2012

Evaluating the Reproductive Ecology of the Diamondback Terrapin in Alabama Salt Marshes: Implications for the Recovery of a Depleted Species

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Recommended Citation

Roberge, Taylor Matthew, "Evaluating the Reproductive Ecology of the Diamondback Terrapin in Alabama Salt Marshes: Implications for the Recovery of a Depleted Species" (2012). *All ETDs from UAB*. 2838.
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EVALUATING THE REPRODUCTIVE ECOLOGY OF THE DIAMONDBACK TERRAPIN IN ALABAMA SALTMARSHES: IMPLICATONS FOR THE RECOVERY OF A DEPLETED SPECIES

by

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A THESIS

Submitted to the graduate faculty of The University of Alabama at Birmingham,
in partial fulfillment of the requirements for the degree of
Master of Science

BIRMINGHAM, ALABAMA

2012

EVALUATING THE REPRODUCTIVE ECOLOGY OF THE DIAMONDBACK TERRAPIN IN ALABAMA SALTMARSHES: IMPLICATONS FOR THE RECOVERY OF A DEPLETED SPECIES

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BIOLOGY

ABSTRACT

The diamondback terrapin (*Malaclemys terrapin*) was once an abundant and economically important species in the salt marshes of Alabama. A variety of threats have impacted this species over the past century, resulting in a drastic decline in the population. Diamondback terrapins are currently considered a “priority one species (highest conservation concern)” in Alabama. The largest nesting aggregation documented to date in Alabama is on the 1.8 km long shell-hash nesting beach bordering the western edge of Cedar Point Marsh (CPM). The current studies address several aspects of the reproductive ecology of the diamondback terrapin in Alabama. Nesting beach surveys were conducted over four nesting seasons to monitor depredated nest abundances and locations on CPM nesting beach from 2008-2011. The mean number of depredated nests recorded each year was 131± 24. Nesting did not show uniform distribution over the length of the nesting beach, with factors such as vegetation and distance from the salt marsh channels possibly playing a role. Mark-recapture data for nesting females from 2011, as well as previously reported data (2006-2010) indicate that approximately 53 adult females utilize the CPM nesting beach. Eighteen adult females were tagged with radio transmitters over the 2010 and 2011 nesting seasons. Radio telemetry data indicate the CPM nesting beach is utilized by not only adult females that remain resident in CPM, but also adult females resident in adjacent marshes in the Heron Bay area. This highlights the importance of CPM and the Heron Bay area as critical habitat for terrapins in Alabama. Female-biased hatchling sex ratios were predicted in the 2011 nesting for the CPM nesting beach; only nests laid early in the nesting produce mixed sex ratios. Surrogate nest studies also showed a female-biased sex ratio, indicating the temperature-based model is a good predictor of sex on this beach. Radioimmunoassay was used to examine the serum testosterone levels of juvenile terrapins as a potential sexing technique. Results indicate that serum testosterone levels are a practical method for sexing juvenile terrapins. The results of this thesis provide critical information for enhancing the management strategy and recovery of the diamondback terrapin in Alabama.

conservation—sex ratios—radio telemetry—sexing technique—nesting characteristics—population estimate—threats

DEDICATION

To my parents, Ray and Laurie Roberge, who have supported the decisions I have made throughout my academic career. The continuation of my education would not have been possible without it.

ACKNOWLEDGEMENTS

First I would like to thank the members of my committee: Thane Wibbels, Ken Marion, and David Nelson. My research would not have been possible without their guidance and advice. I would also like to acknowledge the funding resources for this project: Alabama Center for Estuarine Studies, the Alabama Department of Conservation and Natural Resources, Alabama Academy of Science, the Diamondback Terrapin Working Group, as well as the University of Alabama at Birmingham Department of Biology. I would also like to thank Bud Fischer for always being open to listen to questions, as well as Steve Watts, for his guidance during my graduate studies. In addition, I would like to thank my friends and coworkers who were always willing to volunteer their time on various projects, even when it meant being eaten alive by various insects. Finally, I would like to thank my girlfriend Samantha Haggerty, for putting up with, at times, a crazy schedule including late nights at the lab, and last minute drives to Dauphin Island.

