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Ecology of the Diamondback Terrapin (Malaclemys Terrapin Pileata) in Alabama: Applications of New Technologies

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ECOLOGY OF THE DIAMONDBACK TERRAPIN (*MALACLEMYS TERRAPIN PILEATA*) IN ALABAMA: APPLICATIONS OF NEW TECHNOLOGIES

by

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A THESIS

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2022

ECOLOGY OF THE DIAMONDBACK TERRAPIN (*MALACLEMYS TERRAPIN PILEATA*) IN ALABAMA: APPLICATIONS OF NEW TECHNOLOGIES

FORREST WILLIAM COLLINS

BIOLOGY

ABSTRACT

The Diamondback Terrapin, *Malaclemys terrapin pileata*, inhabits saltmarshes in the Northern Gulf of Mexico including along the coast of Alabama. Due to a variety of factors, this species has declined drastically in Alabama, and it is currently designated as a “species of highest conservation concern”. Understanding the ecology of this species is a prerequisite to the recovery of this population. The current thesis addresses the reproductive and foraging ecology of the Diamondback Terrapin in Alabama. This thesis combines the use of classic ecological methodologies with modern technologies. Between April 2019 to July 2022, wild nesting females were caught at a major nesting beach located on the western border of Cedar Point Marsh in Heron Bay, Alabama. Blood samples were obtained from all of the captured terrapins in order to evaluate the foraging ecology through $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis. The variation in $\delta^{13}C$ and $\delta^{15}N$ exhibited by the samples suggested potential variation in foraging behavior. Additionally, the mean and standard deviation of these values did not overlap with those previously reported for three terrapin populations in southwest Florida. Stable isotope values were also evaluated for a variety of potential prey items and primary producers in the salt marsh habitat. The results revealed significant species-specific and site-specific variation in the stable isotope values of prey and primary producers in both $\delta^{13}C$ and $\delta^{15}N$ and included an isoscape for the Marsh Periwinkle in the eastern portion of the Mississippi

Sound of Alabama. An experimental evaluations of stable isotope values in Head Start terrapins indicated that they could provide a model for resources assimilation by wild Diamondback Terrapin. This study represents the first documentation of $\delta^{13}C$ and $\delta^{15}N$ stable isotope values for the Diamondback Terrapin from a major nesting location in the northern Gulf of Mexico and documents stable isotope values for a variety of prey items in that habitat. The reproductive ecology of the nesting female terrapins was evaluated during the 2021 and 2022 nesting seasons at Heron Bay, Alabama. Captured turtles ranged from 600 to 1385 grams, 67 to 188 mm in plastron length, and 157 to 207 cm in straight carapace length. Gravid females produced an average of 7.7 ± 1.8 eggs. Population estimates revealed that there were 166 individuals during 2021 and 133 individuals during 2022. Radio transmitters were attached to a subset (*n* = 13 for 2021, and *n* = 10 for 2022) of these turtles to monitor post-nesting movements. Telemetry results indicate that many of the terrapins that were nesting in Cedar Point Marsh remained in the Cedar Point Marsh area after nesting, and that terrapins captured in the northern portion of Heron Bay continued to inhabit that area for the period they were tracked post-release. To assess the impact of local predators on terrapin nests, Cedar Point Marsh was surveyed for nesting daily for depredated nests during the 2021 and 2022 nesting seasons. No depredated nests were observed during the 2021 or 2022 terrapin nesting seasons which is in distinct contrast to high nest depredation levels in years previous to a racoon removal program in 2020. Collectively, these data document that the salt marshes and associated beaches in the Heron Bay area represent habitat that is critical for successful reproduction of the Diamondback Terrapin.

Key Words: Conservation, Stable isotopes, Radio telemetry, Blood sampling, Foraging

DEDICATION

I dedicate this to my wife, Dr. Morgan Nicole Collins, for believing and supporting in my works, my dreams, and my desires to further my life in marine sciences. Without you, I do not think I would be in the field that I am today. Your drive to fulfill your own dreams helped push me to go further. Thank you for your constant support and for enjoying my turtle journey along the way. You are a perfect wife, a best friend, an amazing dentist, and a sturdy corner stone.

"As iron sharpens iron, so one person sharpens another"
-Proverbs 27:17

I would also like to dedicate my thesis to the late Dr. Mary Holcombe of the University of Tennessee. Your support as I applied for and entered graduate school will always be a constant encouragement. Being one of the smartest and most loving people I could have ever known, you will always be missed.

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GENERAL INTRODUCTION

GENERAL INTRODUCTION

The Mississippi Diamondback Terrapin (*Malaclemys terrapin pileata*)

The Mississippi Diamondback Terrapin is a primarily brackish water species that ranges throughout the eastern and southern United States (Gibbons, 2018). This species is unique within the Emydidae family since it inhabits brackish water marshes from as far north as Massachusetts to as far south as Texas. Within these different geographical locations, there are seven subspecies: Northern, Mississippi, Carolina, Mangrove, Texas, Ornate, and Florida East Coast (Hart, 2014). The subspecies that is found in Alabama is the Mississippi Diamondback Terrapin, and it ranges from the western portion of the Florida panhandle to the eastern portion of Louisiana.

The Mississippi Diamondback Terrapin was once abundant in the salt marshes in Alabama but has significantly declined in the past century (Carr, 1952; Nelson and Marion, 2004; Coleman, 2011). Its population is currently represented by small, remnant groups in various locations along the coasts of Alabama (Wibbels, 2010). It is currently listed as “a species of highest conservation concern” by the Alabama Department of Conservation and Natural Resources (Nelson and Marion, 2004). The Diamondback Terrapin has endured environmental and human disturbances over the past century that have led to their dramatic decline (Nelson and Marion, 2004). Primary threats that have limited this species recovery in Alabama include depredation of natural nests by predators (in particular raccoons), loss of critical habitat, and crab trap-induced mortality (Bishop, 1983; Wibbels, 2010; Coleman et al. 2011, 2014; Roberge, 2017).

Previous surveys throughout the coastal areas of Alabama have identified a number of nesting areas (Wibbels, 2010; Coleman, 2014; Roberge, 2017; Sirgo, 2020). The largest nesting aggregation that has been identified to-date is at Cedar Point Marsh in Heron Bay which is located in the eastern portion of the Mississippi Sound. Surveys of this beach have periodically documented as many as 151 nests in a single nesting season (Coleman, 2011). Due to its importance as a primary nesting area in Alabama, a long-term mark-recapture program has been conducted at Cedar Point Marsh since 2006 (Wibbels, 2010; Coleman, 2011; Roberge 2017). This program has included the capture and tagging of nesting females as well as a Head Start program using eggs from a subset of the females. This program provides the opportunity to investigate the ecology and conservation status of the Diamondback Terrapin in Alabama. Including the use of classic mark-recapture methodologies, daily nesting beach surveys, as well as the implantation of new technologies.

Classic Ecological Methodologies

The availability of a nesting beach such as Cedar Point Marsh provides the opportunity to continue and extend a long-term mark-recapture study for the Diamondback Terrapin in Alabama. This program has included the use of drift fence-bucket trap sampling system in order to capture adult females while they are on the nesting beach. The capture and tagging of adult females provides an avenue for addressing a wide variety of ecological aspects of the Diamondback Terrapin including topics such as nesting population estimates, growth and longevity, remigration intervals, fecundity, etc. This program has also included regular surveys of the nesting beach during the nesting season which facilitates the documentation of the impact of predators on nests. The availability of gravid adult females also provides the opportunity to obtain eggs and hatchlings for “head starting” as a means of avoiding high depredation rates that have been regularly documented at Cedar Point Marsh. Finally, the ability to capture adult females at Cedar Point Marsh provides an avenue for implementing new technologies for investigating the ecology of the Diamondback Terrapin in Alabama.

Implementation of Technologies

A variety of new technologies have become available in the past several decades that have significantly enhanced the scientific tools available for studying the ecology of turtles in their natural environment. Of particular interest, studies have shown that stable isotope analysis represents a powerful tool for evaluating the ecology of turtles including seasonal movements and foraging behaviors (Hobson, 1998). For example, it can indicate the foraging locations and diet in turtles (Ceriani, 2012). Further, stable isotope analysis can show transitions between environments such as oceanic to neritic shifts in sea turtles (Reich, 2008). Stable isotope analysis has also been shown to reveal temporal aspects in foraging ecology from the analysis of different tissues (e.g., analysis of blood versus shell scute tissues) (Hobson, 1993; Denton et al. 2019). This technology can also be utilized to identify individuals from polluted habitats with high nitrogen contents (Costanzo, 2005). Stable isotope technology has developed to the point that some studies have been predicting the “isoscapes” for turtle populations which reflect the variability of their stable isotope values throughout their ranges and seasons (Cheesman, 2016).

Another technology that directly complements stable isotope studies is tracking telemetry. This technology has been utilized in turtle species to address a wide variety of ecological questions related to foraging behaviors and movements. This has included satellite telemetry, radio telemetry, and sonic telemetry depending on the species, the habitat, and the purpose of the study. In the case of radio telemetry, it has often been used for terrestrial or semi-aquatic species that have relatively small home ranges and/or seasonal movements. Tracking telemetry addresses the ecology of the species relative to daily and seasonal movements, including documentation of home ranges and foraging habitats (Obbard and Brooks, 1981). Further, these studies can document critical habitat for protected species.

Specific Aims of Thesis Research

The current thesis addresses the reproductive and foraging ecology of the Diamondback Terrapin in Alabama. This thesis combines the use of classic ecological methodologies with modern technologies. These tools are utilized to extend our understanding of the ecology and conservation status of the Diamondback Terrapin in Alabama. The thesis includes the continuation and extension of a long-term mark-recapture study for estimating population status and evaluating reproductive ecology. Additionally, stable isotope technology and radio tracking technology are utilized to address the ecology of the adult females captured at Cedar Point Marsh. Collectively, these studies provide base line data that enhance our basic understanding of the ecology and conservation status of the Diamondback Terrapin in Alabama.

Chapter 1 focuses on the foraging ecology of the Diamondback Terrapin by investigating the stable isotopes, specifically the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes. This includes the documentation of values for terrapins in Heron Bay in comparison with previously published values of other terrapin populations. This chapter also includes a comprehensive evaluation of stable isotope values of common prey items including species-specific and location-specific isoscapes. Additionally, an experimental approach is utilized to evaluate the isotopic effect of specific prey items on head start terrapins. Collectively, the results provide insight on the foraging ecology of the Diamondback Terrapin in Alabama.

Chapter 2 focuses on the reproductive ecology and population status of the Diamondback Terrapin nesting in Cedar Point Marsh during 2021 and 2022. Drift fences and pit fall traps were utilized to capture adult female terrapins that were nesting at a major nesting beach in Heron Bay (i.e., Cedar Point Marsh). These turtles were evaluated as part of a long-term mark-recapture study in order to estimate the size of the nesting population. A subset of gravid females were induced to lay eggs as part of an ongoing head start program which was integrated into the stable isotope experimental feeding studies described in Chapter 1. Further, a subset of the adult female turtles were also tagged with radio transmitters in order to monitor their post-nesting movements and document their foraging habitat. Cedar Point Marsh was regularly surveyed for depredated nests during the 2021 and 2022 nesting season in order to document a raccoon removal program in 2020. Collectively, the research in this thesis provides base line information on foraging and reproductive ecology including the documentation of habitat that is essential for several stages of the life history for the Diamondback Terrapin. This information is essential for evaluating the conservation status and developing an effective recovery strategy for the Diamondback Terrapin in Alabama.

USE OF STABLE ISOTOPE TECHNOLOGY FOR INVESTING THE FORAGING ECOLOGY OF THE DIAMONDBACK TERRAPIN IN ALABAMA

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Abstract

The Mississippi Diamondback Terrapin, *Malaclemys terrapin pileata*, inhabits saltmarshes in the Northern Gulf of Mexico including along the coast of Alabama. The current study implemented stable isotope technology to investigate the foraging ecology of this species in the eastern portion of the Mississippi Sound in Alabama. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes were documented in whole blood samples from adult female terrapins collected on a nesting beach at Cedar Point Marsh located north of Dauphin Island, Alabama. The variation in $\delta^{13}C$ and $\delta^{15}N$ exhibited by the samples suggested potential variation in foraging behavior. Additionally, the mean and standard deviation of these values did not overlap with those previously reported for three terrapin populations in southwest Florida. Stable isotope values were also evaluated for a variety of potential prey items and primary producers in this salt marsh habitat. The $\delta^{13}C$ stable isotope values of potential prey items such as the Eastern Oyster (*Crassostrea virginica*), Blue Crab (*Callinectes sapidus*), Marsh Crab (*Sesarma reticulatum*), and Marsh Fiddler Crab (*Uca pugnax*) were similar to those of the Diamondback Terrapin. In contrast, the $\delta^{13}C$ stable isotope values of the Hermit Crab (*Clibanarius vittatus*), Marsh Periwinkle (*Littorina irroratta*), and Olive Nerite (*Vitta usnea*) were statistically different than those of the diamondback terrapin. The relatively high $\delta^{15}N$ values of the terrapins in comparison to the majority of the prey items evaluated are consistent with the hypothesis that terrapins represent a higher trophic level predator within the salt marsh ecosystem. Stable isotope values were also documented for a potential prey item (Marsh Periwinkle) from multiple locations within the eastern portion of the Mississippi Sound. The results revealed significant site-specific variation in the stable isotope values in δ^{13}C and δ^{15}N.

The current study also included the evaluation of δ^{13}C and δ^{15}N stable isotopes from "head start" Diamondback Terrapins that were fed a standard diet of Reptomin®. The results of this experiment show that head start terrapins could provide a model for resources assimilation by the Diamondback Terrapin. This study represents the first documentation of δ^{13}C and δ^{15}N stable isotope values for the Diamondback Terrapin from a major nesting location in the northern Gulf of Mexico. Further, it provides documentation of stable isotope values for a variety of potential prey items, thus providing insight on the foraging ecology of the Diamondback Terrapin, as well as the trophic ecology of the salt marsh habitat in this area.

Key Words: Diamondback Terrapin, Stable Isotopes, Prey Items, Conservation, Foraging Ecology, Alabama, Northern Gulf of Mexico, *Littorina irorrata*

Introduction

Stable isotope technology has become a well-established tool for evaluating trophic level, nutrient flow, and nutrient origins within an ecosystem (DeNiro and Epstein, 1992; Rush et al. 2005; Rosenblatt and Heithaus, 2013). Isotope composition of animals within an ecosystem reflects and is directly dependent upon the resources they assimilate (DeNiro and Epstein, 1978; Hobson and Welch, 1992; Phillips and Eldridge, 2006; Seminoff et al. 2007). Stable isotope analysis encompasses the ratio of heavy isotopes to light isotopes (δ) and how they fractionate and turnover within an organism (Fry, 2006). Now, by examining the ratios of stable isotopes such as carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) in consumers, insight can be gained on their foraging ecology including prey items and trophic levels (Fry, 2006).

Stable isotopes have been used for a variety of studies related to the foraging ecology of turtles (Godley et al. 1998; Seminoff et al. 2007; Reich et al. 2008; Murray and Wolf, 2011; Simona et al. 2012; Denton et al. 2019; Kudman, 2021). Turtles inhabit a wide variety of ecosystems and can exhibit great diversity in foraging ecology depending on the species. Stable isotope studies of turtles have been able to provide insight on diets including spatial and temporal foraging ecology, as well as dietary shifts associated with life history stages (Seminoff et al. 2007; Arthur et al. 2008; Reich et al. 2008, Murray and Wolf, 2011; Denton et al. 2019; Kudman, 2021). These studies have focused on turtles from a few terrestrial, freshwater, marine, and brackish habitats. The Diamondback Terrapin, *Malaclemys terrapin*, provides an interesting consumer for stable isotope studies because it specifically inhabits brackish water coastal habitats, it is an opportunistic feeder, and it has a wide geographic distribution.

Initial studies of the Diamondback Terrapin indicate significant variability in $\delta^{13}C$ and $\delta^{15}N$ stable isotopes between locations and populations (Denton et al 2019; Kudman, 2021). Terrapins from three different locations in Southwest Florida exhibited considerable variation in isotopic values for carbon and nitrogen, variation that appeared to be related to different prey items and different baseline trophic levels in each location (Denton et al. 2019). Similarly, a study of terrapins from three locations in the Northeast (one in New Jersey and two in New York) indicated site-specific variation in isotope values related to site-specific prey consumption and base line trophic levels in each habitat (Kudman, 2021). These studies also utilized analysis of the terrapin's fecal material as an independent method to provide insight on foraging ecology (Reviewed by Tucker et al. 2018). The results of these studies suggested that prey items consumed by terrapin can vary among locations sampled. Other studies that have relied solely on fecal samples have also shown that diets can vary depending on location, as well as factors such as size (head width and body size) of terrapins (Tucker et al. 1995; Reviewed by Tucker et al. 2018). In general, prey items could vary widely but often included gastropods such as the Marsh Periwinkle (*Littorina irrorata*), as well as decapods such as the Marsh Fiddler Crab (*Uca pugnax*), the Marsh Crab (*Sesarma reticulatum*), and the Blue Crab (*Callinectus sapidus*) (Tucker et al. 1995; Tucker et al. 2018). A review of terrapin foraging ecology by Tucker et al. (2018) also indicated the need for additional data related to the foraging ecology of populations in the Gulf of Mexico.

In the current study, the foraging ecology of the Mississippi Diamondback Terrapin, *Malaclemys terrapin pileata*, was evaluated on a nesting beach in the eastern portion of the Mississippi Sound located in Heron Bay, Alabama. Adult female terrapins were collected on the nesting beach and were evaluated for $\delta^{13}C$ and $\delta^{15}N$ stable isotope values measured in whole blood collected from these animals. Fecal samples were also analyzed from a subset of these individuals. Additionally, $\delta^{13}C$ and $\delta^{15}N$ stable isotope values were analyzed for a variety of potential prey items that were collected from the Mississippi Sound area in and adjacent to the Heron Bay area. $\delta^{13}C$ and $\delta^{15}N$ stable isotope values were also analyzed for head start terrapins that were fed standard control diets (i.e., Reptomin®) as well as from heat start terrapins that were fed experimental diets of various prey items. Stable isotope values in terrapins were evaluated relative to those from potential prey items. Additionally, one of the known prey items (the Marsh Periwinkle) was used as a model for developing an "isoscape" for evaluating differences in $\delta^{13}C$ and $\delta^{15}N$ stable isotope values related to variation in foraging and trophic levels between locations in the eastern portion of the Mississippi Sound (Bowen et al. 2010). Collectively, the results provide baseline data on the variation and range of $\delta^{13}C$ and $\delta^{15}N$ stable isotope values of Diamondback Terrapins in the eastern portion of the Mississippi Sound (i.e., Heron Bay, Alabama). Further, the results provide insight on the potential impact of the various prey items on the $\delta^{13}C$ and $\delta^{15}N$ values of terrapins.

Materials and Methods

Capture of Wild Terrapins- Adult female terrapins were captured during the spring and summer of 2019, 2021, and 2022 on a nesting beach at Cedar Point Marsh, located in Heron Bay, Alabama. This site is located in the eastern portion of the Mississippi Sound. Pitfall traps and drift fences were utilized to capture adult female terrapins during their nesting behavior (Coleman, 2011; **Figure 1**). All captured terrapins were determined to be “female” based on head size, tail length, and carapace measurements (curved and straight). The pitfall traps were located adjacent to drift fences and were checked on a daily basis during the nesting season. All terrapins were tagged [shell tag and passive integrated transponder tag (P.I.T)], measured, and had blood samples taken for stable isotope analysis prior to their release. Blood samples were obtained from the subcarapacial vein using a 25-gauge needle attached to 1.0 cc syringe (approximately 25 µl to 100 µl total). Blood samples were stored at 4°C until they were processed for stable isotope analysis. Additionally, scute samples were obtained from a subset of terrapins (n = 7) to study historical feeding by collecting scutes that were in the process of sloughing off from the carapace. Scutes were stored at room temperature until processed for stable isotope analysis (see methodology below).

