 visit to the Georgia Sea Turtle Center?
○ Yes
○ No
○ No, but I have donated to another conservation cause
○ Do not wish to respond

If no, would you be willing to donate to sea turtle conservation in the future (again, this is for research only, we are NOT soliciting donations)?
❍ Yes
❍ No
❍ No, but I would be willing to donate to another conservation cause
❍ Do not wish to respond

Have you volunteered your time to help protect sea turtles since your summer 2015 visit to the Georgia Sea Turtle Center (again, this is for research only, we are NOT soliciting your time)?
❍ Yes
❍ No
❍ No, but I have volunteered at another conservation organization
❍ Do not wish to respond

If no, would you be willing to volunteer to help protect sea turtles in the future (again, we are only interested in your opinions, not in having you sign up as a volunteer at this time)?
❍ Yes
❍ No
❍ No, but I would be willing to volunteer for another conservation cause
❍ Do not wish to respond

The remaining questions will help us to ensure that the people we are surveying are representative of all GSTC visitors. All answers will be kept strictly confidential. If you do not wish to respond, please select that option or leave the question blank if that option is not available.

What is your gender?
❍ Male
❍ Female
❍ Other/do not wish to respond

What is your age (in years)?

Do you have children?
❍ Yes
❍ No

Which of the following categories best describes your race/ethnicity? (Check ALL that apply.)
❑ African American
❑ American Indian
❑ Asian or Pacific Islander
❑ Caucasian
❑ Hispanic, Latino, or Spanish
❑ Other/do not wish to respond

What is the highest level of education you have completed? (Please check ONE box.)
- ❍ High school not completed
- ❍ High school completed or GED
- ❍ Some college or technical school
- ❍ College degree or higher

Please indicate your total household income range before taxes last year. (Please check ONE box.)
- ❍ $25,000 or less
- ❍ $25,001 to $50,000
- ❍ $50,001 to $75,000
- ❍ $75,001 to $100,000
- ❍ $100,001 or more
- ❍ Do not wish to respond

Please provide the state and zip code of your primary residence:

Please use the space provided below for any additional comments.