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GENERAL INTRODUCTION

Brackish Water Habitat

The diamondback terrapin (*Malaclemys terrapin*) is unique in the fact that it is the only emydid turtle in North America that selectively inhabits the brackish waters of estuaries, salt marshes, and bays (Hart and Lee, 2006). Emydid turtles are typically found in freshwater ecosystems. A few emydid turtles have been able to colonize estuarine areas, but most cannot survive in full sea water for more than a few days because they cannot rid themselves of excess salt, and thus become dehydrated. In contrast, diamondback terrapins are able to spend several weeks in full sea water without needing constant access to fresh water (Davenport and Ward, 1993; Davenport, 1992; Hart and Lee, 2006).

Several adaptations have allowed the diamondback terrapin to survive and succeed in the fluctuating salinities of the salt marshes. Similarly to other marine reptiles, terrapins can excrete excess sodium through lachrymal glands near the eyes. However, it has been shown that the gland only contributes partially to the terrapin's ability to live in brackish water habitats (Hart and Lee, 2006). Another physiologic adaptation of the diamondback terrapin is its very low skin permeability to salts and water (Davenport, 1992). Even with all of these adaptations, the terrapin will inevitably become dehydrated in full sea water (e.g. 36ppt) (Davenport, 1992). Because of this, the terrapin has adapted certain behaviors to avoid dehydration and maintain equilibrium. The low turbulence of some areas in the salt marsh allows for stratification of water to occur when there is heavy rainfall. A thin film of fresh water forms on the surface of the brackish water and the terrapins are able to drink from this film using a unique drinking posture. Terrapins have also been known to drink from droplets on plants and even from the curled margin of other turtles' carapaces (Davenport, 1992; Hart and Lee, 2006).

Role in the Salt Marsh Ecosystem

The diamondback terrapin inhabits salt marshes from Cape Cod to Corpus Christi Texas (Hart and Lee, 2006). The terrapin can be considered a possible keystone species by controlling the abundance of the periwinkle snail (*Littorina irrorata*), a favorite prey item. It is suggested that control of *Littorina* through predation is important for the general health and function of the salt marsh ecosystem (Gustafson et al., 2006). Silliman et al. (2005) as well as Gustafson et al. (2006) have both observed negative impacts of increased grazing of *Littorina* on *Spartina* vegetation if left unchecked. Diamondback terrapins are also known to prey on other mollusks, crustaceans, and scavenged fishes (Tucker et al., 1995). Roosenburg et al., 1999 showed that in the absence of abundant *Littorina*, terrapins rely on a diet consisting of more mollusks and crustaceans.

As diamondback terrapins grow they tend to move from specialized feeders on small *Littorina,* to a general diet of other prey items, as well as larger *Littorina*. The choice of prey has been shown to correlate with size, making the overlap of diets smaller between females and males (Tucker et al., 1995). The difference in size also allows for variable habitat usage. Larger mature females will use a more open water area of habitat, while smaller males and juveniles will stay close to shallow water habitats (Roosenburg *et al*., 1999). Terrapins seem to show home site fidelity, with some even being recaptured within 100 meters of previous capture (Gibbons et al., 2001).

Reproductive Biology and Life History

Mating is often observed in the spring as the salt marsh gradually warms (Seigel, 1980). Starting in early May to early June, the female will lay one to three clutches of approximately 5-12 eggs per clutch over the nesting season (Davenport, 1992; Hart and Lee, 2006; Mann, 1995). The nests are dug above the high tide line at a depth of 16 - 25 cm (Roosenburg, 1994). The required incubation period is a range of 45-86 days depending on the temperature (Burger, 1976; Jeyasuria et al., 1994). Diamondback terrapins display temperature-dependent sex determination (TSD), where the sex of the turtle is determined by the incubation temperature of the eggs' surroundings, with relatively warm temperatures producing females and relatively cool temperatures producing males (Jeyasuria et al., 1994; Roosenburg and Place, 1995). Thus, specific beach characteristics can influence the sex ratios, where cool shady beaches would produce mostly males and warm open beaches would produce predominately females. Because of TSD, it is of utmost importance that terrapins have enough variable nesting beaches in an area so that enough turtles of each sex are produced each nesting season (Hart and Lee, 2006). The rate of mortality is extremely high during the incubation and hatching period. Predators such as raccoons, skunks, fish crows, ghost crabs, and foxes prey on the eggs and hatchlings (Burger, 1977; Butler et al., 2004; Davenport, 1992). Most diamondback terrapins emerge during the day and head for the nearest vegetation instead of the open horizon (Burger, 1976; Coleman, 2012). This behavior quickly provides protective cover in order to avoid many predators such as birds (Davenport, 1992; Burger, 1976).