Terrapin Fecal Samples and Examination- A subset of the captured terrapins were held in isolated containers for approximately 24 hours. Fecal samples were collected from these terrapins after the 24 hour period. The fecal samples were then washed with distilled water and dried in a vacuum oven at 60°C for 24 hours. Remnant shells and other food items were identified to the nearest taxonomic ranking to identify prey. Percent dry mass was calculated based on the nearest 100 µ grams (Marion et al. 1991).

Raising and Sampling of Head Start Terrapins- Due to high depredation of nests by racoons (*Procyon lotor*) at Cedar Point Marsh, terrapin eggs were obtained from a subset of the adult female terrapins from that beach and were used to produce hatchlings. Hatchlings were then “head-started” for two-to-three years.

The adult females were induced to lay eggs by injecting oxytocin (approximately 10 I.U.) into a caudal sinus using a 25-gauge needle attached to 1.0 cc syringe. Following oviposition, eggs were collected and transferred to a custom laboratory incubator that maintained constant incubation temperature ± 1/10°C. Eggs from each clutch were split into two groups with one group being incubated at 26°C (i.e., male producing), and the other group incubated at 31°C (i.e., female producing). The hatchlings were retained in captivity for approximately two to three years in captivity and subsequently released at Cedar Point Marsh. While in captivity, terrapins were fed Reptomin® on a daily basis.

Prior to their release, “head-started” terrapins were shell and P.I.T tagged, measured, and released back into Cedar Point Marsh. Blood samples (see methodology above) were also collected from head-started terrapins. Blood samples were stored at 4°C until they were processed for stable isotope analysis (see methodology below). Additionally, prior to their release, a subset of head-started terrapins were used in the feeding experiments described below.

Collection of Prey Items and Primary Producers for Stable Isotope Analysis- A variety of potential prey items were collected from May 2021 to March 2022 at known locations where terrapins have been collected in previous years (**Figure 2** and **Figure 3**). The prey items collected have been historically known prey items for the Mississippi Diamondback Terrapin (Carr, 1952; Nelson and Marion, 2004; Coleman, 2011). The prey items included Blue Crabs (*Callinectes sapidus*), Eastern Oysters (*Crassostrea virginica*), Marsh Crabs (*Sesarma reticulatum*), Marsh Fiddler Crabs (*Uca pugnax*), Hermit Crabs

(*Clibanarius vittatus*), Marsh Periwinkles (*Littorina irroratta*), and Olive Nerites (*Vitta usnea*). Samples of the primary marsh grasses in the Mississippi Sound, Saltmarsh Cordgrass (*Spartina alterniflora*) and Black Needlerush (*Juncus roemerianus*), were collected from various potential terrapin foraging grounds. Tissues from the prey items and primary producers were processed for stable isotope analysis (see methodology below).

In order to evaluate variation of isotope values in a known prey item, Marsh Periwinkles were collected at multiple locations in the Mississippi Sound (**Figure 2**). Tissues from the Marsh Periwinkles were processed for stable isotope analysis (see methodology below).

Experimental Evaluation of the Impact of Natural Diets on Stable Isotopes- The current study included an experimental approach that utilized the head start terrapins to evaluate the effects of specific natural prey items on δ^{13}C and δ^{15}N values. Subsets (*n* = 4 - 9 per subset) of head start terrapins (approximately 2 years old and 400 grams) were fed a diet consisting of potential prey items that occur in, or adjacent to, the Mississippi Sound. Feeding experiments were conducted in a controlled environment that regulated air temperature (25°C), light cycles (12-hour cycle, 9:00 A.M. – 9:00 P.M.), and feeding schedules. Each experimental group was fed a specific type of prey item: House Crickets (*Acheta domesticus*, 1.5 to 2.5 grams per turtle), Common Periwinkles (*Littorina littorea*, 3 to 5 snails per turtle with an average diameter approximately 2.5 cm), Blue Crabs (*Callinectes sapidus*, 36.00 g, wet weight, per turtle), or Marsh Periwinkles (*Littorina irroratta*, 3 to 5 snails per turtle with an average diameter approximatley 2.5 cm). The control group was fed commercial pelleted food (Reptomin®) that was regularly utilized in the head starting of the terrapins (15 to 20 pellets). Both experimental and control groups were fed their specific diets over a course of two weeks. Samples of whole blood were collected on a weekly or bi-weekly basis during the study (see methodology above). Blood samples were stored at 4°C until they were processed for stable isotope analysis (see methodology below).

Tissue Sampling and Processing for Stable Isotope Analysis- For each sample of whole blood collected, an aliquot (7 - 10 µl) was blotted onto a 4 mm x 4 mm sectioned square of GE Whatman™ Fiberglass Filter Paper and placed within a 5 mm x 9 mm tin capsule. For prey items and primary producers, the tissue samples were macerated, weighed, and then 7 mg was deposited directly into the tin capsule. All samples were dried for 24 hours at 80°C in a vacuum oven. Each tin capsule was placed in a 96-well plate prior to shipment to a stable isotope analysis facility.

Statistics- "R" programming was used for statistical analysis. Linear regression, correlation, and t-test models were used to evaluate differences in the $\delta^{13}C$ and $\delta^{15}N$ between scute and blood samples collected from terrapins. A t-test was used to compare stable isotope values for the wild terrapins versus head start terrapins. ANOVA was used to compare stable isotope values to different locations within the Mississippi Sound, to different locations within Cedar Point Marsh, and to their prey items. I applied a repeated-measures ANOVA to evaluate stable isotope values in each of the four feeding experiments. Prior to ANOVA testing, normality and equality of variance was determined through Q-Q plots. For all statistical tests, $\alpha \leq 0.05$ was considered representative of statistical significance.

Figure 1. Examples of the drift fences and pit fall traps laid out in Cedar Point Marsh during terrapin nesting seasons

Figure 2. Locations where prey items have been collected in the Mississippi Sound.

Figure 3. Exact locations within Cedar Point Marsh where Marsh Periwinkles (*Littorina irroratta*) were collected.

Results

Wild Terrapin Analysis

A total of 41 adult female terrapins were collected in Heron Bay, Alabama, during the nesting seasons (April - August) of 2019, 2021, and 2022. Whole blood from each terrapin was analyzed to determine the stable isotope values for $\delta^{13}C$ and $\delta^{15}N$ (**Figure 4**). The mean value for $\delta^{13}C$ was -23.32 ‰ ± 1.56 and the mean value for $\delta^{15}N$ was 9.66 ‰ ± 1.04 (**Table 1**).

In addition to whole blood, scute samples were analyzed for a subset (*n* = 7) of these adult female terrapins. The mean value for the scute samples were $\delta^{13}C$ was -22.81 ‰ ± 2.61 and the mean value for $\delta^{15}N$ was 8.9 ‰ ± 1.44. Correlation coefficients (r) of the scute versus whole blood values were 0.46 for $\delta^{13}C$, and 0.25 for $\delta^{15}N$. Scute samples are shown relative to corresponding whole blood samples in Figure 6. Scute versus whole blood linear regression (R^2) values were 0.21 for $\delta^{13}C$ (see **Figure 7**), and 0.06 for $\delta^{15}N$ (see **Figure 8**). Comparison of the $\delta^{13}C$ and the $\delta^{15}N$ values for scute versus whole blood samples did not reveal any significant variation (dependent t-test; $t_6 = 0.55$, $p = 0.60$ and $t_6 = 0.30$, $p = 0.77$ respectfully).

Fecal samples were collected and evaluated to identify prey item from a subset of wild terrapins (*n* = 9); Table 3. The majority of the fecal matter was composed of shell fragments from the Ribbed Mussel, *Geukensia demissa,* (45.8%), and the Marsh Periwinkle (45.3%).

Prey and Primary Producer Analysis

A variety of potential prey items and the two predominant marsh grasses, Saltmarsh Cordgrass and Black Needlerush, were collected and analyzed over a two-year period. The $\delta^{13}C$ and $\delta^{15}N$ values for the marsh grasses and the potential prey items are shown in Table 2 and Figure 9. $\delta^{13}C$ and $\delta^{15}N$ values for the primary producers and the potential prey items were compared using ANOVA. There was a significant variation within the group's $\delta^{13}C$ ($F_{8,\,122} = 18.75$, $p < 0.01$) and $\delta^{15}N$ ($F_{8,\,122} = 12.87$, $p < 0.01$). Bonferroni post-hoc test revealed which primary producers and potential prey items were significantly different than one another in regard to $\delta^{13}C$ (**Table 4**) and $\delta^{15}N$ (**Table 5**) values.

$\delta^{13}C$ and $\delta^{15}N$ values of the wild terrapins were compared to the values of the potential prey items using ANOVA (**Figure 10**). There was a significant variation within the group's $\delta^{13}C$ ($F_{7,\,142} = 41.0$, $p < 0.01$) and $\delta^{15}N$ ($F_{7,\,142} = 30.52$, $p < 0.01$). Bonferroni post-hoc test revealed that wild terrapins' $\delta^{13}C$ values were not significantly different than those of Blue Crab ($p = 1.0$), Marsh Fiddler Crab ($p = 1.0$), Marsh Crab ($p = 1.0$), and Eastern Oyster ($p = 1.0$). However, the $\delta^{13}C$ values of the Hermit Crab ($p < 0.01$), Olive Nerite ($p < 0.01$) and Marsh Periwinkle ($p < 0.01$) were significantly different than those of the wild terrapins. The $\delta^{15}N$ values for the wild terrapins were not significantly different than those of Blue Crab ($p = 1.0$), Marsh crab ($p = 0.90$) and Eastern oyster ($p = 0.71$). However, the $\delta^{15}N$ values of the Marsh Fiddler Crab ($p < 0.01$), Hermit Crab ($p < 0.01$), Olive Nerite ($p < 0.01$), and Marsh Periwinkle ($p < 0.01$) were significantly different from those of the wild terrapins. The results of the ANOVA are summarized in Table 6.

Prey Isoscape Analysis

The Marsh Periwinkle was used to develop an isoscape model to evaluate variation in site-specific isotope values for a potential prey item in the Mississippi Sound of Alabama. Marsh Periwinkles were sampled from nine different locations (**Figures 2**, **3,** and **11**) and the values from each location are summarized in Table 7. Results indicated significant site-specific variation in both δ^{13}C and δ^{15}N values (δ^{13}C, $F_{8, 36} = 9.15$, $p < 0.01$; δ^{15}N, $F_{8, 36} = 15.55$, $p < 0.01$). Bonferroni post-hoc test revealed significant difference in δ^{13}C (**Table 8**) and δ^{15}N (**Table 9**) among some locations.

Comparison of Marsh Periwinkle Isoscape to Wild Terrapins

The δ^{13}C and δ^{15}N values of the wild terrapins that were caught in Heron Bay are shown in Figure 12 in comparison with the Marsh Periwinkle isoscape values. ANOVA revealed significant variation between the δ^{13}C and δ^{15}N values for the wild terrapins and the Marsh Periwinkle isoscape locations (δ^{13}C, $F_{9, 76} = 45.03$, $p < 0.01$; δ^{15}N, $F_{9, 76} = 64.07$, $p < 0.01$). Bonferroni post-hoc test for δ^{13}C indicated significant variation between the values of the wild terrapins and those of all isoscape locations with the exception of Fowl River (Airport Marsh, $p < 0.01$; Bayou Coden, $p < 0.01$; Bayou La Batre, $p < 0.01$; Cedar Point Marsh- North, $p < 0.01$; Cedar Point Marsh- South, $p < 0.01$; Jemison Marsh, $p < 0.01$; Lightning Point, $p < 0.01$; Fowl River, $p = 1.0$). Bonferroni post-hoc test for δ^{15}N indicated significant variation between the values of the wild terrapins and those of all isoscape locations (Airport Marsh, $p < 0.01$; Bayou Coden, $p < 0.01$; Bayou La Batre, $p < 0.01$; Cedar Point Marsh- North, $p < 0.01$; Cedar Point Marsh- South, $p < 0.01$; Fowl River, $p < 0.01$; Jemison Marsh, $p < 0.01$; Lightning Point, $p < 0.01$).

Head Start Terrapin Analysis

Head start terrapins had a mean $\delta^{13}C$ value of -23.7 ‰ ± 0.67 and a mean $\delta^{15}N$ value of 8.36 ‰ ± 0.84 (**Table 1**). Significant variation was detected when comparing the $\delta^{13}C$ and $\delta^{15}N$ values for the head start versus the wild terrapins ($\delta^{13}C$ independent t-test was $t_{55.5} = 6.05$, $p < 0.05$, and $\delta^{15}N$ was $t_{75.25} = -6.08$, $p < 0.05$) (**Figure 14**). Wild terrapins had significantly lower $\delta^{13}C$ values than head-started terrapins, and significantly higher $\delta^{15}N$ values than head-started terrapins (**Figure 15**).

Analysis of the food items (House Crickets, Common Periwinkles, Blue Crabs, Marsh Periwinkles, and Reptomin®) revealed a significant variation for $\delta^{13}C$ ($F_{4, 30} = 29.21$, $p < 0.01$) and $\delta^{15}N$ ($F_{4, 30} = 6.73$, $p < 0.01$) seen in Figure 17. Bonferroni post-hoc test for $\delta^{13}C$ and $\delta^{15}N$ indicated a significant variation in food items seen in Table 10 and Table 11, respectfully.

Statistical Analysis of Feeding Experiments- The results from the four-feeding experiment are shown in Table 12 and Figures 18, 19, 20, and 21. Analysis of the results of the 14-day feeding experiments reveal statistically significant variation with any of the diets: House Cricket feeding experiment, $\delta^{13}C$ ($F_{2, 10} = 0.05$, $p = 0.96$) and $\delta^{15}N$ ($F_{2, 10} = 0.39$, $p = 0.69$); Common Periwinkle feeding experiment, $\delta^{13}C$ ($F_{2, 6} = 1.39$, $p = 0.32$) and $\delta^{15}N$ ($F_{2, 6} = 2.64$, $p = 0.15$); Blue Crab feeding experiment, $\delta^{13}C$ ($F_{1, 8} = 2.64$, $p = 0.14$) and $\delta^{15}N$ ($F_{1, 8} = 0.65$, $p = 0.44$); Marsh Periwinkle feeding experiment, $\delta^{13}C$ ($F_{1, 8} = 1.22$, $p = 0.30$) and $\delta^{15}N$ ($F_{1, 8} = 0.89$, $p = 0.37$).

Table 1. Mississippi Diamondback Terrapin (*Malaclemys terrapin pileata*), primary producers, and potential prey items δ^{13}C and δ^{15}N values in "‰." Values in the table are mean ± standard deviation (range).

Group	***n***	**δ^{13}C**	**δ^{15}N**
***Malaclemys terrapin pileata* (Mississippi Diamondback Terrapin)**			
Wild	41	-23.32 ± 1.56 (-27.44 to -19.76)	9.66 ± 1.04 (8.21 to 12.48)
Head Start	37	-21.70 ± 0.67 (-22.55 to -19.04)	8.36 ± 0.84 (6.6 to 10.36)
Vegetation			
***Spartina alterniflora* (Saltmarsh Cordgrass)**	18	-14.90 ± 3.86 (-25.58 to -12.57)	6.33 ± 1.83 (2.1 to 9.28)
***Juncus gerardi* (Blackneedle Rush)**	4	-27.16 ± 1.35 (-29.00 to -26.09)	5.77 ± 0.23 (5.45 to 5.98)
Potential Prey Items			
***Callinectes sapidus* (Blue Crab)**	15	-23.64 ± 0.90 (-24.04 to -22.10)	8.32 ± 1.59 (6.24 to 10.03)
***Sesarma reticulatum* (Marsh Crab)**	2	-23.78 ± 0.03 (-23.80 to -23.76)	6.91 ± 0.18 (6.78 to 7.04)
***Uca pugnax* (Marsh Fiddler Crab)**	2	-22.36 ± 1.62 (-23.50 to -21.21)	3.19 ± 0.16 (3.08 to 3.30)
***Clibanarius vittatus* (Hermit Crab)**	6	-19.40 ± 3.23 (-22.42 to -13.59)	13.40 ± 6.87 (7.00 to 22.56)
***Littorina irroratta* (Marsh Periwinkle)**	72	-16.63 ± 2.43 (-23.43 to -11.71)	5.77 ± 1.14 (3.27 to 8.10)
***Vitta usnea* (Olive Nerite)**	17	-18.74 ± 2.74 (-22.45 to -17.17)	7.17 ± 1.85 (5.42 to 10.02)
***Crassostrea virginica* (Eastern Oyster)**	5	-24.00 ± 0.57 (-24.75 to -23.43)	7.78 ± 0.50 (6.98 to 8.29)

Table 2. δ^{13}C and δ^{15}N values of the Mississippi Diamondback Terrapin from Alabama (*Malaclemys terrapin pileata*) versus Mangrove Diamondback Terrapin from Florida (*Malaclemys terrapin rhizophorarum*). Values from Florida derived from Denton et al. (2019) and are mean ± standard deviation (range).

Group	**Location**	**δ^{13}C**	**δ^{15}N**
Malaclemys terrapin pileata	Heron Bay, AL	-23.32 ± 1.56 (-27.44 to -19.76)	9.66 ± 1.04 (8.21 to 12.48)
Malaclemys terrapin rhizophorarum	Big Sable Creek, FL	-24.00 ± 0.90 (-25.90 to -22.3)	6.20 ± 1.00 (3.90 to 7.70)
	Florida Bay, FL	-20.60 ± 1.70 (-24.60 to -18.20)	7.20 ± 0.90 (5.50 to 8.70)
	Key West, FL	-17.20 ± 0.70 (-18.80 to -15.70)	4.70 ± 0.70 (2.80 to 5.90)

Table 3. Prey items found in the fecal contents of adult female *Malaclemys terrapin pileata* ($n = 9$).

Category	**Percent Dry Mass**
Geukensia demissa	45.8
Littoraria irrorata	45.3
Unidentified Bivalves	5.8
Other	3.1

Table 4. Comparison of δ^{13}C values of the primary producers and potential prey items using ANOVA (NS = not statistically significant, * = $p > 0.05$, ** = $p > 0.01$, *** = $p > 0.001$).

δ^{13}C	***Spartina alterniflora* (Saltmarsh Cordgrass)**	***Juncus gerardi* (Blackneedle Rush)**	***Callinectes sapidus* (Blue Crab)**	***Sesarma reticulatum* (Marsh Crab)**	***Uca pugnax* (Marsh Fiddler Crab)**	***Clibanarius vittatus* (Hermit Crab)**	***Littorina irroratta* (Marsh Periwinkle)**	***Vitta usnea* (Olive Nerite)**	***Crassostrea virginica* (Eastern Oyster)**
***Spartina alterniflora* (Saltmarsh Cordgrass)**	NA	***	***	***	**	*	NS	**	***
***Juncus gerardi* (Blackneedle Rush)**	***	NA	NS	NS	NS	***	***	***	NS
***Callinectes sapidus* (Blue Crab)**	***	NS	NA	NS	NS	NS	***	NS	NS
***Sesarma reticulatum* (Marsh Crab)**	***	NS	NS	NA	NS	NS	**	NS	NS
***Uca pugnax* (Marsh Fiddler Crab)**	**	NS	NS	NS	NA	NS	NS	NS	NS
***Clibanarius vittatus* (Hermit Crab)**	*	***	NS	NS	NS	NA	NS	NS	NS
***Littorina irroratta* (Marsh Periwinkle)**	NS	***	***	**	NS	NS	NA	NS	***
***Vitta usnea* (Olive Nerite)**	**	***	NS	NS	NS	NS	NS	NA	**
***Crassostrea virginica* (Eastern Oyster)**	***	NS	NS	NS	NS	NS	***	**	NA

Table 5. Comparison of $\delta^{15}N$ values of the primary producers and potential prey items using ANOVA (NS = not statistically significant, * = *p* > 0.05, ** = *p* > 0.01, *** = *p* > 0.001).

$\delta^{15}N$	***Spartina alterniflora* (Saltmarsh Cordgrass)**	***Juncus gerardi* (Blackneedle Rush)**	***Callinectes sapidus* (Blue Crab)**	***Sesarma reticulatum* (Marsh Crab)**	***Uca pugnax* (Marsh Fiddler Crab)**	***Clibanarius vittatus* (Hermit Crab)**	***Littorina irroratta* (Marsh Periwinkle)**	***Vitta usnea* (Olive Nerite)**	***Crassostrea virginica* (Eastern Oyster)**
***Spartina alterniflora* (Saltmarsh Cordgrass)**	NA	NS	NS	NS	NS	***	NS	NS	NS
***Juncus gerardi* (Blackneedle Rush)**	NS	NA	NS	NS	NS	***	NS	NS	NS
***Callinectes sapidus* (Blue Crab)**	NS	NS	NA	NS	NS	***	NS	NS	NS
***Sesarma reticulatum* (Marsh Crab)**	NS	NS	NS	NA	NS	**	NS	NS	NS
***Uca pugnax* (Marsh Fiddler Crab)**	NS	NS	NS	NS	NA	***	NS	NS	NS
***Clibanarius vittatus* (Hermit Crab)**	***	***	***	**	***	NA	***	***	***
***Littorina irroratta* (Marsh Periwinkle)**	NS	NS	NS	NS	NS	***	NS	NS	NS
***Vitta usnea* (Olive Nerite)**	NS	NS	NS	NS	NS	***	NS	NA	NS
***Crassostrea virginica* (Eastern Oyster)**	NS	NS	NS	NS	NS	***	NS	NS	NA

Table 6. Comparison of $\delta^{13}C$ and $\delta^{15}N$ values of the potential prey items versus wild terrapins using ANOVA (NS = not statistically significant, * = *p* > 0.05, ** = *p* > 0.01, *** = *p* > 0.001).

Wild Terrapin Stable Isotope	**Blue Crab**	**Marsh Fiddler Crab**	**Marsh Crab**	**Hermit Crab**	**Marsh Periwinkle**	**Olive Nerite**	**Eastern Oyster**
$\delta^{13}C$	NS	NS	NS	**	***	***	NS
$\delta^{15}N$	NS	***	NS	***	***	***	NS

Table 7. Marsh Periwinkle Isoscape of δ^{13}C and δ^{15}N values in "‰" from different Mississippi Sound locations. Values in the table are mean ± standard deviation (range).

Location	*n*	δ^{13}C	δ^{15}N
Airport Marsh	5	-14.91 ± 0.95 (-16.04 to -14.00)	5.14 ± 0.23 (4.82 to 5.43)
Bayou Coden	5	-19.49 ± 3.11 (-23.22 to -15.07)	4.34 ± 1.10 (3.27 to 5.94)
Bayou La Batre	5	-15.39 ± 0.69 (-16.23 to -14.51)	3.84 ± 0.62 (3.35 to 4.80)
Cedar Point Marsh-North	5	-15.59 ± 1.04 (-16.87 to -14.11)	5.30 ± 0.15 (5.20 to 5.55)
Cedar Point Marsh-South	5	-16.64 ± 0.66 (-17.55 to -15.70)	6.47 ± 0.21 (6.26 to 6.71)
Fowl River	5	-21.58 ± 1.35 (-23.43 to -20.27)	5.00 ± 0.22 (4.76 to 5.26)
Jemison Marsh	5	-17.61 ± 2.03 (-19.46 to -14.51)	6.32 ± 0.61 (5.33 to 6.86)
Lightning Point	5	-16.32 ± 1.23 (-17.47 to -14.42)	6.76 ± 0.81 (6.04 to 7.44)
Point Aux Pines	5	-16.66 ± 1.66 (-18.82 to -14.58)	6.15 ± 0.37 (5.56 to 6.53)

Table 8. Analysis of δ^{13}C values of Marsh Periwinkle sampled in different locations within the Mississippi Sound using ANOVA (NS = not statistically significant, * = $p > 0.05$, ** = $p > 0.01$, *** = $p > 0.001$).

δ^{13}C	Airport Marsh	Bayou Coden	Bayou La Batre	Cedar Point Marsh-North	Cedar Point Marsh-South	Fowl River	Jemison Marsh	Lightning Point	Point Aux Pines
Airport Marsh	NA	**	NS	NS	NS	***	NS	NS	NS
Bayou Coden	**	NA	**	*	NS	NS	NS	NS	NS
Bayou La Batre	NS	**	NA	NS	NS	***	NS	NS	NS
Cedar Point Marsh-North	NS	*	NS	NA	NS	***	NS	NS	NS
Cedar Point Marsh-South	NS	NS	NS	NS	NA	***	NS	NS	NS
Fowl River	***	NS	***	***	***	NA	*	***	***
Jemison Marsh	NS	NS	NS	NS	NS	*	NA	NS	NS
Lightning Point	NS	NS	NS	NS	NS	***	NS	NA	NS
Point Aux Pines	NS	NS	NS	NS	NS	***	NS	NS	NA

Table 9. Comparison of δ^{15}N values of Marsh Periwinkle sampled in different locations within the Mississippi Sound using ANOVA (NS = not statistically significant, * = $p > 0.05$, ** = $p > 0.01$, *** = $p > 0.001$).

δ^{15}N	**Airport Marsh**	**Bayou Coden**	**Bayou La Batre**	**Cedar Point Marsh-North**	**Cedar Point Marsh-South**	**Fowl River**	**Jemison Marsh**	**Lightning Point**	**Point Aux Pines**
Airport Marsh	NA	NS	*	*	NS	NS	NS	**	NS
Bayou Coden	NS	NA	NS	NS	***	NS	***	***	***
Bayou La Batre	*	NS	NA	*	***	NS	***	***	***
Cedar Point Marsh-North	NS	NS	*	NA	NS	NS	NS	**	NS
Cedar Point Marsh-South	*	***	***	NS	NA	**	NS	NS	NS
Fowl River	NS	NS	NS	NS	**	NA	*	***	NS
Jemison Marsh	NS	***	***	NS	NS	*	NA	NS	NS
Lightning Point	**	***	***	**	NS	***	NS	NA	NS
Point Aux Pines	NS	***	***	NS	NS	NS	NS	NS	NA

Table 10. Comparison of δ^{13}C values of the food items from the feeding experiments using ANOVA (NS = not statistically significant, * = $p > 0.05$, ** = $p > 0.01$, *** = $p > 0.001$).

δ^{13}C	***Callinectes sapidus* (Blue Crab)**	***Littorina littorea* (Common Periwinkle)**	***Acheta domesticus* (House Crickets)**	***Littorina irrorata* (Marsh Periwinkle)**	**Reptomin®**
***Callinectes sapidus* (Blue Crab)**	NA	***	NS	***	NS
***Littorina littorea* (Common Periwinkle)**	***	NA	***	NS	***
***Acheta domesticus* (House Crickets)**	NS	***	NA	***	NS
***Littorina irrorata* (Marsh Periwinkle)**	***	NS	***	NA	***
Reptomin®	NS	***	NS	***	NA

Table 11. Comparison of δ^{15}N values of the food items from the feeding experiments using ANOVA (NS = not statistically significant, * = $p > 0.05$, ** = $p > 0.01$, *** = $p > 0.001$).

δ^{15}N	***Callinectes sapidus* (Blue Crab)**	***Littorina littorea* (Common Periwinkle)**	***Acheta domesticus* (House Crickets)**	***Littorina irrorata* (Marsh Periwinkle)**	**Reptomin®**
***Callinectes sapidus* (Blue Crab)**	NA	**	***	***	***
***Littorina littorea* (Common Periwinkle)**	**	NA	NS	NS	NS
***Acheta domesticus* (House Crickets)**	***	NS	NA	NS	NS
***Littorina irrorata* (Marsh Periwinkle)**	***	NS	NS	NA	NS
Reptomin®	***	NS	NS	NS	NA

Table 12. δ^{13}C and δ^{15}N values in ‰ for the four feeding experiments. Values in the table are mean ± standard deviation (range).

			Day 0		**Day 7**
Feeding Experiment	**Group**	***n***	**δ^{13}C**	**δ^{15}N**	**δ^{13}C**
House Cricket	Reptomin	6	-22.20 ± 0.41 (-22.55 to -21.65)	8.37 ± 0.59 (7.40 to 9.08)	-21.78 ± 0.32 (-22.28 to -21.50)
	House Cricket	6	-21.73 ± 0.37 (-22.37 to -21.40)	8.16 ± 0.66 (7.05 to 8.72)	-21.70 ± 0.22 (-21.89 to -21.38)
Common Periwinkle	Reptomin	4	-21.70 ± 0.46 (-21.80 to -21.53)	8.22 ± 0.46 (7.78 to 8.73)	-21.84 ± 0.20 (-22.08 to -21.52)
	Common Periwinkle	4	-21.52 ± 0.29 (-21.81 to -21.16)	8.03 ± 0.59 (7.01 to 8.76)	-21.31 ± 0.59 (-21.96 to -20.62)
Blue Crab	Reptomin	9	-21.47 ± 0.75 (-22.18 to -20.18)	8.93 ± 0.92 (7.72 to 10.36)	
	Blue Crab	9	-21.56 ± 0.96 (-22.21 to -19.04)	7.88 ± 0.74 (7.04 to 9.09)	
Marsh Periwinkle	Reptomin	9	-21.47 ± 0.75 (-22.18 to -20.18)	8.93 ± 0.92 (7.72 to 10.36)	
	Marsh Periwinkle	9	-21.88 ± 0.37 (-22.35 to -21.23)	8.25 ± 1.35 (5.57 to 9.69)	
			Day 7	**Day 14**	
			δ^{15}N	**δ^{13}C**	**δ^{15}N**
House Cricket	Reptomin	6	7.89 ± 0.48 (7.42 to 8.70)	-21.70 ± 0.28 (-22.04 to -21.39)	8.20 ± 0.57 (7.64 to 8.84)
	House Cricket	6	7.99 ± 0.35 (7.47 to 8.53)	-21.78 ± 0.47 (-22.60 to -21.28)	8.31 ± 0.65 (7.43 to 9.22)
Common Periwinkle	Reptomin	6	8.48 ± 0.33 (8.02 to 8.80)	-21.70 ± 0.13 (-21.95 to -21.59)	8.51 ± 0.52 (7.83 to 9.08)
	Common Periwinkle	4	8.30 ± 0.34 (7.93 to 8.68)	-21.14 ± 0.28 (-21.33 to -20.74)	8.67 ± 0.16 (8.49 to 8.83)
Blue Crab	Reptomin	9		-21.13 ± 0.78 (-21.91 to -19.68)	8.65 ± 0.80 (7.51 to 9.96)
	Blue Crab	9		-21.11 ± 0.30 (-22.58 to -21.56)	8.28 ± 1.40 (5.79 to 10.08)
Marsh Periwinkle	Reptomin	9		-21.13 ± 0.78 (-21.91 to -19.68)	8.65 ± 0.80 (7.51 to 9.96)
	Marsh Periwinkle	9		-21.75 ± 0.24 (-22.05 to -21.40)	8.71 ± 1.03 (7.22 to 10.6)

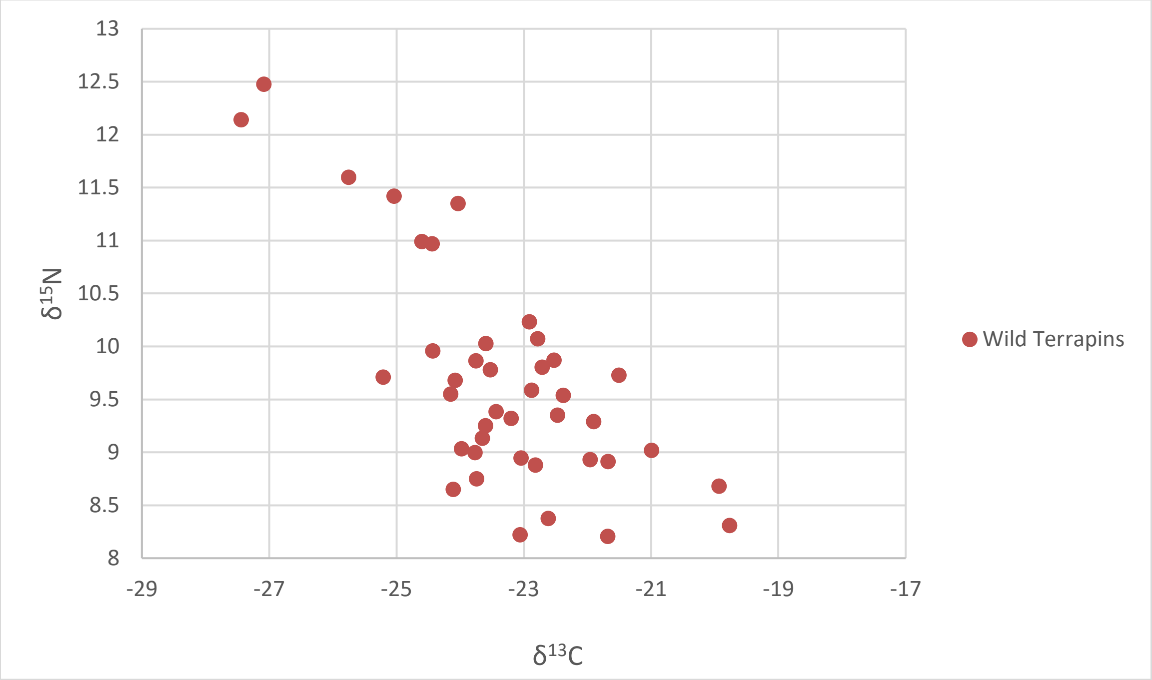

Figure 4. δ^{13}C and δ^{15}N isotopic plot for wild terrapins (*n* = 41) caught across three nesting seasons (2019, 2021, and 2022) within the Mississippi Sound.

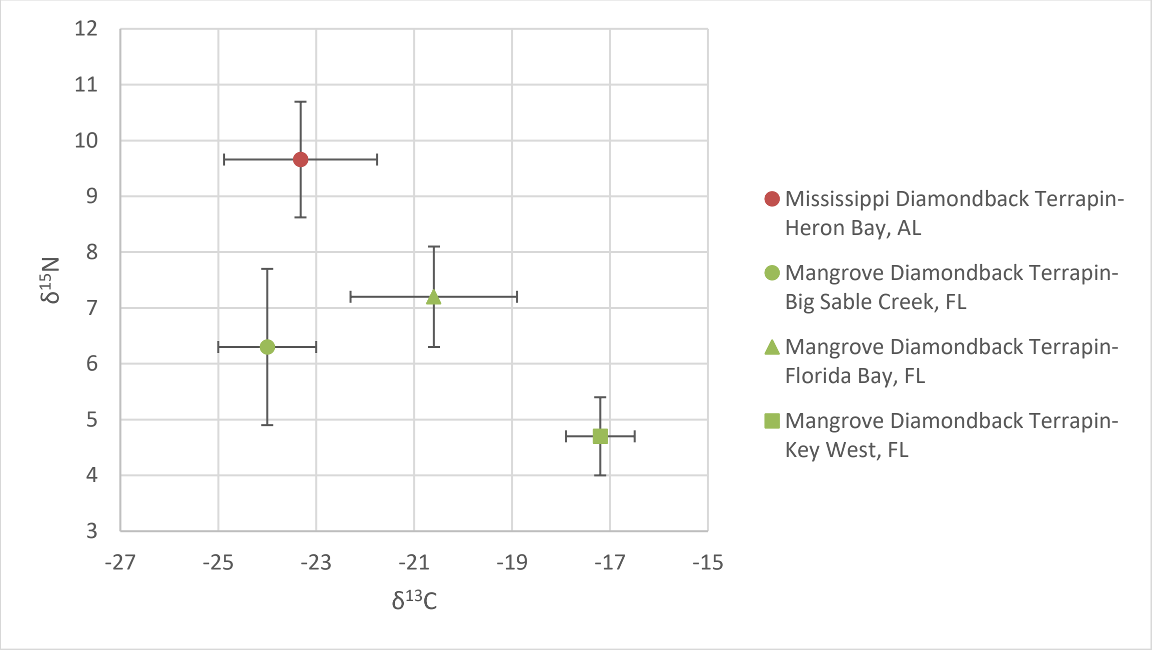

Figure 5. δ^{13}C and δ^{15}N isoscape (mean and standard deviation) of wild terrapins across Alabama and Florida (Mississippi Diamondback Terrapin (*Malaclemys terrapin pileata*) at Cedar Point Marsh (*n* = 41), Alabama, and the Mangrove Diamondback Terrapin (*Malaclemys terrapin rhizophorarum*) at Big Sable Creek (*n* = 21), Florida Bay (*n* = 18), and Key West (*n* = 23), Florida). Values from Florida derived from Denton et al. (2019).

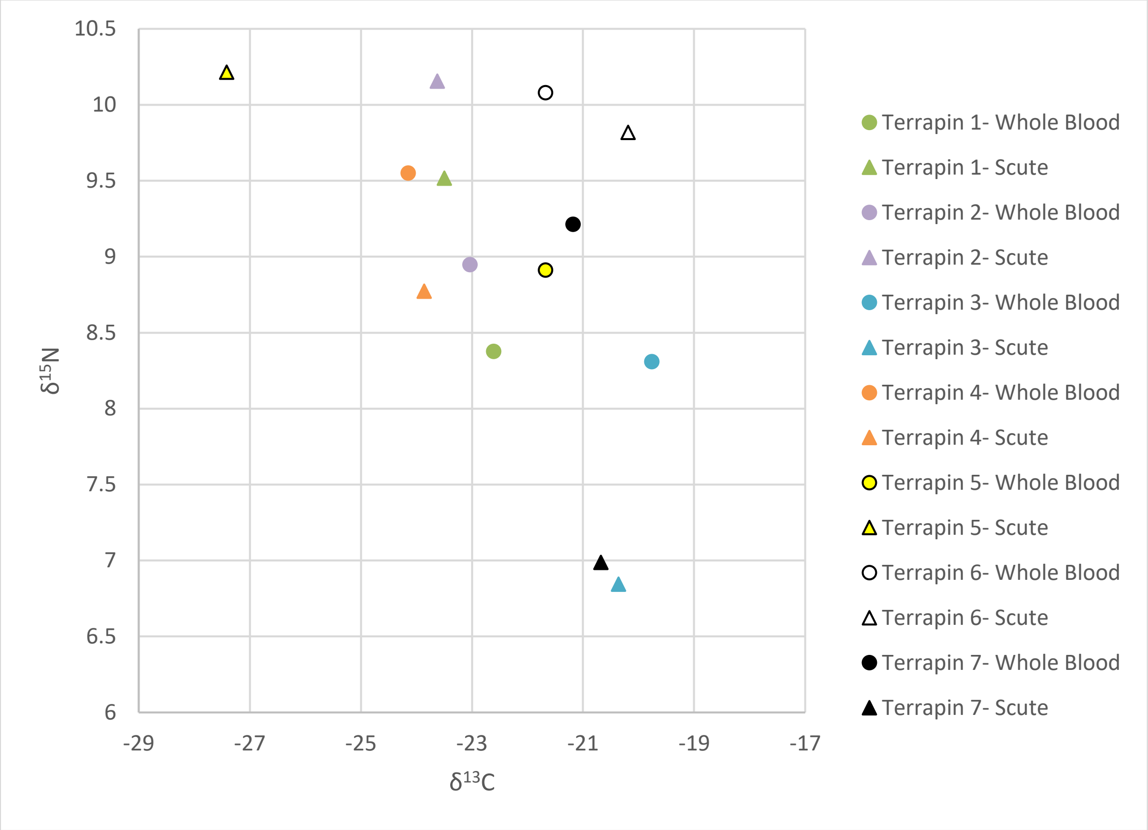

Figure 6. $\delta^{13}C$ and $\delta^{15}N$ isotopic plot for Mississippi Diamondback Terrapin (*Malaclemys terrapin pileata*) whole blood samples and their corresponding carapace scute samples (*n* = 7 individuals sampled).