Hatchling and post-hatchling terrapins are believed to reside within the tidal rack and associated terrestrial portions of the salt marsh, feeding on small invertebrates (Lovich et al., 1991). Current estimates indicate that females will mature in approximately 7 years, where males may mature in approximately 4 years at a smaller size (Hart and Lee, 2006). During this time period, terrapins make a transition from feeding on small invertebrates in the tidal rack to larger invertebrates in the salt marsh (Lovich et al., 1991; Tucker et al., 1995).

Cultural History

The diamondback terrapin has played a significant cultural role in the history of America. In colonial America, these turtles were an important food item for the continental army and later a major protein source for slaves on tidewater plantations. In the late 1800's and through the Great Depression, terrapins were an exclusive sought-after item in markets and restaurants, as well as an important food source for those living in secluded coastal areas. This turtle became so fashionable as a delicacy during the early 1900s, that special bowls and forks became a part of the flatware of the wealthy (Hart and Lee, 2006). The demand in the Northeast grew so much that terrapins were even shipped from the Gulf Coast by the thousands to satisfy demands in the Northeast (Carr, 1952). The demand for terrapin stew resulted in a population crash and an effort was made by the U.S. Federal Bureau of Fisheries, from 1909 to 1940, to rear terrapins for restocking and commercial use (Hart and Lee, 2006). Factors such as the economic decline in the early 1900’s, as well as the loss of one of the main ingredients, sherry, during prohibition contributed to the decline of the commercial harvest of terrapin (Hart and Lee, 2006). Although a limited commercial demand still existed for terrapins in the late 1900’s, it was far less than that from a century prior. Unfortunately, a commercial demand for the terrapin in Chinese markets has appeared and is putting pressure on populations that have still not fully recovered from the historical over-harvesting (Brennessel, 2006).

History in Alabama

Recorded anecdotes show that the now depleted population located in Alabama was once very abundant. During the late 1800’s, Alabama supported one of the largest diamondback terrapin farms in the United States. It was located at Cedar Point Marsh, next to the present causeway leading to Dauphin Island (New York Times article, 1881). It was said to contain 25,000 terrapins in a 3-acre salt marsh farm. Every year, twelve thousand terrapins were shipped by rail to Savannah, Georgia, to be loaded onto steam boats headed to New York. The Farm also obtained up to eight thousand new terrapins each year that were collected locally at a price of 3$ per dozen (New York Times article, 1881). Comments from a fisherman who lived on Little Dauphin Island in the early 1900’s indicated that a twenty minute tow with a drag seine could capture a “corn sack” full of turtles (J.W. Barber, Dauphin Island, Alabama, pers. comm.). The economic importance of this animal during the 1900’s can be seen by the fact that Alabama enacted a terrapin tax that charged 5 cents for every commercially-harvested terrapin (Brennessel, 2006). The population seemed to decrease substantially in the late 1900’s, correlating with the increase of the crab fishery and coastal development (J.W. Barber, Dauphin

Island, Alabama, pers. comm.). By the late 1900's, surveys indicated that the terrapin population in Alabama was drastically depleted, and in 2004 it was designated a "Priority One Species (Highest Conservational Concern)" (Marion and Nelson, 2004).