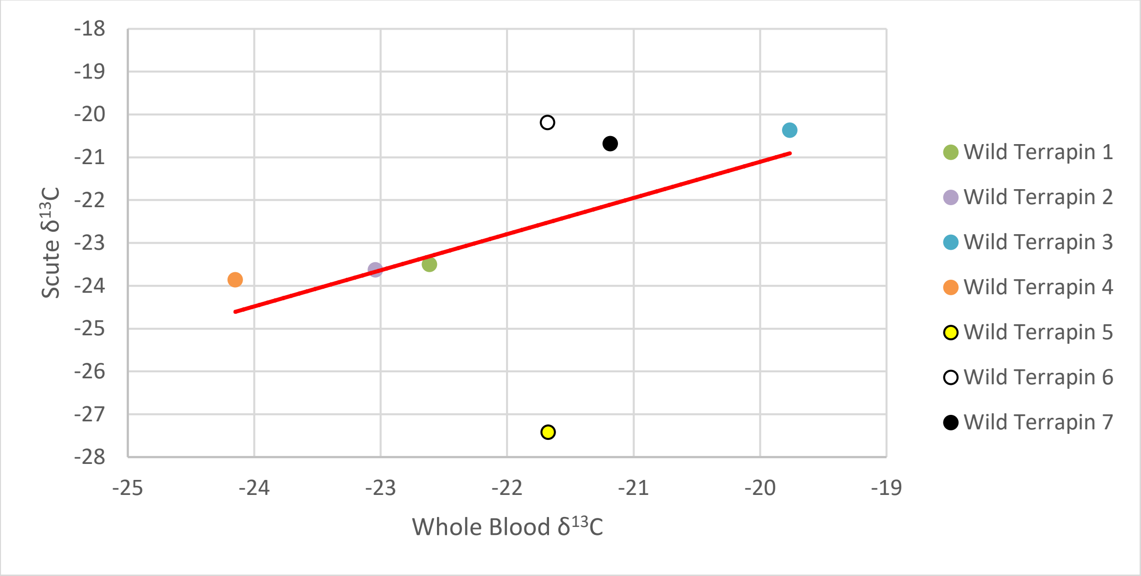

Figure 7. Linear regression ($R^2 = 0.21$) plot for $\delta^{13}C$ of whole blood and scute sampled collected from Mississippi Diamondback Terrapin (*Malaclemys terrapin pileata*) ($n = 7$ individuals sampled).

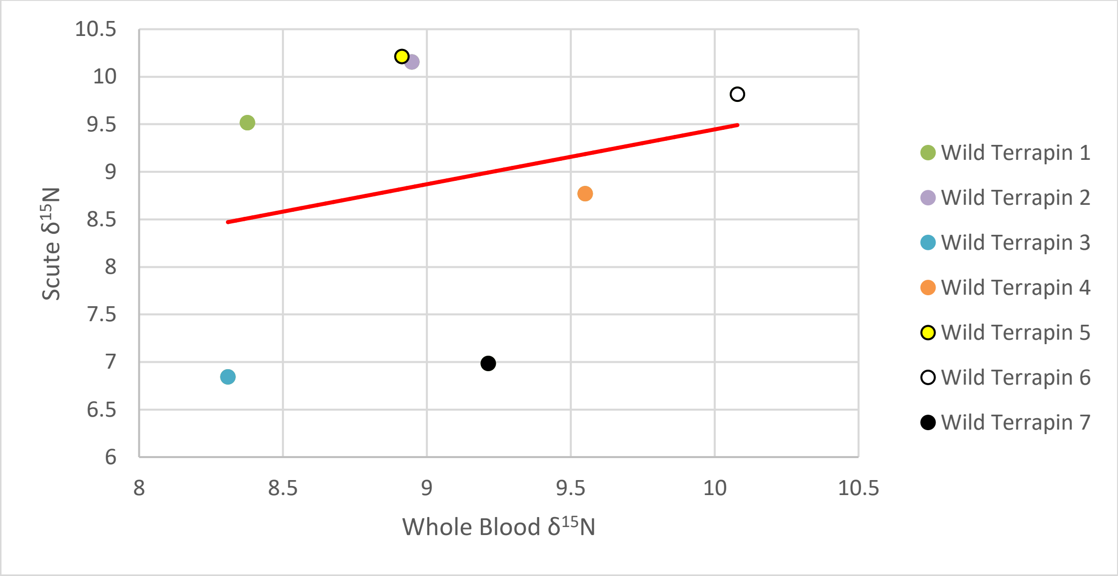

Figure 8. Linear regression plot ($R^2 = 0.06$) for $\delta^{15}N$ of whole blood and scute sampled collected from Mississippi Diamondback Terrapin (*Malaclemys terrapin pileate*) ($n = 7$ individuals sampled).

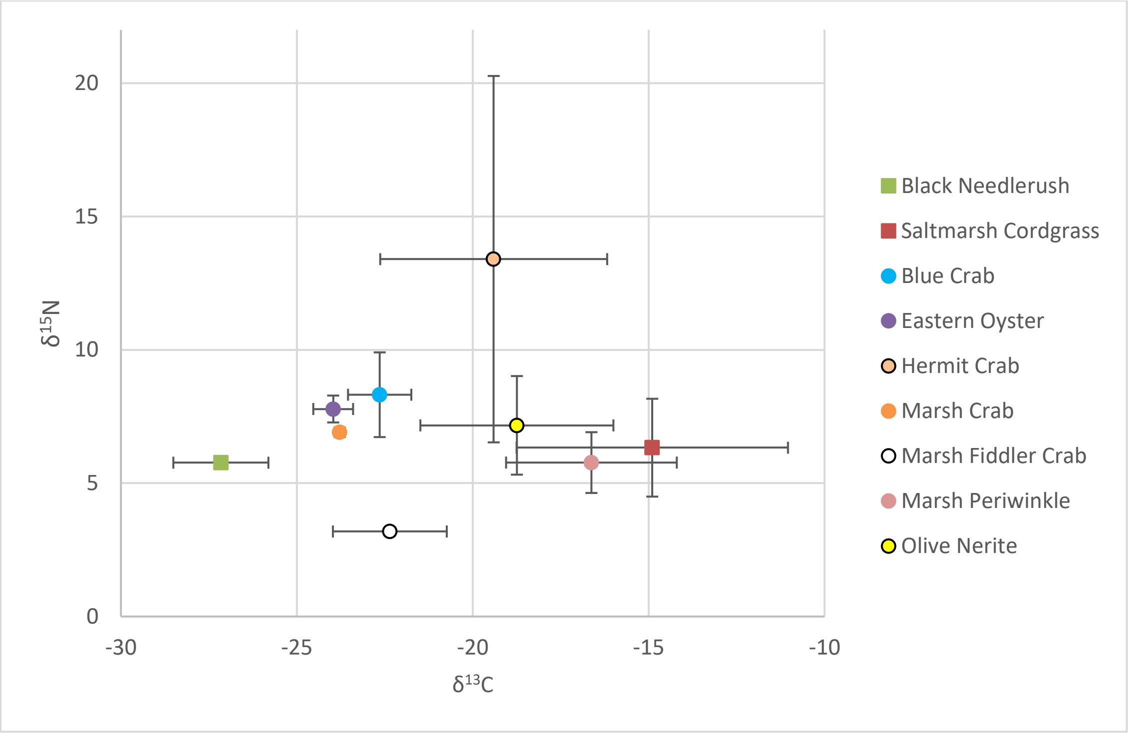

Figure 9. δ^{13}C and δ^{15}N overall mean and standard deviation for primary producers and common prey items of the Mississippi Diamondback Terrapin (*Malaclemys terrapin pileata*) sampled a prey items within Heron Bay [Black Needlerush (*Juncus gerardi*, *n* = 4), Saltmarsh Cordgrass (*Spartina alterniflora*, *n* = 38), Blue Crabs (*Callinectes sapidus*, *n* = 5), Eastern Oysters (*Crassostrea virginica*, *n* = 5), Hermit Crabs (*Clibanarius vittatus*, *n* = 6), Marsh Crabs (*Sesarma reticulatum*, *n* = 2), Marsh Fiddler Crabs (*Uca* pugnax, *n* = 2), Marsh Periwinkles (*Littorina irroratta*, *n* = 72), and Olive Nerites (*Vitta usnea*, *n* = 17)].

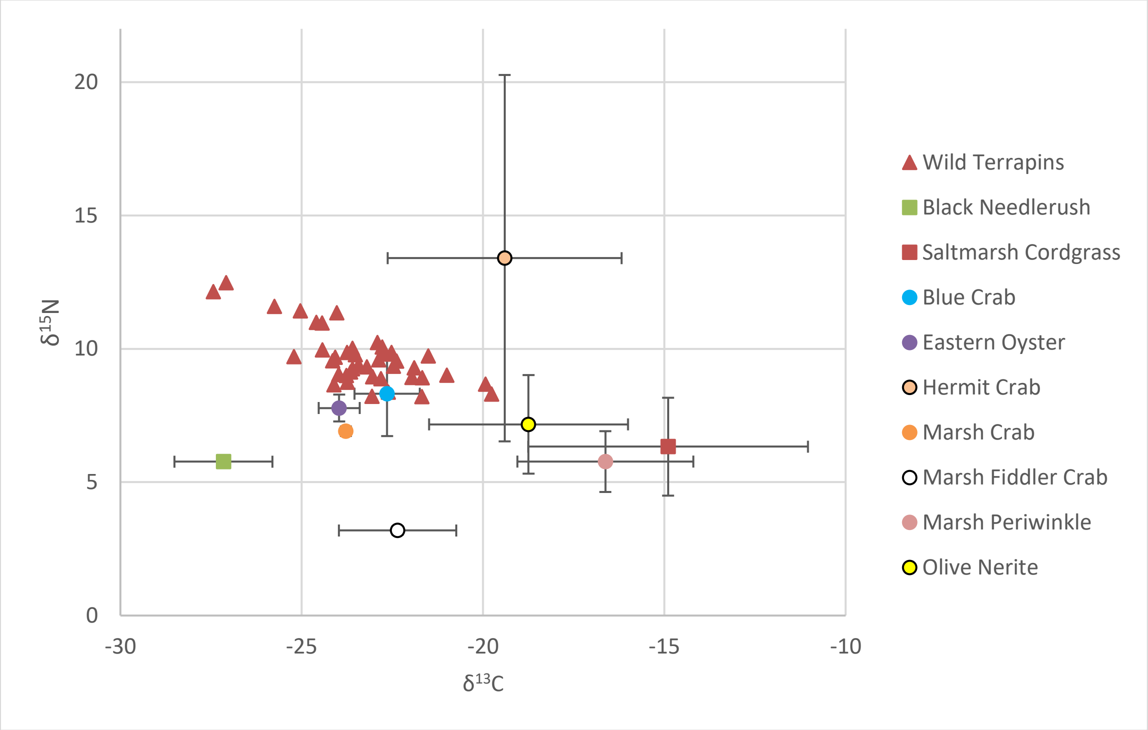

Figure 10. $\delta^{13}C$ and $\delta^{15}N$ overall mean and standard deviation for common salt marsh grasses and terrapin prey items compared with wild terrapins [Mississippi Diamondback Terrapins (*Malaclemys terrapin pileata*, *n* = 41), Black Needlerush (*Juncus gerardi*, *n* = 4), Saltmarsh Cordgrass (*Spartina alterniflora*, *n* = 38), Olive nerites (*Vitta usnea*, *n* = 17), Blue crabs (*Callinectes sapidus*, *n* = 5), Eastern oysters (*Crassostrea virginica*, *n* = 5), Marsh fiddler crabs (*Uca pugnax*, *n* = 2), Marsh crabs (*Sesarma reticulatum*, *n* = 2), Hermit crabs (*Clibanarius vittatus*, *n* = 6), and Marsh Periwinkles (*Littorina irroratta*, *n* = 72)].

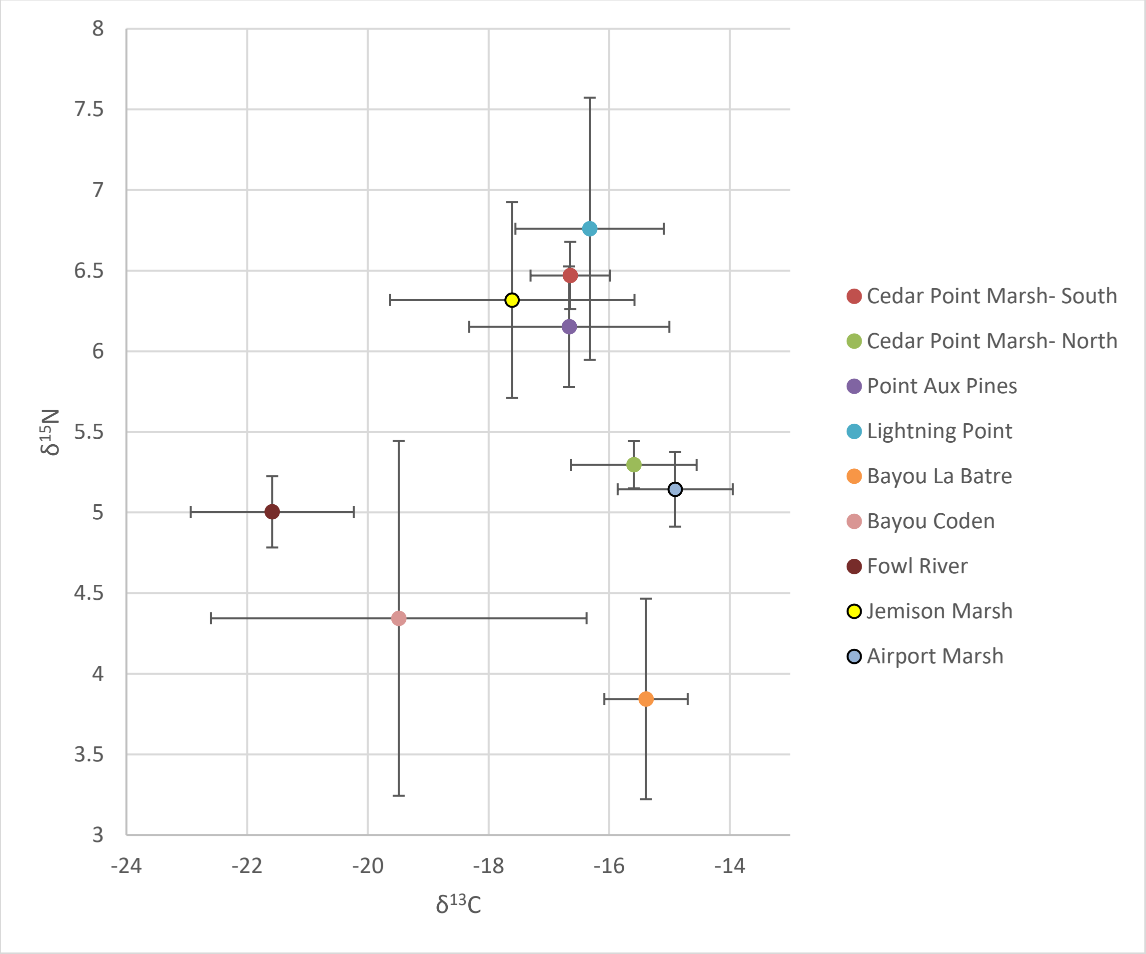

Figure 11. $\delta^{13}C$ and $\delta^{15}N$ overall mean and standard deviation of Marsh Periwinkles (*Littorina irroratta*) captured throughout the Mississippi Sound [Cedar Point Marsh-South (*n* = 5), Cedar Point Marsh- North (*n* = 5), Point Aux Pines (*n* = 5), Lightning Point (*n* = 5), Bayou La Batre (*n* = 5), Bayou Coden (*n* = 5), Fowl River (*n* = 5), Jemison Marsh (*n* = 5), and Airport Marsh (*n* = 5)].

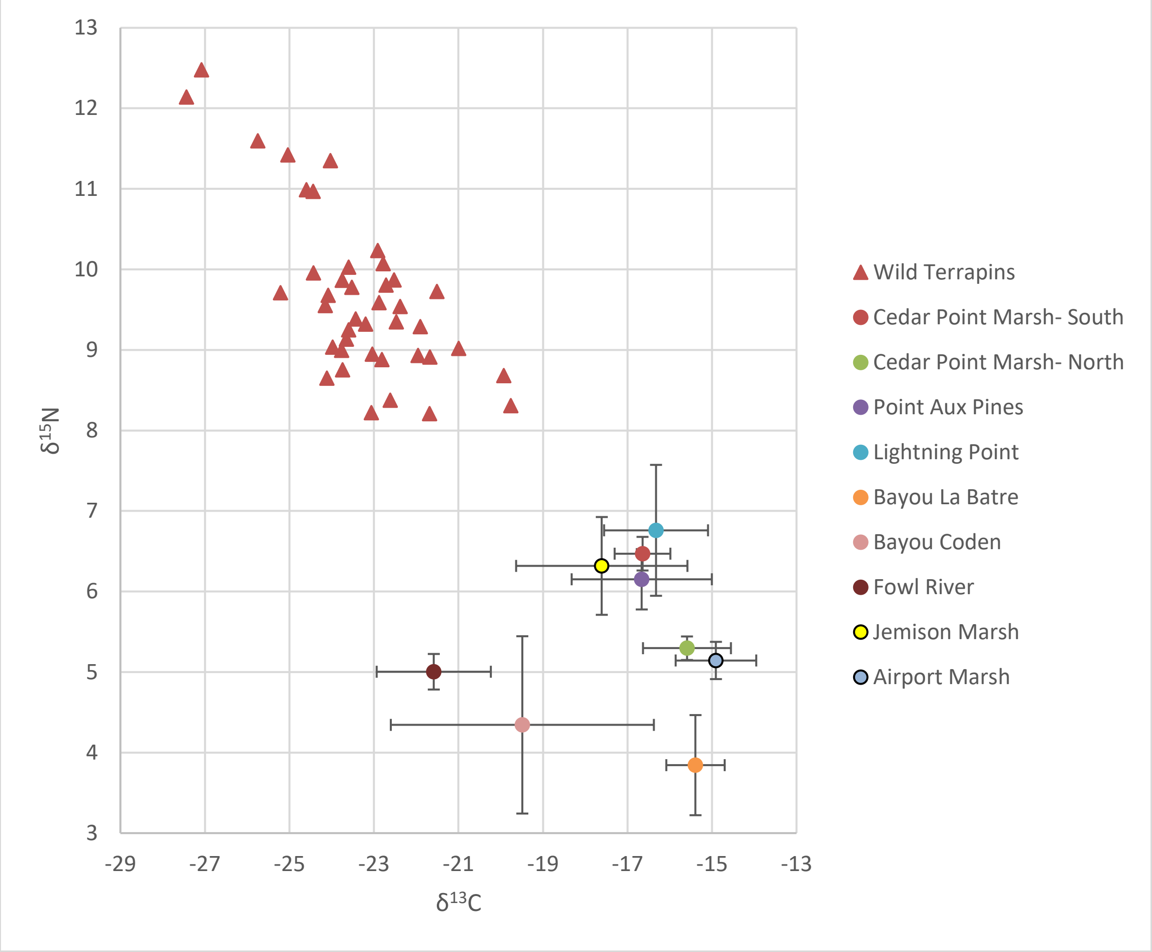

Figure 12. $\delta^{13}C$ and $\delta^{15}N$ overall mean and standard deviation of Marsh Periwinkles (*Littorina irroratta*) captured throughout the Mississippi Sound [Cedar Point Marsh- South (*n* = 5), Cedar Point Marsh- North (*n* = 5), Point Aux Pines (*n* = 5), Lightning Point (*n* = 5), Bayou La Batre (*n* = 5), Bayou Coden (*n* = 5), Fowl River (*n* = 5), Jemison Marsh (*n* = 5), and Airport Marsh (*n* = 5)] and Mississippi Diamondback Terrapins (*Malaclemys terrapin pileata*) caught throughout Heron Bay (*n* = 41).

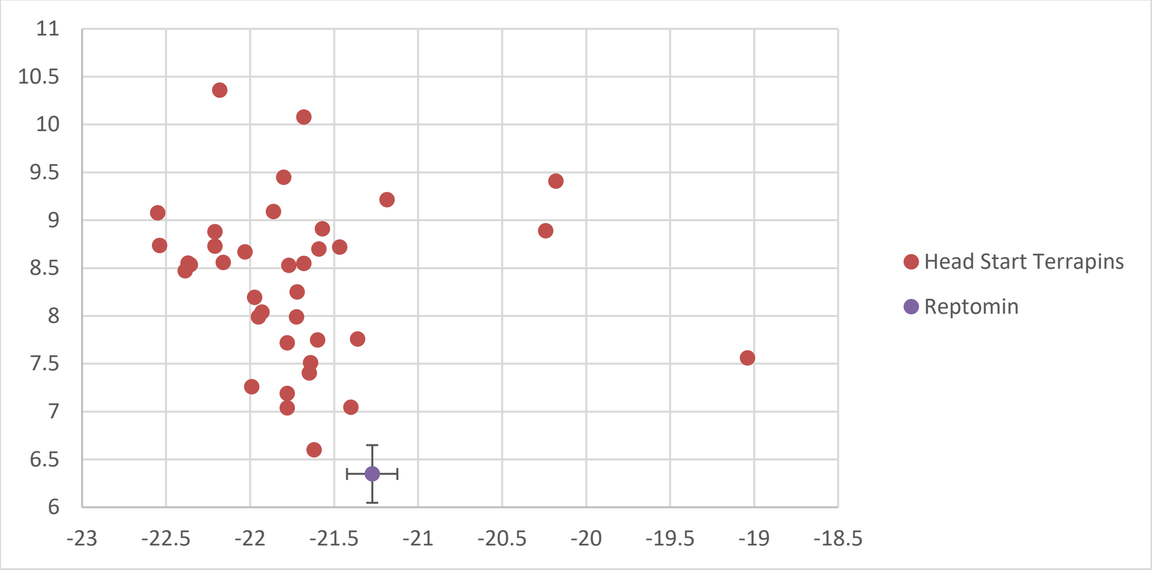

Figure 13. $\delta^{13}C$ and $\delta^{15}N$ isotopic plot for head start Mississippi Diamondback Terrapins (*Malaclemys terrapin pileata*; *n* = 37) versus their food, Reptomin® (*n* = 7).

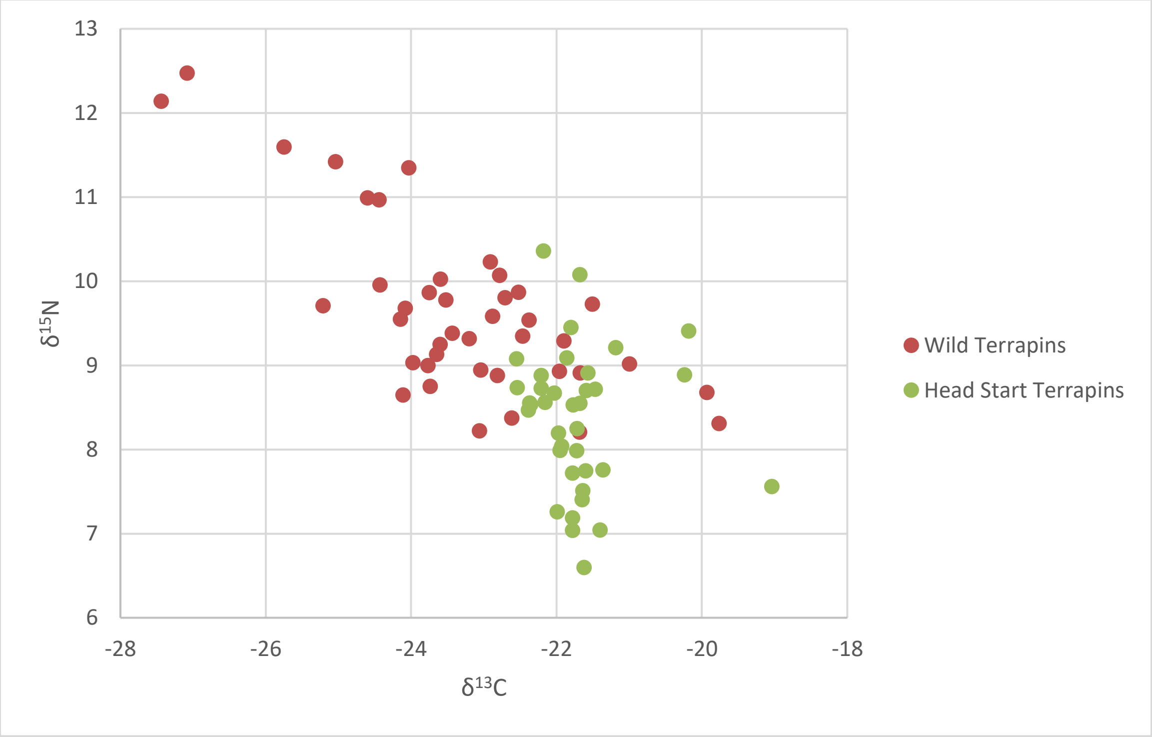

Figure 14. $\delta^{13}C$ and $\delta^{15}N$ isotopic plot for Diamondback Terrapins (*Malaclemys terrapin pileata*; *n* = 41) and head-started Diamondback Terrapins (*n* = 37) over three nesting seasons (2019, 2021, and 2022).

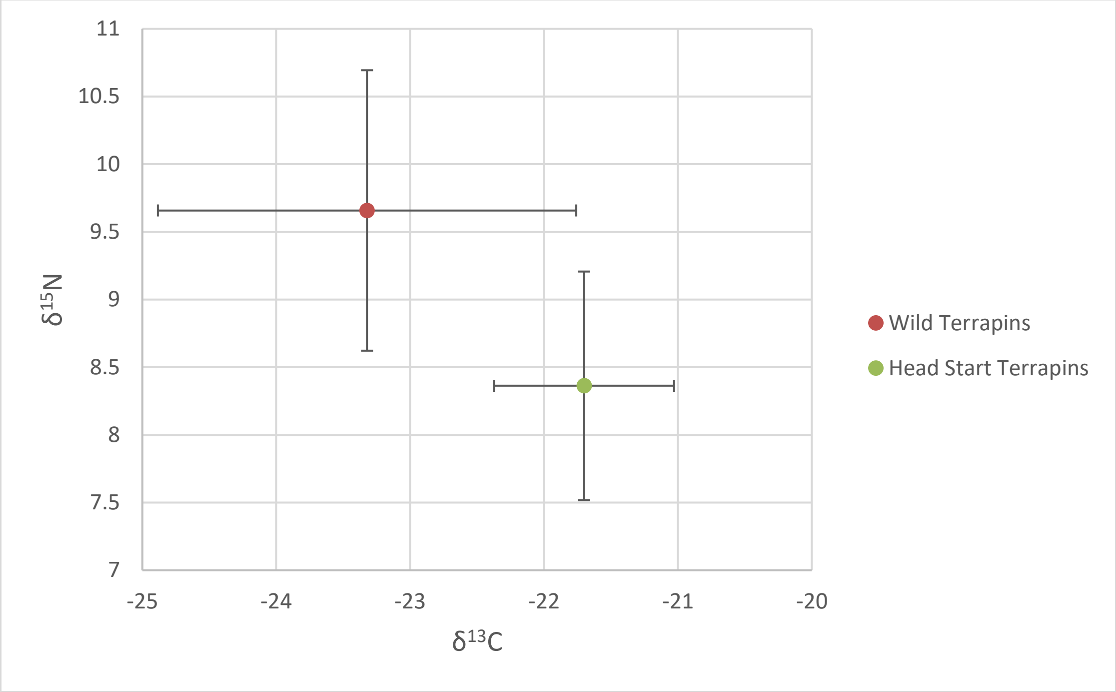

Figure 15. $\delta^{13}C$ and $\delta^{15}N$ mean and standard deviation for Diamondback Terrapins (*Malaclemys terrapin pileata*; *n* = 41) and head-started Diamondback Terrapins (*n* = 37) over two nesting seasons (2019, 2021, and 2022).

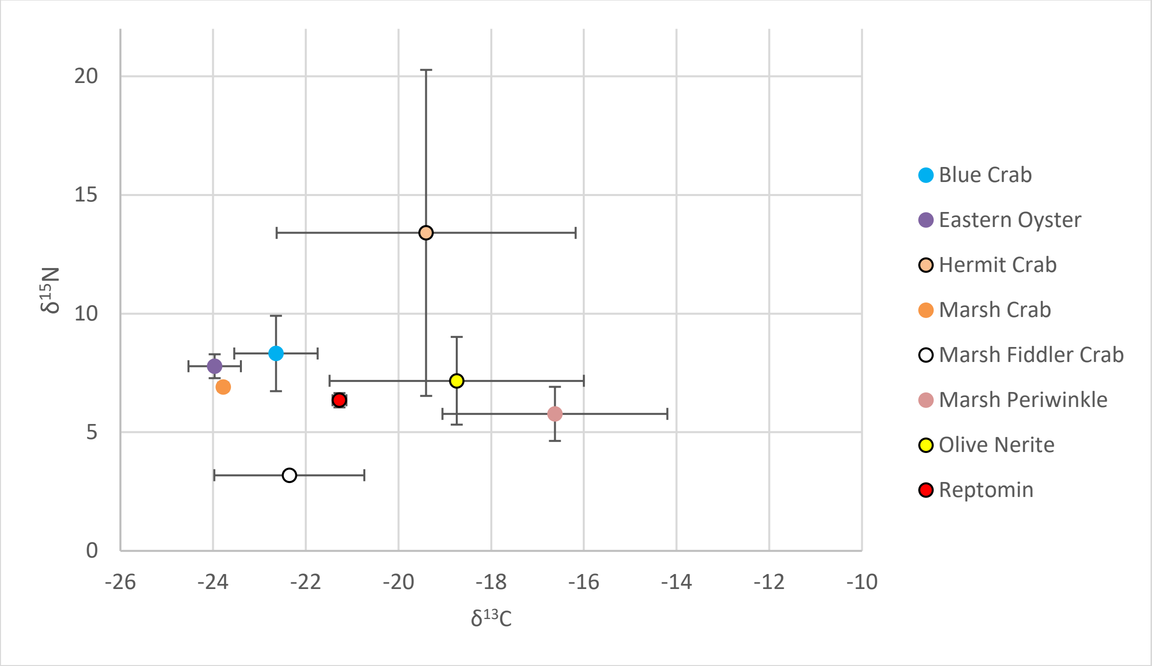

Figure 16. δ^{13}C and δ^{15}N mean and standard deviation of Reptomin® (*n* = 7) versus the the potential prey items [Olive nerites (*Vitta usnea*, *n* = 17), Blue crabs (*Callinectes sapidus*, *n* = 5), Eastern oysters (*Crassostrea virginica*, *n* = 5), Marsh fiddler crabs (*Uca pugnax*, *n* = 2), Marsh crabs (*Sesarma reticulatum*, *n* = 2), Hermit crabs (*Clibanarius vittatus*, *n* = 6), and Marsh Periwinkles (*Littorina irroratta*, *n* = 72)].

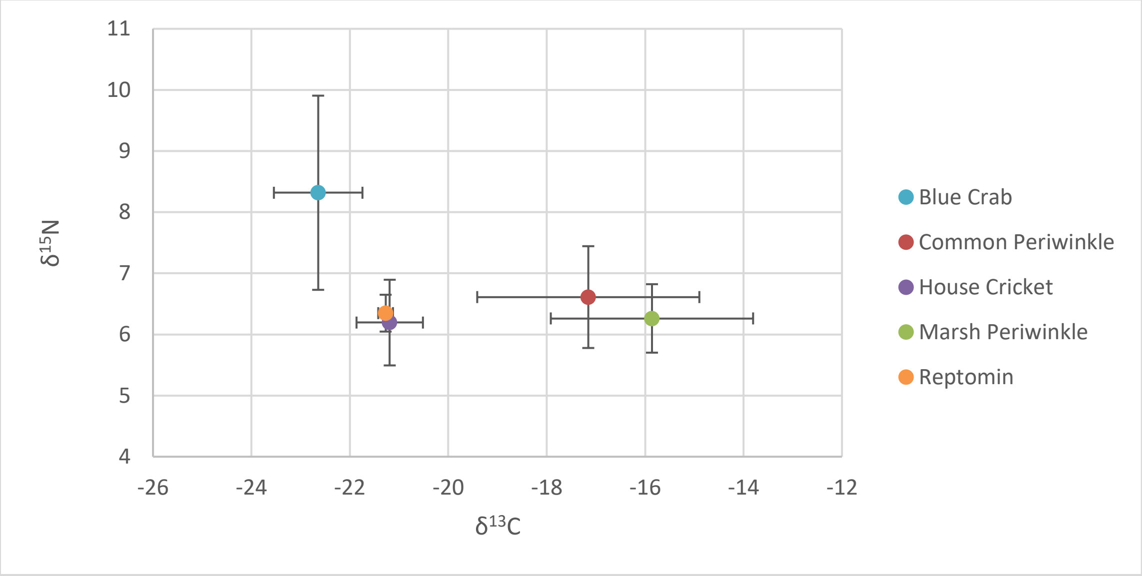

Figure 17. δ^{13}C and δ^{15}N mean and standard deviation for the food items used in the feeding experiments [Blue crabs (*Callinectes sapidus*, *n* = 5), Common Periwinkles (*Littorina littorea*, *n* = 5), House Crickets (*Acheta domesticus*, *n* = 8), Marsh periwinkles (*Littorina irrorate*, *n* = 10), and Reptomin® (*n* = 7)].

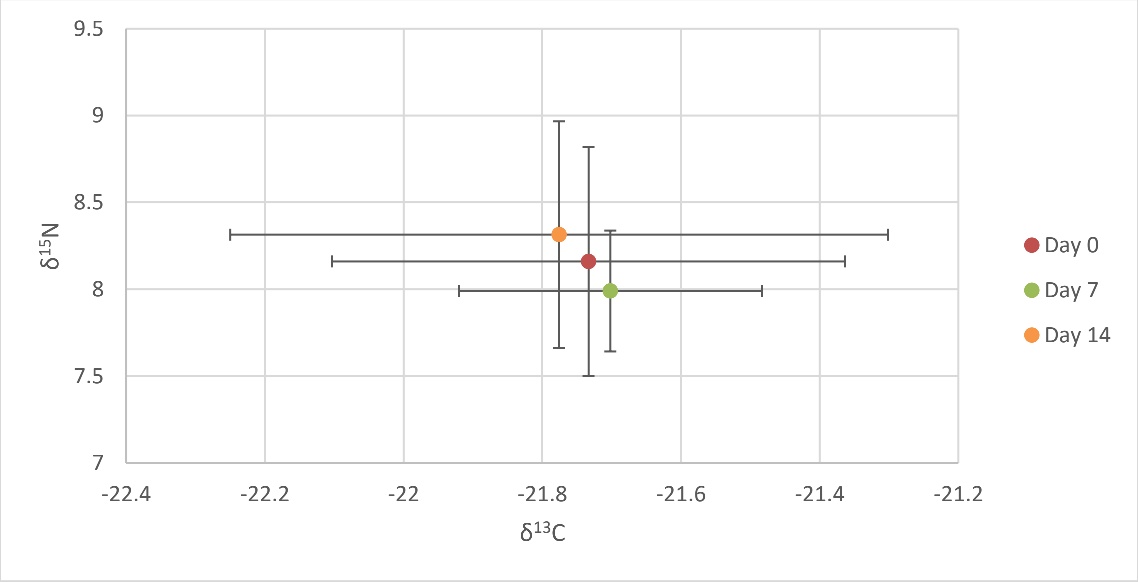

Figure 18. δ^{13}C and δ^{15}N mean and standard deviation of the 14-day House Cricket (*Acheta domesticus*) feeding experiment performed from August 28th, 2020, to September 3rd, 2020 (House Cricket Group, *n* = 6).

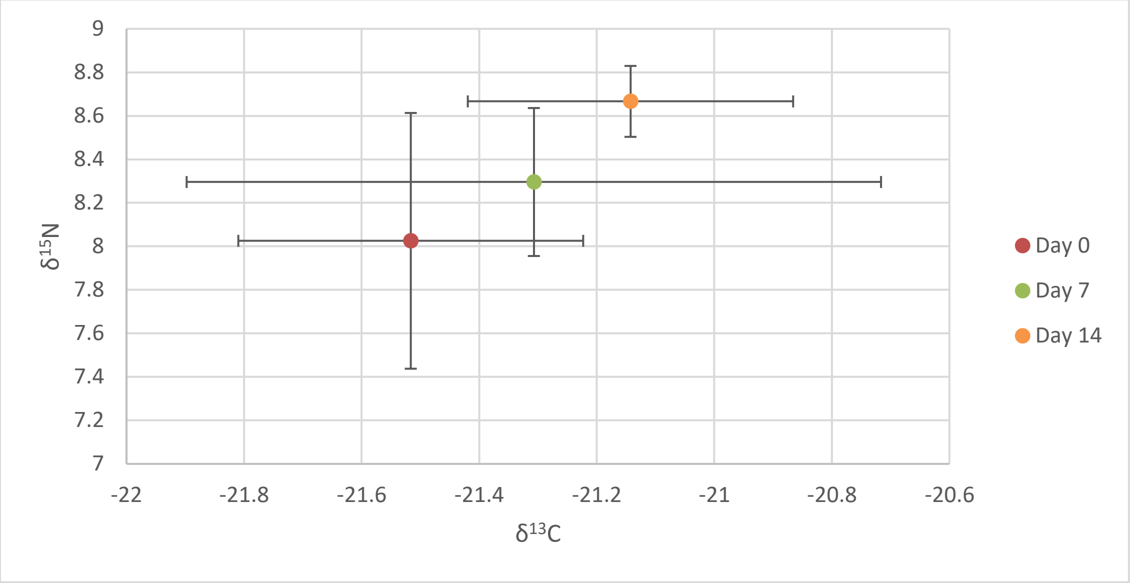

Figure 19. $\delta^{13}C$ and $\delta^{15}N$ mean and standard deviation of the two-week Common Periwinkle (*Littorina littorea*) feeding experiment performed from February 9th, 2021, to March 5th, 2021 (Common Periwinkle Group, *n* = 4).