Threats/ Reasons for Decline

There are many different threats present throughout the range of the diamondback terrapin. Many of these threats are of anthropogenic origin and therefore can possibly be reduced. These threats include incidental capture and drowning of terrapins in crab traps, predation of eggs and hatchlings on the nesting beach, road mortality of females during nesting migrations, habitat loss through coastal development, as well as injuries sustained by boat strikes (Hart and Lee, 2006; Gibbons et al., 2001; Roosenburg et al., 1995; Wood, 1997; Wood and Herlands, 1997).

Many of the diamondback terrapins' preferred nesting sites have been impacted by coastal development. Roadways built crossing or parallel to salt marshes, the primary nesting habitat, are now heavily trafficked. Females attempting to nest may cross these roads and have a significant chance of being struck by motor vehicles (Wood and Herlands, 1997). The selective killing or injury of mature females is particularly detrimental to the population's recovery because they are individuals with high reproductive value.

Another major threat to the recovery of the terrapin populations is the incidental capture and drowning of individuals in crab traps. Because crab traps are fully submerged, once a terrapin enters and it cannot reach the surface for air, and will drown. Trap-induced mortality is common throughout most of the extensive coastal range of the terrapins, and the number of total annual drownings can have a significant impact on the entire species (Wood, 1997). In addition to commercial crab traps (that are checked on a regular basis), “ghost,” or derelict, crab traps (that have been abandoned and remain submerged indefinitely) may actually be doing more damage by continuously drowning terrapins year round. For example, a single derelict crab trap in North Carolina contained 29 dead terrapins; 49 terrapins were found in a ghost trap in the Chesapeake Bay (Roosenburg et al., 1997); and the remains of 94 terrapins were found in a single crab trap in Georgia (Grosse et al., 2009). For example, the mortality from a single derelict crab trap can represent up to 2.8% of the population in a single area (Roosenburg, 1990). There is a size/sex bias in crab trap mortality. The opening of the crab trap excludes the largest terrapins (females), which can result in an increased capture rate of adult males in comparison to larger adult females (because of sexual size dimorphism). Males remain vulnerable to trap-mortality for their entire lives whereas females may no longer be able to enter traps around the age of 8 years (Roosenburg et al., 1997). The use of TEDs (Terrapin Excluder Devices) has been shown to significantly reduce the capture of terrapins (Wood, 1997; Coleman, 2011). However, they have been implemented only in a few areas throughout the range of the diamondback terrapin.

The loss of both suitable nesting beach and salt marsh habitat has also been problematic for recovering terrapin populations. The act of placing bulkheads to stabilize the shoreline functions as a fence the keeps terrapins from accessing areas above the high tide line, resulting in inundation and drowning of the eggs. Even seemingly non-destructive erosion control procedures, such as planting beach grasses, can be detrimental to the eggs and nesting of terrapins. The rhizomes of the grasses can enter the eggs and kill them, as well as entangle the hatchlings (causing them to die underground). Beach grasses can also change the microclimate of the beach, resulting in different temperatures and soil moisture (Roosenburg, 1990).

Boat strikes that cause major shell damage can reduce the survivorship and the reproductive output of terrapin populations (Cecala et al., 2008). Mature females are the most susceptible to these types of shell damage, because they are the size class that is best able to venture into deeper waters where watercrafts are more common (Roosenburg et al., 1999).

Predation on nesting beaches also presents a major threat to terrapin populations in several life stages. Terrapin eggs, hatchlings, and adult terrapins all experience predation (Feinberg and Burke, 2003). Egg and hatchling predators include stripped skunks, ghost crabs, crows, laughing gulls, and the most common predator in most nesting areas, raccoons (Feinburg and Burke, 2003; Butler et al., 2004). Dead adult terrapins have been found where predation, most likely from raccoons, appeared to be the primary cause of mortality; however, no predation was actually observed. This predation has a bias towards adult females caused by the need to climb onto shore to nest. This bias toward female mortality can have severe consequences on a population’s ability to survive and recover (Feinburg and Burke, 2003). Raccoons have been reported to be the primary source of nest predation in many areas. Feinberg and Burke (2004) found that in their study area 98% of the depredated nests counted had evidence of predation by raccoons. In some cases late in the nesting season, raccoons consumed the entire egg including the shell. Birds can also be major nest predators. While raccoons tend to depredate all eggs in a nest, birds seem to take only a few eggs from each nest (Feinburg and Burke, 2003). When birds take only a few eggs from nests, they leave the nests exposed for other predators (e.g. raccoons) (Butler et al., 2004). Predation by raccoons on eggs, hatchlings and adult females may cause the greatest non-human threat to a population and can be detrimental to its future success and recovery.