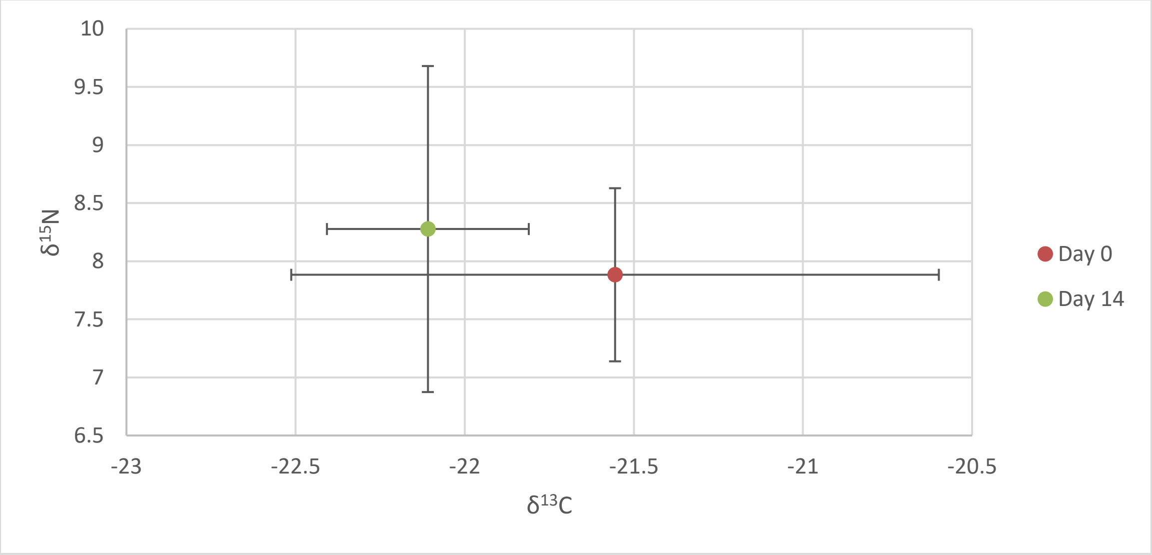

Figure 20. $\delta^{13}C$ and $\delta^{15}N$ mean and standard deviation of the 14-day Blue Crab (*Callinectus sapidus*) feeding experiment performed from November 30th, 2021, to December 30th, 2021 (Blue Crab Group, *n* = 9).

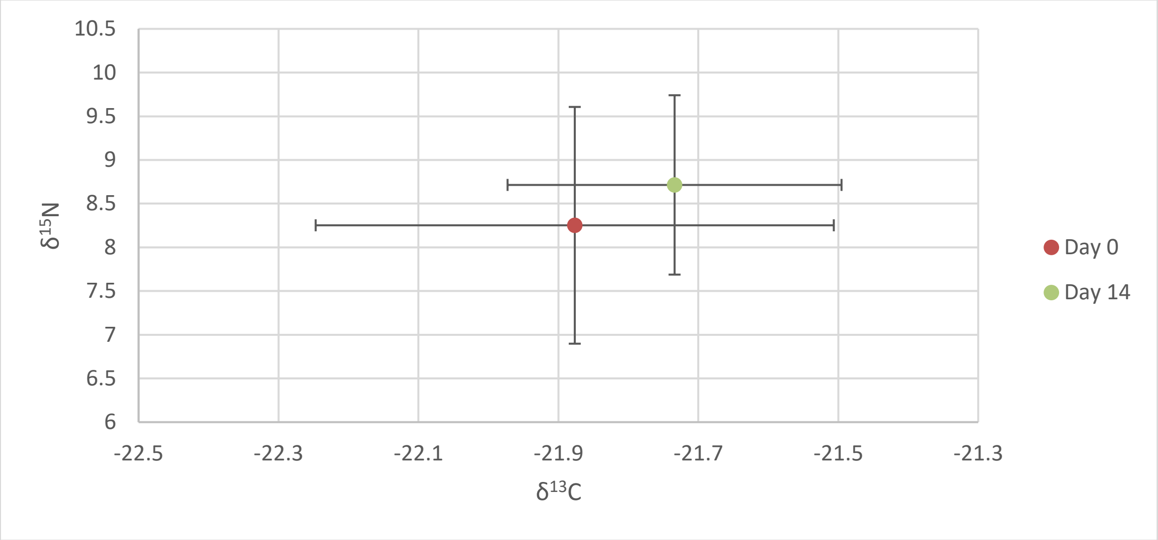

Figure 21. δ^{13}C and δ^{15}N mean and standard deviation of the 14-day Marsh Periwinkle (*Littorina irrorata*) feeding experiment performed from November 30th, 2021, to December 16th, 2021 (Common Periwinkle Group, *n* = 9).

Discussion

Stable Isotope Analysis of Wild Terrapins

Stable isotopes have been shown to provide an accurate method for evaluating foraging ecology in a variety of turtle species. For example, stable isotopes have been used to evaluate foraging behavior (Ceriani et al. 2012, 2014; Williams et al. 2013; Denton et al. 2019; Haywood et al. 2019), trophic level organization (Arthur et al. 2008; Reich et al. 2011; Kudman, 2021), transition in life history stages (Seminoff et al. 2007; Arthur et al. 2008; Reich et al. 2008, Murray and Wolf, 2011; Denton et al. 2019; Kudman, 2021). and metabolic turn over and residence timing (Seminoff et al. 2007; Reich et al. 2008; Murray and Wolf, 2011; Vander Zanden et al. 2014). Stable isotope values have been addressed in two previous studies of the Diamondback Terrapin. These studies documented carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope values for two subspecies of terrapins. In one study, *Malaclemys terrapin rhizophorarum* was sampled at three locations in Southwest Florida (Denton et al, 2019), and the other study, *Malaclemys terrapin terrapin* was evaluated in two locations in New Jersey and one location in New York (Kudman, 2021). These studies were the first to provide baseline data on the stable isotope values of Diamondback Terrapins. The results of those studies revealed differences in stable isotope values that potentially reflected different foraging locations and prey items (Denton et al, 2019; Kudman, 2021). The purpose of the current study was to document baseline data on stable isotope values of a third subspecies of Diamondback Terrapin, *Malaclemys terrapin pileata*, that inhabits a distinctly different location and habitat, the saltmarshes of the Northern Gulf of Mexico.

The results of the current study provide the magnitude and ranges of $\delta^{13}C$ and $\delta^{15}N$ values for adult female terrapins from a population of Diamondback Terrapins in Heron Bay, Alabama, located in the eastern portion of the Mississippi Sound. The findings are primarily based on whole blood analysis. Whole blood was chosen because it can reflect relatively recent dietary input, is easy to collect and process in the field, and provides a stable tissue for stable isotope analysis (Tieszen et al. 1983; Hobson, 1997; Seminoff et al. 2007; Reich et al. 2008; Denton et al. 2019). Further, the results are comparable to those from Denton et al. since they utilized whole blood during their stable isotope analysis.

The results revealed a unique range of stable isotope, $\delta^{13}C$ and $\delta^{15}N$, values for the Diamondback Terrapin population in Heron Bay in comparison to those reported for terrapins in Southwest Florida (**Table 1** and **Figure 4**). The Heron Bay population was notably higher in $\delta^{15}N$ values whereas the $\delta^{13}C$ values were similar to at least one of the locations in Southwestern Florida (**Table 2** and **Figure 5**). The previous study of terrapins in Southwest Florida found that the stable isotope values of terrapins and prey items varied between locations. That study inferred that baseline differences in primary productivity and prey items between the three different habitats influenced the stable isotope values of the terrapins (Denton et al. 2019). The unique $\delta^{13}C$ and $\delta^{15}N$ values of the Diamondback Terrapins in the current study reflect the differences in the baseline isotopic values for primary producers and prey items in the eastern portion of the Mississippi Sound. It indicates that the Diamondback Terrapins from Heron Bay could be feeding on different prey items compared to the terrapins in Southwest Florida.

Scute samples were taken from a subset of wild terrapins for $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis. Statistical analysis revealed no significant variation between the $\delta^{13}C$ and $\delta^{15}N$ values of the scute and blood samples (**Figure 6**, **7**, and **8**). Previous studies have suggested that diet-derived stable isotope values occur at varying rates depending on tissue specific metabolic activity (Seminoff et al. 2007; Vander Zanden et al. 2010, 2014; Denton et al. 2019). Tissues with relatively high metabolic activity (e.g., whole blood) reflect more recent dietary history while tissues with lower metabolic activity (e.g., scute) reflect long-term integration of the dietary history (Hobson and Welch, 1992; Godley et al. 1998; Seminoff et al. 2007; Reich et al. 2007; Vander Zanden et al. 2010; Denton et al. 2019). Since no significant variation was detected from $\delta^{13}C$ and $\delta^{15}N$ values from the scute and whole blood, this suggests that the adult terrapins in the current study may have had a consistent long-term diet. However, some studies have also suggested that stable isotope turnover rates in scutes may not be significantly lower than some of the other tissues, including blood (Reich et al. 2008; Vander Zanden et al 2010).

Analysis of potential prey items and primary producers from the Mississippi Sound revealed a variety of species-specific stable isotope values and ranges (**Table 1** and **Figure 9**). The average $\delta^{13}C$ values of the potential prey items ranged from -16.63 ‰ (Marsh Periwinkle) to -24.00 ‰ (Eastern Oyster). This range in stable isotope values overlap with those values reported for potential prey items in a previous study of terrapins in other habitats by Denton et al. (2019) Additionally, the range in stable isotope values overlap with values reported for several of the same species of potential prey items in a habitat adjacent to the current study site (Rush et al. 2010). The $\delta^{13}C$ values of the two primary salt marsh grasses, Saltmarsh Cordgrass and Black Needlerush, represented the highest and lowest average values, respectively of all the prey items and primary producers (**Table 1** and **Figure 10**). These values overlap with those previously reported for in a habitat adjacent to the current study site (Rush et al. 2010). The relatively high values of the Saltmarsh Cordgrass in comparison of the low values of the Black Needlerush would be anticipated based on stable isotope differences between C_3 photosynthetic versus C_4 photosynthetic plant characteristics (Stribling and Cornwell, 1997; Rush et al. 2010).

$\delta^{13}C$ values from certain prey items (i.e., Eastern Oyster, Blue Crab, Marsh Crab, and Marsh Fiddler Crab) were not significantly different from the values of the terrapins. The similarities in the stable isotope values indicates that terrapins were foraging on those prey items. The $\delta^{13}C$ values from the Hermit Crab, the Marsh Periwinkle, and the Olive Nerite varied significantly from those of the terrapins. Fecal analysis indicated that some terrapins were feeding primarily on Marsh Periwinkles and Ribbed Mussels (**Table 2**). The stable isotope values of Ribbed Mussels were not evaluated within the current study but a previous study of Ribbed Mussel in the adjacent habitats; Pascagoula, MS, and Grand Bay, MS (Rush et al. 2010). The $\delta^{13}C$ values for Ribbed Mussels (ranging from -27.2 ‰ to -21.2 ‰) reported in that study (Rush et al. 2010) overlapped with those values of the terrapins in the current study. The $\delta^{13}C$ values of the Marsh Periwinkles in the current study varied significantly from the terrapins. Its plausible that foraging on the Marsh Periwinkle could result in a shift in the terrapins' stable isotope values but not enough to result in statistically similar $\delta^{13}C$ values (Fry, 2006; Hart and Hunter, 2014; Tucker et al. 2018; Denton et al. 2019).

The results of the current study also documented $\delta^{15}N$ values for a variety of primary producers and consumers from the terrapin habitat in the Mississippi Sound of Alabama. Previous work with stable isotopes have shown that $\delta^{15}N$ values may indicate trophic levels within an ecosystem due to stepwise increase and enrichment of nitrogen with increasing trophic level (Hobson and Welch, 1992). The relatively high $\delta^{15}N$ values of the terrapins in comparison to the majority of the prey items (**Figure 10**) are consistent with the hypothesis that terrapins represent a higher trophic level predator within the salt marsh ecosystem (Hobson and Welch, 1992; Fry, 2006). The prey items with $\delta^{15}N$ values that were closest to the terrapins were the Eastern Oyster, the Blue Crab, and the Marsh Crab (i.e., no significant difference), while other potential prey items had significantly lower values (Marsh Fiddler Crabs, Olive Nerites, and Marsh Periwinkles) or had significantly higher values (Hermit Crabs). The Saltmarsh Cordgrass and the Black Needlerush evaluated in the current study had some of the lower $\delta^{15}N$ values as would be expected from primary producers.

Marsh Periwinkle Isoscape Model

An isoscape was developed for the Marsh Periwinkle as a model for evaluating variation in stable isotope values at different locations in the eastern portion of the Mississippi Sound. A previous study suggested that baseline isotopic values can vary between terrapin habitats and could cause site-specific variation in terrapin stable isotope values (Denton et al. 2019). The Marsh Periwinkle was chosen for the current study because it has been a previously reported food item for the Diamondback Terrapin (Reviewed by Tucker et al. 1995; also see Hart and Hunter, 2014; Denton et al. 2019).

Further, several studies have indicated that terrapins may represent a keystone species for some saltmarsh habitats because of their foraging on the Marsh Periwinkle (Silliman and Zieman, 2001; Silliman and Bertness, 2002). These studies indicated that terrapin foraging prevented the over-abundance of Marsh Periwinkle populations which was essential for maintaining the healthy growth of Saltmarsh Cordgrass in the saltmarsh ecosystem. Additionally, Marsh Periwinkle shell fragments were one of the primary prey items found within the fecal samples of terrapins in this study (**Table 2**).

For the Marsh Periwinkle isoscape study, all samples were collected on the same day to avoid any potential variation related to the day of collection. The results revealed a relatively wide variation in δ^{13}C and δ^{15}N values depending on the location of the sampling (**Table 7**). δ^{13}C values for the samples from Fowl River and Bayou Coden were the most depleted, whereas the values from Airport Marsh, Bayou La Batre, and Cedar Point Marsh-North were the most enriched. The Marsh Periwinkle has been reported to feed directly on saltmarsh grasses as well as grazing on the fungus and senescent materials associated with the Saltmarsh Cordgrass (Silliman and Zieman, 2001). Differences in the δ^{13}C values could also relate to differences of the fungal processing of carbon from the different locations (Henn and Chapela, 2000). Further, it has been suggested that terrestrially derived nitrogen could potentially affect the foraging of Marsh Periwinkles on Saltmarsh Cordgrass (Silliman and Bertness, 2002; Rush et al. 2010).

A variety of location-specific δ^{15}N values were recorded for the Marsh Periwinkle. The samples with the most enriched δ^{15}N values were recorded at Cedar Point Marsh-South, Jemison Marsh, Lightning Point, and Point Aux Pines. The most depleted δ^{15}N values were recorded at two locations that were adjacent to one another:

Bayou Coden and Bayou La Batre. The basis for the variability is currently unclear, but previous studies have shown that factors such as river effluent can alter or enrich the levels of ^{15}N in the estuaries which appear in various trophic levels of the food web (McClelland et al. 1997; Costanzo, 2005). The current study area is located in the eastern Mississippi Sound which has a variety of freshwater input sources that could represent potential point sources for the delivery of nitrogen into the estuaries, including Mobile Bay and associated river systems (Fowl River, Bayou Coden, and Bayou La Batre). For example, the highest $\delta^{15}N$ values recorded were from Lightning Point, which is adjacent to the channel of Bayou La Batre. Additionally, several of the high values were from locations that were adjacent to Mobile Bay (Cedar Point Marsh-South and Jemison Marsh). In the case of locations with relatively lower $\delta^{15}N$ values, its plausible the marsh ecosystem itself could be acting as a natural filter (Dardeu et al. 1992; Nelson and Zavelata, 2012). For example, the sampling areas at Bayou Coden and Bayou La Batre were from isolated areas located within the marsh.

Although the specific basis of the variability is unclear, the results indicate that the Marsh Periwinkle could exhibit a variety of $\delta^{13}C$ and $\delta^{15}N$ values within this limited area of the Mississippi Sound. Comparison of the Marsh Periwinkle isoscape with the stable isotope values obtained from the terrapins provides insight on the potential influence of this prey item on the $\delta^{13}C$ and $\delta^{15}N$ values of Diamondback Terrapins. Considering the distribution of terrapin and Marsh Periwinkle stable isotope values, it would be anticipated that a diet rich in Marsh Periwinkles from these various locations would result in an enrichment in ^{13}C and a decrease in ^{15}N in comparison to the majority of the potential prey items analyzed (**Figure 10**). Based on the Marsh Periwinkle isoscape recorded in the current study, certain locations may have varying degrees of influence on enriching the $\delta^{13}C$ values, while as other locations may have varying degrees on decreasing $\delta^{15}N$ values (**Figure 12**). The variability in stable isotope values for Marsh Periwinkles from different locations provides signatures that could be transferred to higher trophic levels, thus providing insight on the feeding ecology of the Diamondback Terrapin in the Mississippi Sound.

Head Start Terrapin Analysis

The $\delta^{13}C$ values for the head start terrapins were similar to the values of the Reptomin® (**Figure 13**). The current study is a rare example in which stable isotopes values are examined in a consumer which has been on the same controlled diet (Reptomin®) for its entire life (approximately 2-to-3-years). Thus, the stable isotope values of these terrapins represent the stable isotope contribution of the Reptomin® along with the fractionation during food assimilation (Tieszen et al. 1983; Hobson and Clark, 1993; Phillips and Eldridge, 2006;). The age of these terrapins (i.e., 2-to-3 years old on the same diet) would indicate that they were at isotopic equilibrium (**Figure 13**), which is also supported by previous long-term studies with the Red Ear Slider (Seminoff et al. 2007) and the Loggerhead Sea Turtle (Reich et al. 2008). The mean $\delta^{13}C$ value of the head start terrapins was within approximately 0.43‰ of the average of the Reptomin® (-21.70‰ vs -21.27‰, respectively). This exemplifies the impact of diet on the $\delta^{13}C$ stable isotope values of the consumer (Hobson and Welch, 1992; Ceriani et al. 2012). It has been suggested that there are minor differences of $\delta^{13}C$ values (e.g., 1‰) between trophic levels (DeNiro and Epstein, 1978; Hobson and Welch 1992). The $\delta^{15}N$ values for the head start terrapins were consistently above the δ^{15}N values of the Reptomin® diet. This could relate to isotopic fractionation during the digestion and incorporation of the Reptomin® (DeNiro and Epstein, 1978, 1981). For example, A previous study has suggested that consumer tissues are enriched in ^{15}N by approximately 2 - 5‰ over their food source (DeNiro and Epstein, 1981).

The δ^{13}C and δ^{15}N values of the wild terrapins were significantly different than those of the head start terrapins (**Figure 14** and **15**) and reflect the natural diet and habitat, see discussion above (Gibbons, 2018). The variation between the δ^{13}C values of head start versus wild terrapins suggests that the wild terrapins' prey items are more depleted in δ^{13}C in comparison to Reptomin® (**Figure 16**). For example, certain potential prey items examined in the current study (Blue Crab, Eastern Oyster, Marsh Fiddler Crab, and Marsh Crab) are depleted in δ^{13}C in comparison to Reptomin® whereas others were enriched in comparison to Reptomin® (Hermit Crab, Oliver Nerite, and Marsh Periwinkle). The higher δ^{15}N values in wild terrapins versus head start terrapins could potentially reflect higher δ^{15}N values in natural prey items versus the Reptomin®. In the current study, the mean δ^{15}N values of the Blue Crab, Eastern Oyster, Hermit Crab, Marsh Crab, and Olive Nerite were higher than the mean δ^{15}N value of Reptomin®.

The difference in the isotope values of head start versus wild terrapins also provides a novel approach for evaluating adaptation of head start terrapins following their release into the wild. It is anticipated that if head start terrapins adapt and survive following their release, their δ^{13}C and δ^{15}N stable isotope values will gradually transition to reflect their new natural diet, potentially shifting in the direction of the values documented for the wild terrapins in the current study.

Feeding Experiment Analysis

The four experimental food items had a variety of differing δ^{13}C values (**Figure 18, 19, 20,** and **21**), with two of the food items having significantly enriched carbon (Common Periwinkles and Marsh Periwinkles), one having a similar value (House Crickets), and the third food item (Blue Crabs) having a depleted carbon in comparison to Reptomin® (**Figure 17**). In all four of the experiments (Blue Crabs, Common Periwinkle, House Cricket, and Marsh Periwinkle), the δ^{13}C values following the 14-day feeding period did not significantly differ from the day-0 control. In the case of the δ^{15}N values, all four food items resulted in increased δ^{15}N values, but these values were not significantly different than those from the Reptomin® control diet. Based on these results, its plausible a longer-feeding study would indicate if these various diets would result in significant shifts in δ^{13}C and δ^{15}N values in comparison to the Reptomin® diet. Previous studies indicate that the turnover rate for δ^{13}C and δ^{15}N stable isotopes is variable between turtle species and tissues (Seminoff et al. 2007; Reich et al. 2008), and that some rates for some tissues can extend well-beyond a 14-day period. Furthermore, in all four of the current experiments (Blue Crabs, Common Periwinkle, House Cricket, and Marsh Periwinkle), all shifted in the direction of the carbon source, but they were not significantly different after day-14. Thus, its plausible that a longer experimental period could produce significant variation. For example, based on Reich et al. evaluation on hatchling Loggerheads, a resident time took approximately 36 days for whole blood.

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EVALUATION OF THE REPRODUCTIVE ECOLOGY OF THE DIAMONDBACK TERRAPIN OF HERON BAY, ALABAMA

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Abstract

The reproductive ecology of the Diamondback Terrapin, *Malaclemys terrapin pileata*, was evaluated during the 2021 and 2022 nesting seasons at Heron Bay, Alabama. Drift fences and pit fall traps were utilized to capture adult female terrapins that were nesting at a major nesting beach located on the eastern border of Heron Bay (i.e., Cedar Point Marsh). These turtles were tagged with VHF radio transmitters, measured, and a subset of these terrapins were induced to lay eggs via oxytocin injections. Captured turtles ranged from 600 to 1385 grams, 6.7 to 18.8 cm in plastron length, and 15.7 to 20.7 cm in straight carapace length. Gravid females produced an average of 7.7 ± 1.8 eggs. Population estimates based on mark-recapture estimated that there were 166 nesting females during 2021 and 133 nesting females during 2022 in this nesting aggregation. Radio transmitters were attached to a subset (*n* = 9 for 2021, and *n* = 10 for 2022) of these turtles to monitor post-nesting movements. Radio tracking was conducted upon release and continued at approximately one-to-two-week intervals during the nesting season. The length of successful tracking varied from 4 to 285 days, with an average of 38 days. Telemetry results indicate that many of the terrapins that were nesting in Cedar Point Marsh remained in the Cedar Point Marsh area after nesting, and that terrapins captured in the northern portion of Heron Bay continued to inhabit that area for the period they were tracked post-release. To assess the impact of local predators on nests, Cedar Point Marsh was surveyed daily for depredated nests during the 2021 and 2022 nesting seasons. No depredated nests were observed during the 2021 or 2022 terrapin nesting seasons. These findings were in distinct contrast to surveys conducted prior to a USDA raccoon removal program conducted in 2020 at Cedar Point nesting beach.Collectively, these data document that the salt marsh areas and the Cedar Point Marsh nesting beach in Heron Bay represent nesting and inter-nesting habitat that is critical for the survival of the Diamondback Terrapin in Alabama.

Key Words: Mark-Recapture, Population Estimate, Radio Tracking, Nest Depredation

Introduction

The Diamondback Terrapin inhabits estuarine environments along the Atlantic and Gulf coasts of the United States (Lovich et al. 2018). The subspecies that is found in Alabama is the Mississippi Diamondback Terrapin, *Malaclemys terrapin pileata*, and it ranges from the Florida panhandle to the eastern portions of Louisiana (Lovich et al. 2018). This species was once abundant in the salt marshes in Alabama, but this population has significantly declined in the past century (Carr, 1952; Coleman et al. 2011, 2014; Roberge, 2012, 2017; Sirgo, 2020), leading to be listed as "a species of highest conservation concern" by the Alabama Department of Conservation and Natural Resources (Nelson and Marion, 2004). The primary threats that have limited this species recovery in Alabama include depredation of nests by predators (such as raccoons), loss of essential habitat, and crab trap-induced mortality (Bishop, 1983; Roberge, 2012, 2017; Coleman, 2014; Wibbels, 2010). Due to the concerning status of the Diamondback Terrapin, it is imperative to gain a better understanding of the ecology of this animal in Alabama. Surveys of saltmarsh and estuarine systems in Alabama indicate that the Diamondback Terrapin is currently represented by small, remnant groups in various locations along the coast (Wibbels, 2010; Roberge, 2012, 2017; Coleman, 2014). The largest nesting aggregation to be identified is located in Heron Bay on the western border of Cedar Point Marsh (Roberge, 2012, 2017; Coleman, 2014; Sirgo, 2020). That location includes a relatively elevated nesting beach that is directly adjacent to Heron Bay to the west and Cedar Point Marsh to the east. Surveys of that nesting area for over a decade have consistently documented relatively large number of nests each nesting season (Roberge, 2012, 2017; Coleman, 2014; Sirgo, 2020). Surveys of that nesting beach have also consistently documented a relatively high level of nest depredation each nesting season (Roberge, 2012, 2017; Coleman, 2014; Sirgo, 2020).

Radio tracking has been shown to be an effective technology for monitoring habitat use and home range for Diamondback Terrapins (Munscher et al. 2012; Roberge, 2012, 2017; Sirgo, 2020). This has included initial studies of terrapin movements in Heron Bay, Alabama which have indicated that this area may represent the home range for many terrapins in this area (Roberge, 2012, 2017; Sirgo, 2020).

Through the present study, I address the reproductive ecology of Diamondback Terrapins that utilized the Cedar Point Marsh nesting beach. As indicated above, this is the most important nesting area for Diamondback Terrapins that has been identified to-date in Alabama.

Materials and Methods

Study Site- I focused my work on Cedar Point Marsh because it has been previously documented as an important nesting site for the Diamondback Terrapin in the eastern portion of the Mississippi Sound, north of Dauphin Island, Alabama. Cedar Point Marsh forms the eastern margin of Heron Bay. The Cedar Point Marsh nesting beach forms the western border of the marsh. Cedar Point Marsh stretches approximately 1.8 kilometers from north to south and 0.5 kilometers from east to west. It is a saltmarsh that is primarily composed of two marsh grasses: the Saltmarsh Cordgrass (*Spartina alterniflora*) and the Black Needlerush (*Juncus roemerianus*). Historically, this location has been documented to have up to 100 or more Diamondback Terrapin nests per year (Roberge, 2012, 2017; Coleman, 2014).

Capture Methods- Adult female terrapins were captured on the nesting beach using “pitfall” traps located adjacent to drift fences on the western shoreline of Cedar Point Marsh (**Figure 1**). Each drift fence ranged from 50-to-100 yards and contained 4-to-8 pitfall traps. For the 2021 nesting season, 3 drift fences were placed with 18 pitfall traps (**Figure 2**). For the 2022 nesting season, 4 drift fences were placed with 14 pitfall traps (**Figure 3**). These drift fences and pitfall traps were placed towards the center portion of the nesting beach where the majority of the nesting has been detected from previous years. Pitfall traps were placed on alternating sides of the drift fence to capture terrapins arriving to the nesting beach from the bay side or the marsh side. Once installed, drift fences and pitfall traps were checked on a daily basis for captured terrapins. Typically, drift fences were installed during the middle of May, towards the start of the nesting season, and were removed in late July/early August, after the nesting season. Additionally, Diamondback Terrapins were captured in the northern portion of Heron Bay (4 terrapins in 2021) by Auburn University’s Biological Sciences Department (Dr. Iwo Gross and Dr. Matthew Wolak). These terrapin were captured north of Cedar Point Marsh in a marsh system that has been named “Jemison Marsh” based on the local fishing market parallel to it.

Daily Surveys- The Cedar Point Marsh nesting beach was surveyed on a daily basis for depredated nests, terrapins, signs of predators, and to check the pitfall traps. In 2021, the beach was surveyed from April 23rd to August 27th. In 2022, the beach was surveyed from March 22nd to July 17th.

Processing of Captured Terrapins- All captured terrapins were examined for previous tags, weighed, measured, and palpated to determine if they were gravid. Oxytocin was used to induce oviposition if eggs were detected (Ewert and Legler, 1978). All captured terrapins were tagged with shell tags in the rear marginal scutes and P.I.T tags were inserted in the left inguinal area under the plastron bridge. After processing, all terrapins were released back into Cedar Point Marsh.

Population Estimates- I estimated the size of the nesting population utilizing the Cedar Point Marsh nesting beach were estimated for 2021 and 2022 nesting seasons based on the Schnabel method (Schnabel, 1938):

$$\frac{\Sigma(C_t \times M_t)}{\Sigma(R_t) + 1}$$

Where C_t represents the number of captures at time t;

M_t represents the total number of marked individuals in the population at time t;

and R_t represents the number of recaptures at time t.

The number of tagged terrapins in the nesting population was based on previous tagging studies performed at the Cedar Point Marsh nesting beach from 2006 to 2019 (Coleman, 2011; Roberge, 2012, 2017; Sirgo, 2020). For the population estimates, the annual survivability was taken into account for estimating the total number of tagged individuals in the nesting population during the 2021 and 2022 nesting seasons. An annual survivability factor of 78% was utilized from based on data from three previous population studies of Diamondback Terrapins (Tucker et al. 2011; King and Ludlam, 2014; Witczak et al. 2014).

Radio Tracking- A subset of the adult female terrapins that were captured in pitfall traps were tagged with a radio transmitter prior to release. The VHS radio transmitters (Lotek™) were attached to anterior portion of the carapace along the vertebral scutes. The attachment area was cleaned and lightly sanded prior to using JB Weld™ Epoxy to attach the transmitter (**Figure 4**). The transmitters are relatively small (18 grams), have a battery life of approximately one year, and generate a pulse rate of approximately 10 pules per minute. Each transmitter has its own unique frequency ranging from 165 to 167 MHz. A SRX800 radio receiver (Lotek™) attached to a Yagi antenna was used to locate the terrapins following their release. During radio tracking sessions, the receiver was used manually from multiple locations in Heron Bay. Once a terrapin was detected, the antenna was moved in a systematic fashion in order to identify and record the direction of maximal signal strength. During a tracking session, multiple locations were utilized in an attempt to triangulate the location of any terrapins that were detected.

Figure 1. Pitfall traps and drift fences placed in Cedar Point Marsh during the nesting season.

Figure 2. Drift fence locations (*n* = 3) with bucket locations (*n* = 18) within Cedar Point Marsh for the 2021 nesting season.

Figure 3. Drift fence locations (*n* = 4) with bucket locations (*n* = 14) within Cedar Point Marsh for the 2022 nesting season.

Figure 4. A Mississippi Diamondback Terrapin (*Malaclemys terrapin pileata*) that has a Lotek™ transmitter attached to its carapace.

Results

Capture of Adult Female Terrapins

During the 2021 nesting season, a portion of the drift fences were destroyed by Tropical Storm Claudette and were replaced within approximately 1-to-2 weeks after the tropical storm. A total of 16 terrapins were captured in Cedar Point Marsh during the 2021 nesting season (11 were captured in pitfall traps and 5 were captured by hand while surveying the beach). During the 2022 nesting season, I captured 13 terrapins in Cedar Point Marsh (11 were captured in pitfall traps and 2 were captured by hand while surveying the beach). During 2021, the capture dates ranged from May 20th, 2021, to July 9th, 2021, and during 2022, the capture dates ranged from May 10th, 2022, to July 1st, 2022 (**Table 1**). The number of terrapins captured each month is shown in Figure 5 for both nesting seasons.

Mass of terrapins (**Table 1**) captured during the 2021 and 2022 season ranged from 600 to 1385 g (mean mass: 1,004.1 ± 219.1 g). The straight carapace length (SCL) of these terrapins ranged from 15.7 to 20.7 cm (mean SCL: 18.9 ± 1.3 cm). The plastron length (PL) of these terrapins captured ranged from 14.6 to 18.8 cm (mean PL: 16.8 ± 1.1 cm). Of the 29 captured terrapins, 20 were successfully induced to lay eggs (the number of eggs laid ranged from 5 to 12 and had a mean of 7.7 ± 1.8 eggs).

Of the 29 terrapins captured, 5 were recaptures from previous years (0574 was caught twice, 1015, 1427, and 1429). Terrapin 0574 was originally captured by hand at Cedar Point Marsh on May 13th, 2014, with a PL of 17.0 cm, a SCL of 19.4 cm, and a weight of 1,085 g. Since 2014, this terrapin was recaptured two more times: May 22nd, 2021, and May 22nd, 2022. Its last capture was in Cedar Point Marsh in a pitfall trap on

May 22nd, 2022, and the terrapin had a PL of 17.8 cm, a SCL of 20.4 cm, and a weight of 1,205 g. Terrapin 1015 was originally captured in a pitfall trap at Cedar Point Marsh on May 27th, 2019, and it had a PL of 16.7 cm, a SCL of 18.6 cm, and a weight of 1095 g. It was captured in the current study at Cedar Point Marsh in a pitfall trap on May 27th, 2021, and the terrapin had a PL of 17.0 cm, a SCL of 18.9 cm, and a weight of 1090 g. Terrapin 1427 (originally tagged as 0226) was originally captured in a pitfall trap at Cedar Point Marsh on July 14th, 2006, and the terrapin had a PL of 16.9 cm, a SCL of 18.2 cm, and a weight of 1,164 g. Since 2006, this terrapin has been recaptured three more times: June 24th, 2009; May 20th, 2011; and May 15th, 2022, with the last capture by hand in Cedar Point Marsh on May 15th, 2022. The terrapin had a PL of 17.5 cm, a SCL of 19.4 cm, and a weight of 1,200 g. Terrapin 1429 (originally 0783) was a captive-reared terrapin that was hatched in the summer of 2013 and released at approximately two years of age into Cedar Point Marsh on September 8th, 2015, with a PL of 7.2 cm, a SCL of 8.1 cm, a weight of 133.5 g. During the current study, this terrapin was recaptured in Cedar Point Marsh by a pitfall trap on June 7th, 2022, and the terrapin had a PL of 16.1 cm, a SCL of 18.6 cm, and a weight of 895 g.

Population Estimate

Based on the Schnabel method, the nesting population of adult female terrapins at the Cedar Point Marsh nesting beach was estimated to be approximately 163 individuals during the 2021 nesting season, and 133 individuals during the 2022 nesting season.

Radio Tracking

2021 Nesting Season- 13 adult female terrapins were tagged with radio transmitters (**Table 2** and **Figure 6**, **7**, and **8)**. Of these 13 terrapins, 4 were captured in Jemison Marsh while the remaining 9 were captured in Cedar Point Marsh. All were originally released into a tidal channel in Cedar Point Marsh directly adjacent to the middle portion of the nesting beach (30.319691°, -88.143789°). Radio tracking was conducted upon release and continued at approximately 1 to 2-week intervals during the nesting season. Additionally, tracking was infrequently conducted a few times during the winter and spring due to weather and time of day.

The locations of the four terrapins that were captured within Jemison Marsh and released into Cedar Point Marsh are shown in Figure 6 and Figure 8. Within a month after their release, all four terrapin's locations were found to be back in Jemison Marsh. Furthermore, each of these terrapins were documented additional times in Jemison Marsh during the month of June (**Table 2** and **Figure 8**). Radio tracking in the Jemison Marsh area during July and August did not detect any of the transmitters either from maximum transmitter-receiver distance or from damaged receivers from Tropical Storm Claudette. The radio tracking locations of the terrapins that were captured on the Cedar Point nesting beach are shown in Figure 6 and 7. Of these 9 terrapins released with radio transmitters, 1 was not located after the day of release. The remaining 8 terrapins remained in Cedar Point Marsh and were relocated from 2 to 7 times, with total tracking periods ranging from 4 to 285 days.

2022 Nesting Season- 10 adult female terrapins were tagged with radio transmitters (**Table 2** and **Figure 9)**. Of these 10 terrapins, 1 was captured by hand crossing the road in the northern portion of Heron Bay while the remaining 9 were captured on the nesting beach in Cedar Point Marsh. All were released into a tidal channel in Cedar Point Marsh directly adjacent to the middle portion of the nesting beach (30.319691°, -88.143789°). Radio tracking was conducted upon release and continued at approximately one-to-two-week intervals during the nesting season. Figure 6 show the release locations and triangulated locations of each terrapin during 2022.

The terrapin that was captured while crossing the road was found to be in northern Heron Bay 2 weeks after her release [**Figure 9**; 1428 (white)]. The radio tracking locations of the 9 terrapins that were captured on the Cedar Point nesting beach are shown in Figure 6. Of the 9 terrapins released with radio transmitters, one was not located after the day of release. The remaining 8 terrapins were relocated in Cedar Point Marsh from 2 to 9 times, with total tracking periods ranging from 9 to 50 days.

Nest Depredation Surveys

The number of beach surveys for depredated nests per month are shown in Table 3 for the 2021 and 2020 nesting seasons. During the primary months of the nesting season (i.e., May – July) surveys were typically conducted at 1-to-3-day intervals. Additionally, surveys were conducted periodically during several of the months pre- or post-nesting season. Following the racoon removal program, no depredated nests were observed during any of the surveys conducted during 2021 and 2022. Additionally, during the beach surveys of 2021 and 2022, there were no observations of potential indicators (i.e., tracks and scat) of racoons (a previously identified primary predator on the terrapin nests on Cedar Point Marsh).

Table 1. List of all captured terrapins throughout the 2021 – 2022 nesting seasons with corresponding shell tags, capture dates, estimated ages, plastron lengths, straight carapace lengths, weights, eggs laid, and recapture status.

Nesting Season	Shell Tag	Date Captured	Estimated Age	Plastron Length (cm)	Straight Carapace Length (cm)	Weight (g)	Number of Eggs	Recapture: Initial Year of Release
2021	1158	5/20/2021	8	15.7	17.2	680	5	
2021	1160	5/20/2021	7	16.3	18.4	900	0	
2021	1159	5/20/2021	7	15.6	17.2	665	6	
2021	1162	5/22/2021	9	18.4	20.6	1295	6	
2021	0574	5/22/2021	25	17.8	20.4	1205	0	2014
2021	1163	5/22/2021	10	16.4	18.3	925	7	
2021	1164	5/25/2021	9	16.7	18.4	895	8	
2021	1015	5/27/2021	10	17.0	18.9	1090	10	2019
2021	1165	5/27/2021	9	18.0	19.7	1165	9	
2021	1166	6/7/2021	10	18.8	20.4	1365	8	
2021	1167	6/7/2021	8	14.6	15.7	600	5	
2021	1168	6/12/2021	10	17.3	19.8	965	10	
2021	1169	6/17/2021	9	17.2	18.6	1090	9	
2021	1170	7/4/2021	7	16.2	18.0	955	0	
2021	1371	7/9/2021	6	16.0	18.4	840	7	
2021	1372	7/9/2021	9	15.7	18.0	825	0	
2022	1423	5/10/2022	7	17.3	19.4	960	7	
2022	1427	5/15/2022	10	17.5	19.4	1200	6	2006
2022	1424	5/16/2022	8	16.6	18.5	905	0	
2022	1425	5/20/2022	9	16.8	19.2	1100	9	
2022	1426	5/21/2022	7	15.1	17.3	710	6	
2022	1428	6/4/2022	7	15.4	16.4	705	7	
2022	0574	6/6/2022	10	17.9	20.6	1385	0	2014
2022	1429	6/7/2022	9	16.1	18.6	895	0	2015 (Head Start)
2022	1430	6/8/2022	10	17.9	20.7	1230	12	
2022	1431	6/9/2022	10	18.0	20.3	1340	9	
2022	1432	6/11/2022	10	17.6	19.7	1085	0	
2022	1433	6/20/2022	10	17.8	19.9	1095	0	
2022	1434	7/1/2022	9	16.3	19.0	1050	8	

Table 2. List of all radio tracked terrapins with corresponding shell tags, frequencies, locations of capture, release dates, duration of tracking, number of times located, end locations for the 2021 and 2022 nesting season, and I.D number for radio tracking.