Current status of the diamondback terrapin in Alabama

Marion and Nelson (2004) reported that the diamondback terrapin population in Alabama was depleted and it is considered a species of “highest conservation concern”. Therefore, comprehensive surveys were initiated in the summers of 2004 and 2005 by researchers at the University of Alabama at Birmingham and the University of South Alabama to evaluate the abundance and distribution of this species in the salt marshes of Alabama. These surveys indicated that the diamondback terrapin in Alabama is represented by small aggregations in a few specific locations. The surveys also identified Cedar Point Marsh as the most important nesting beach in Alabama that contains the largest aggregation of terrapins. Interestingly, this is the same area that contained the terrapin farm from the late 1800’s. The surveys have also verified that the population has declined to the point that its classification as a “Priority One” species of “Highest Conservation Concern” is justified (Marion and Nelson, 2004; Coleman, 2011).

Specific aims of Thesis Research

The primary goal of this thesis was to evaluate various aspects of the reproductive ecology and physiology, as well as the conservation status of the diamondback terrapin in Alabama. The ultimate goal was to utilize these data in order to enhance the recovery of this depleted population.

The study described in chapter 1 is an evaluation of the nesting ecology of the diamondback terrapin at Cedar Point Marsh. This included the sampling of nesting females throughout two nesting seasons and evaluating their fecundity. Additionally, depredation of natural nests were monitored for two nesting seasons. This study includes the most comprehensive estimate to date of the size of the nesting population at this location. It also includes an evaluation of spatial and temporal characteristics of nesting. Finally, it quantifies the level of depredation on this important nesting beach in Alabama.

In chapter 2, the inter-nesting and post-nesting movements of adult female terrapins nesting at Cedar Point Marsh are examined. These data address the extent to which terrapins from the Heron Bay Area utilize the Cedar Point Marsh nesting beach. Additionally the results provide insight in identifying post-nesting critical habitat for these terrapins in the Heron Bay area.

In chapter 3, the natural effects of temperature-dependent sex determination are evaluated on the Cedar Point Marsh nesting beach. Nesting beach temperature profiles at Cedar Point Marsh were recorded at representative locations for two full nesting seasons. These data provide a basis for developing a model for predicting the sex ratios produced from nests at various times and locations throughout the nesting season. This study also included a natural experiment in which eggs from a surrogate species with a similar temperature-dependent sex determination characteristics was used to provide ground-truth for sex ratios produced on the natural nesting beach.

In chapter 4, a sexing technique for juvenile terrapins was evaluated. Serum testosterone levels were examined in captive-reared juvenile terrapins via radioimmunoassay. These turtles were obtained from eggs incubated under either male or female temperatures and raised for 2 to3 years. The results were analyzed to determine if serum testosterone levels represents an accurate and practical method for sexing juvenile terrapins.

Collectively, the results from this thesis provide information on the diamondback terrapin that increases our understanding of its reproductive biology and conservation status, thus facilitating our ability to enhance the recovery of this depleted species in Alabama.