Nesting Season	Shell Tag	Transmitter Frequency	Location of Capture	Date of Release	Duration (Days)	Number of Times Located	Ending Location	Terrapin I.D.
2021	0574	165.680	Cedar Point Marsh	6/1/2021	14	3	Cedar Point Marsh	1
2021	1151	165.939	Jemison Marsh	5/7/2021	27	3	Jemison Marsh	2
2021	1152	165.981	Jemison Marsh	5/7/2021	49	7	Jemison Marsh	3
2021	1157	165.692	Jemison Marsh	5/19/2021	37	6	Jemison Marsh	4
2021	1158	166.866	Cedar Point Marsh	5/25/2021	0	1	Cedar Point Marsh	5
2021	1159	166.767	Cedar Point Marsh	6/1/2021	10	3	Cedar Point Marsh	6
2021	1160	165.617	Cedar Point Marsh	6/1/2021	285	3	Cedar Point Marsh	7
2021	1161	165.831	Jemison Marsh	5/7/2021	27	4	Jemison Marsh	8
2021	1162	165.880	Cedar Point Marsh	6/1/2021	71	4	Cedar Point Marsh	9
2021	1163	165.568	Cedar Point Marsh	6/1/2021	14	3	Cedar Point Marsh	10
2021	1164	165.655	Cedar Point Marsh	6/7/2021	8	2	Cedar Point Marsh	11
2021	1165	165.818	Cedar Point Marsh	6/11/2021	4	3	Cedar Point Marsh	12
2021	1166	166.567	Cedar Point Marsh	6/7/2021	4	2	Cedar Point Marsh	13
2022	0574	165.550	Cedar Point Marsh	6/14/2022	0	1	Cedar Point Marsh	14
2022	1423	165.100	Cedar Point Marsh	5/26/2022	50	9	Cedar Point Marsh	15
2022	1424	165.200	Cedar Point Marsh	5/26/2022	40	5	Cedar Point Marsh	16
2022	1425	165.250	Cedar Point Marsh	5/26/2022	40	5	Cedar Point Marsh	17
2022	1426	165.300	Cedar Point Marsh	5/27/2022	7	5	Cedar Point Marsh	18
2022	1427	165.150	Cedar Point Marsh	5/26/2022	40	6	Cedar Point Marsh	19
2022	1428	165.350	Jemison Marsh	6/14/2022	9	2	Jemison Marsh	20
2022	1429	165.390	Cedar Point Marsh	6/14/2022	21	3	Cedar Point Marsh	21
2022	1430	165.450	Cedar Point Marsh	6/14/2022	21	2	Cedar Point Marsh	22
2022	1431	165.490	Cedar Point Marsh	6/14/2022	21	2	Cedar Point Marsh	23

Table 3. Total number of surveys (depredated nests) by month for the 2021 and 2022 nesting seasons.

	March	April	May	June	July	August
2021	0	1 (0)	11 (0)	14 (0)	11 (0)	3 (0)
2022	1 (0)	1 (0)	21 (0)	25 (0)	13 (0)	3 (0)

Table 4. Total number of published surveyed depredated nests per year from 2006 to 2011 (Coleman, 2011; Roberge, 2012), 2018 to 2019 (Sirgo, 2020), and 2021 to 2022 (current study).

Cedar Point Marsh	2006	2007	2008	2009	2010	2011	2018	2019	2021	2022
Depredated Nests	110	65	97	146	151	131	60	32	0	0

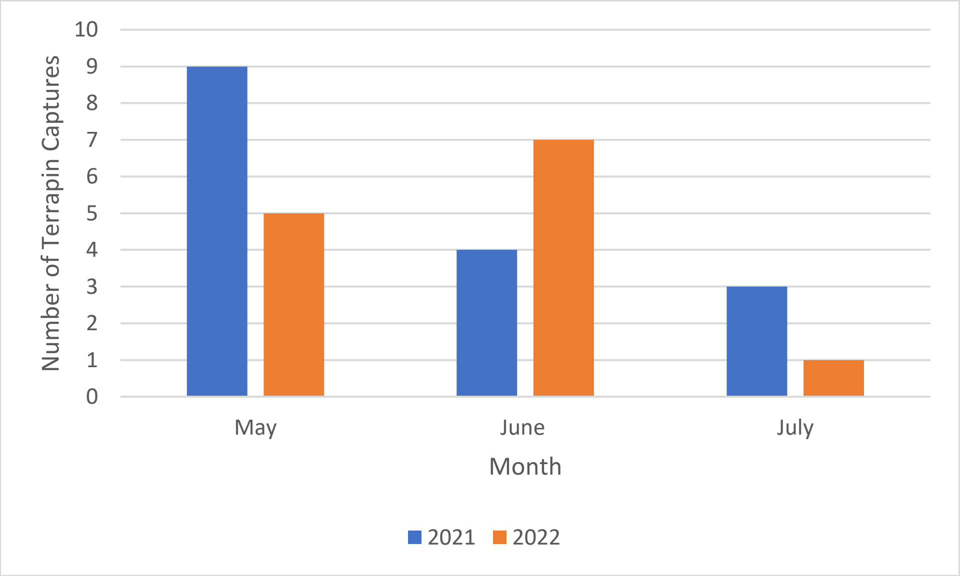

Figure 5. Number of captures per month for the 2021 and 2022 nesting seasons.

Figure 6. All 13 Mississippi Diamondback Terrapins (*Malaclemys terrapin pileata*) that were tracked in Cedar Point Marsh during the 2021 nesting season (white pins) and were found within Heron Bay from June 2021, to March 2022. All terrapins were released at a singular "Release Point" (red star). Reference **Table 2** for specific terrapin location.

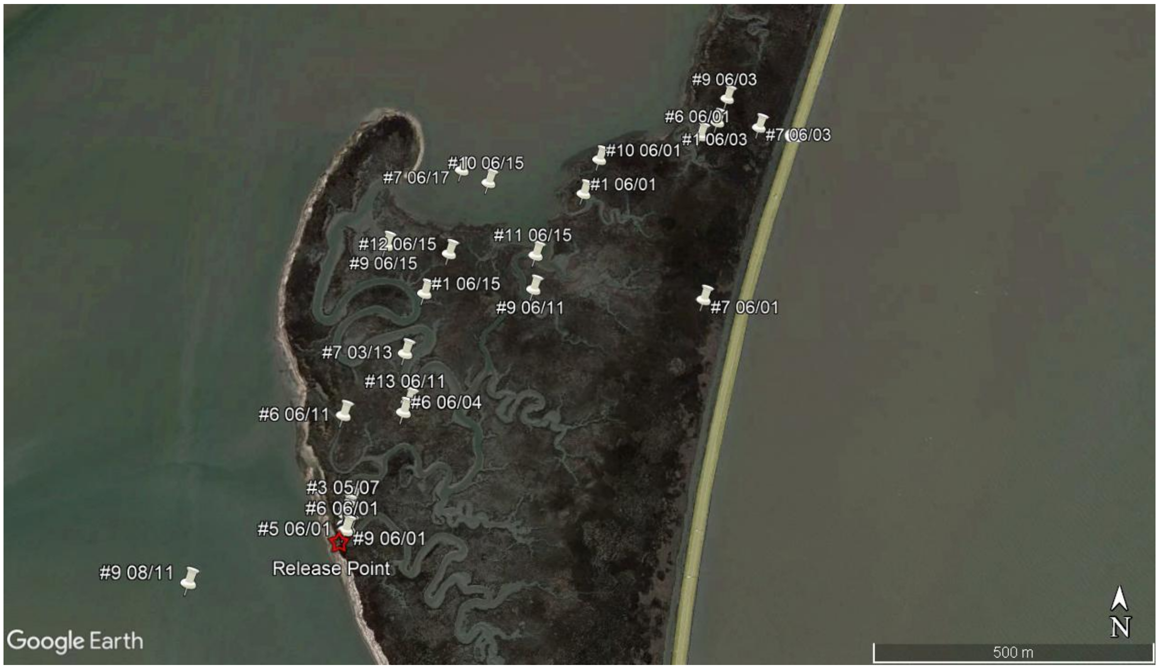

Figure 7. 9 Mississippi Diamondback Terrapins (*Malaclemys terrapin pileata*) that were tracked in Cedar Point Marsh during the 2021 nesting season (white pins) and were found within Heron Bay from June 2021, to March 2022. All terrapins were released at a singular “Release Point” (red star). Reference **Table 2** for specific terrapin location.

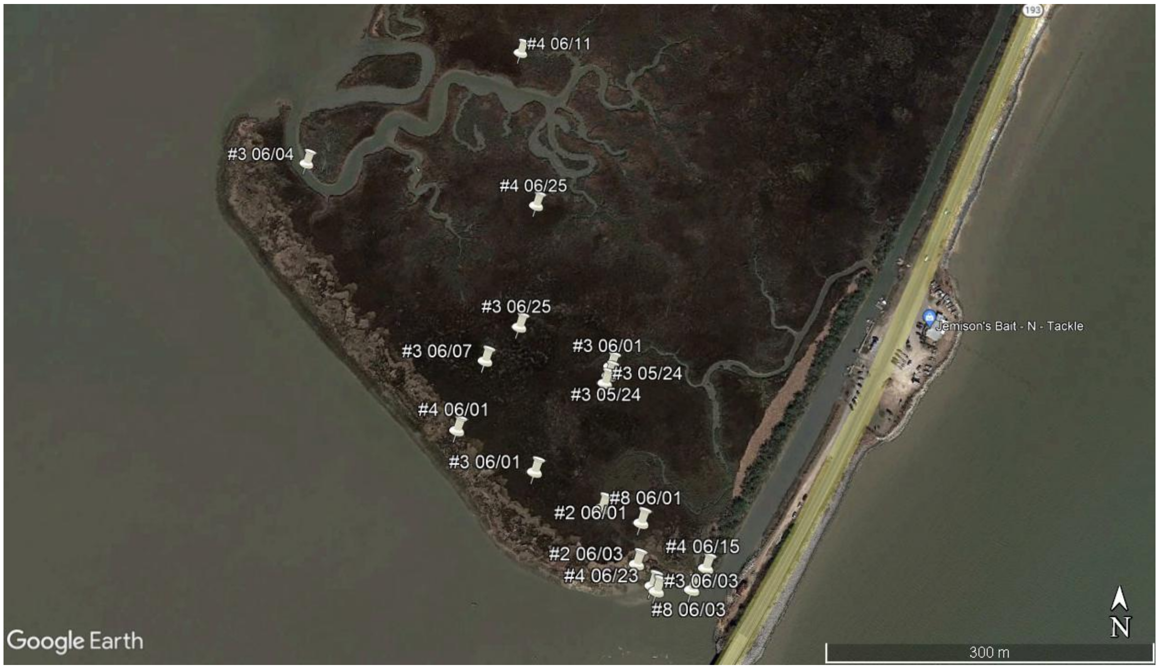

Figure 8. 4 Mississippi Diamondback Terrapins (*Malaclemys terrapin pileata*) that were caught in Jemison Marsh during the 2021 nesting season and were tracked within Heron Bay from June 2021, to March 2022 (white pins). Reference **Table 2** for specific terrapin location.

Figure 9. 10 Mississippi Diamondback Terrapins (*Malaclemys terrapin pileata*) that were caught during the 2022 nesting season and were tracked within Heron Bay from May 2022, to July 2022 (white pins). All terrapins were released at a singular "Release Point" (red star). Reference **Table 2** for specific terrapin location.

Discussion

Sampling of Terrapins on Cedar Point Marsh Nesting Beach

Consistent with previous nesting seasons, the results of the current study indicate that the shell hash beach of the western margin of Cedar Point Marsh is utilized annually as a major nesting area for terrapins in Alabama. The current results indicate a nesting season that spans from early May through at least the end of July, with nesting activity peaking during May and June. These findings are consistent with previous studies at Cedar Point Marsh (Coleman et al. 2011, 2014; Roberge, 2012; Sirgo, 2020).

The average size of the nesting terrapins (e.g., average PL length = 16.8 ± 1.1 cm) was consistent with those captured from previous years at Cedar Point Marsh (Coleman et al. 2011, 2014; Sirgo, 2020). A review by Coleman et al. (2014) indicates that nesting terrapins from the southeastern United States may be smaller than those in the northeast regions of the terrapin range. It was hypothesized by Coleman et al. (2014) that this size difference could potentially make these terrapins more susceptible to being captured in crab traps. The average-induced clutch size during the current study was 7.7 ± 1.8 eggs (20 clutches). This value is higher than the average values reported in previous years in Cedar Point Marsh and may reflect variability associated with oxytocin-induced oviposition (Coleman, 2011; Sirgo, 2020).

Of the 29 terrapins captured on the nesting beach at Cedar Point Marsh during the 2021 and 2022 nesting seasons, 5 were recaptures. The results for the recaptures indicate that terrapins can repeatedly use Cedar Point Marsh as a nesting area. The results also support that some of the terrapins can nest in consecutive nesting seasons and that one of the terrapins was documented over a nine-year period at the Cedar Point Marsh nesting beach (e.g., 0574). Finally, one of the recaptures from the Cedar Point Marsh nesting beach was a terrapin that was hatched in 2013 from an egg laid by a terrapin captured at Cedar Point Marsh (e.g., 1429). It was captively reared for two years at the University of Alabama at Birmingham and released in 2015 at Cedar Point Marsh.

Population Estimates

The results from the current study provide estimates ranging from 133 (2022) to 166 (2021) individuals (for the 2022 and 2021 nesting seasons, respectively). This was based on mark-recapture data from the current study and from previous studies from 2006 to 2019 (Coleman, 2011; Roberge, 2012, 2017; Sirgo, 2020). The population size estimates for the current study are within the range of values estimated previously in three previous studies of this nesting population ranging from 2006 to 2016; 54 (Roberge, 2012), 83 (Coleman, 2011), and 222 (Roberge, 2017). The population size of these nesting terrapins is distinctly smaller than the population sizes seen on the east coast of the United States (Coleman, 2011; Roberge, 2012, 2017; Lovich and Hart, 2018; Lovich at el. 2018). These results support the Mississippi Diamondback Terrapin’s population status of being of “highest conservation concern” in the state of Alabama (Nelson and Marion, 2004; Lovich and Hart, 2018).

Radio Tracking

The findings from the radio tracking studies during 2021 and 2022 indicate several general aspects of the ecology of terrapins nesting at Cedar Point Marsh nesting beach. Many of the terrapins were shown to reside in Cedar Point Marsh for several weeks to several months after their release. This suggests that Cedar Point Marsh is not only of importance for essential nesting terrapins but is also important for foraging. This supports how Cedar Point Marsh supports all stages in the life history of the Diamondback Terrapin. For example, a previous study have indicated that terrapins for that nesting beach orient towards the marsh following nest emergence, revealing the importance of the marsh system for early development and forward (Coleman, 2011). The terrapins that were captured within the northern portion of Heron Bay during the 2021 and 2022 nesting seasons were relocated back north within a few weeks of release from Cedar Point Marsh. This could represent a home range for terrapins, suggesting that they are returning to optimal foraging sites or adhere to a site fidelity for future seasons (Yearicks et al. 1981; Lamont et al. 2021).

Nest Depredation Surveys

As indicated in Table 4, relatively large number of depredated nests have been consistently recorded on the Cedar Point nesting beach between 2006 and 2019 (Coleman, 2011; Roberge, 2012; Sirgo, 2020). Previous studies have indicated that predator removal programs can be an effective means of increasing nest survivorship on turtle nesting beaches (Christiansen and Gallaway, 1984; Spencer and Thompson, 2003; reviewed by Munscher et al. 2012) Due to the previously documented relatively high number of depredated nests at Cedar Point Marsh, the USDA APHIS initiated a racoon removal program at that location during the winter and spring of 2020 (Stephens and Buckley, 2022). Their results suggested a relatively small number of racoons had inhabited that area and were subsequently removed during the winter and spring of 2020. During the current study, the Cedar Point Marsh nesting beach was frequently surveyed for depredated nests throughout the 2021 and 2022 nesting seasons. In contrast to the previous surveys reviewed in Table 4, no depredated nests were detected during 2021 and 2022 nesting seasons (**Table 3**). These results indicate that racoon removal programs can represent an effective means of decreasing nest depredation on Diamondback Terrapin nesting beaches. A previous study by Munscher et al. (2012) also indicated that a racoon removal program significantly decreased the level of nest depredation on a terrapin nesting area in northeast Florida. During that study, racoons repopulated the area and nest depredation increased the year after the predator removal program was terminated. The results from the current study indicate that the predator removal during 2020 was an effective management tool that has significantly decreased nest depredation for two nesting seasons. This could be potentially be due to the isolated nature of Cedar Point Marsh (e.g., connected to the mainland and to Dauphin Island via bridges) which could delay the repopulation of the area by racoons. It will be of interest to continue monitoring the Cedar Point Marsh nesting beach to evaluate the chronology of racoon repopulation and recruitment in that area.

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GENERAL DISCUSSION

The current thesis addresses the reproductive and foraging ecology of the Diamondback Terrapin in Alabama by combing the use of classic ecological methodologies with modern technologies. The findings of this thesis extend our understanding of the ecology and conservation status of the Diamondback Terrapin in Alabama.

Chapter 1 of this thesis implemented stable isotope technology to investigate the foraging ecology of this species in the eastern portion of the Mississippi Sound in Alabama. Stable isotope values were documented in for adult female terrapins collected on a major nesting beach located at Cedar Point Marsh. The variation in $\delta^{13}C$ and $\delta^{15}N$ stable isotope values suggested potential individual variation in foraging behavior. Additionally, the stable isotope values from Cedar Point Marsh appeared unique in comparison to values reported for three terrapin populations in southwest Florida. The foraging ecology of the terrapins was further evaluated by analysis of stable isotopes values for prey items and primary producers in the eastern Mississippi Sound. The results indicated species-specific and site-specific variation in $\delta^{13}C$ and $\delta^{15}N$ values which provide insight on the foraging ecology of the Diamondback Terrapin. The results document baseline isotopic values in terrapins, terrapin prey items, and primary producers in salt marsh habitat in the eastern Mississippi Sound. This sort of information is essential to understanding the foraging ecology for the Diamondback Terrapin in Alabama. Further, this study represents the first documentation of $\delta^{13}C$ and $\delta^{15}N$ stable isotope values for the Diamondback Terrapin from a major nesting location in the northern Gulf of Mexico.

Chapter 1 also includes the development and evaluation of an experimental system for evaluating the impact of specific prey items on the stable isotopes of terrapins. This approach utilized head start terrapins that were fed natural prey items. The findings of the experiment show that head start terrapins can provide a model for evaluating resource assimilation in the Diamondback Terrapin.

Chapter 2 evaluated the reproductive ecology and conservation status of the Diamondback Terrapin during the 2021 and 2022 nesting seasons at Heron Bay, Alabama. Adult female terrapins captured at Cedar Point Marsh nesting beach were utilized in a long-term mark-recapture study in order to estimate the nesting population size. The findings indicate that although Cedar Point Marsh represents a major nesting location for Diamondback Terrapins in Alabama, it supports a relatively small population of nesting females in Alabama. The movements of a subset of the adult females captured on the nesting beach or in Heron Bay area were monitored through radio tracking technology. The results indicated that many of these turtles remained in the Heron Bay area, including Cedar Point Marsh (bordering the eastern portion of the Heron Bay) and Jemison Marsh (bordering the northern portion of Heron Bay).

Chapter 2 also included an evaluation of the magnitude of nest depredation on the Cedar Point Marsh nesting beach during the 2021 and 2022 nesting seasons. The resulting data was of a particular importance because it followed a raccoon removal program in 2020 by the USDA. In contrast to year prior to 2020, no depredated nests were detected during the 2021 and 2022 nesting seasons. These results indicate that raccoon removal represents an effective management strategy at Cedar Point Marsh nesting beach. Further, the results indicate that the duration of the impact is at least two years suggesting a prolonged period for raccoons to repopulate the Cedar Marsh area.

Collectively, the research completed in this thesis provides base line information on foraging and reproductive ecology including the documentation of habitat that is essential for several stages of the life history for the Diamondback Terrapin. This information is essential for evaluating the conservation status and developing an effective recovery strategy for the Diamondback Terrapin in Alabama.

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