CHAPTER 1

NESTING CHARACTERISTICS AND ABUNDANCE OF FEMALE DIAMONDBACK TERRAPINS UTILIZING THE CEDAR POINT MARSH NESTING BEACH

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In preparation for Journal of Herpetology
Format adapted for thesis

Abstract.—The diamondback terrapin (*Malaclemys terrapin*) was once an abundant and economically important species in the salt marshes of Alabama. A variety of threats have impacted this species over the past century, resulting in a drastic decline in the population. The diamondback terrapin is currently considered a “priority one species (highest conservation concern)” in Alabama. Surveys during recent years indicate that the diamondback terrapin is now represented by only small aggregations in specific salt marshes along the Alabama coast. The largest nesting aggregation documented to date in Alabama is on the 1.8 km long shell-hash nesting beach bordering the western edge of Cedar Point Marsh (CPM). The current study addresses several aspects of the nesting ecology of the diamondback terrapin at CPM. Nesting beach surveys were conducted over four nesting seasons to monitor depredated nest abundances and locations. Nesting typically begins in late April to early May and extends to early August. The number of depredated nests ranged from approximately 97 to 151 per nesting season, with a mean of 131± 24. The locations of depredated nests recorded were not distributed evenly over the entire nesting beach, with the more frequented areas consistently in similar locations from year to year. Additionally, drift fences equipped with pitfall traps were used to sample nesting females during the 2011 nesting season. A total of 13 females were captured, 11 of them were over 10 years in age and the remaining two were 7-8 years in age. Ten of these were successfully induced to lay eggs. The mean clutch size was 7.1± 1.7. The 72 resulting hatchlings have been successfully reared for over 9 months, with no mortality, as part of a head-start conservation program for the recovery of the diamondback terrapin in Alabama. Based on mark-recapture data from the nesting females together with previously-reported data (2006-2010), the results suggest a total of approximately 53 nesting females utilizing the Cedar Point Marsh nesting beach. Previous surveys suggest that the CPM nesting beach is the major nesting beach for diamondback terrapins in Alabama. The current results indicate that this species is significantly depleted in Alabama, and its recovery is dependent on a comprehensive management strategy.

Key words: Alabama; Depredated nests; Schnabel method; *Malaclemys terrapin*; Nest Distribution

Introduction

Diamondback terrapins (*Malaclemys terrapin*) range from approximately Cape Cod, MA to Corpus Christi, TX. They are the only emydid turtle in North America to exclusively inhabit the brackish waters of estuaries, salt marshes and bays. They have been suggested to be a keystone predator that helps stabilize the salt marsh ecosystem by feeding on the salt marsh periwinkle *Littorina sp.* (Tucker et al., 1995; Silliman et al., 2005; Gustafson et al. 2006). *Malaclemys terrapin pileata* is the subspecies that inhabits the salt marshes of Alabama. Historical data indicate that terrapins were once very abundant in the coastal waters of Alabama (Carr, 1952; Jackson and Jackson, 1970; Nelson and Marion, 2004). In fact, it was a commercially valuable species in Alabama and was exported heavily to the northeast for making terrapin stew (New York Times, 1881; Brennessel, 2006). During the late 1800s the state of Alabama boasted about the largest terrapin farm in the country (New York Times, 1881).

Despite the abundance of salt marsh habitat and state protection for the species, recent surveys indicate that this once abundant species has become scarce in Alabama salt marshes, estuaries and bays (Nelson and Marion, 2004; Coleman, 2011). The diamondback terrapin has declined in Alabama to the point that it is considered a "Priority One" species of "Highest Conservation Concern" (Mirarchi et al., 2004). Surveys over the past few years indicate that the diamondback terrapin is represented by small aggregations in specific salt marshes along the coast of Alabama. The largest aggregation identified to date is located in Cedar Point Marsh, which is located north of Dauphin Island (Wibbels et al., 2009; Coleman, 2011).

The purpose of the current study was to evaluate the location and abundance of nesting at Cedar Point Marsh. This study includes the most comprehensive estimate to date of the total number of adult females that utilize the CPM nesting beach, the most important nesting beach, identified to date, for diamondback terrapins in Alabama.

Materials and Methods

Adult Female Capture and Estimation of Abundance.—Six drift fences, 100 ft long, were set out running parallel on the nesting beach of Cedar Point Marsh. Four pit fall traps were placed in an alternating pattern along each length of fence. Shades were placed over each trap to prevent captured turtles from overheating. Drift fence traps were checked daily for captured terrapins and to make any necessary repairs to the fence and shades. When a capture was made, the terrapin was transported to the laboratory where morphological measurements could be taken and gravid females were induced to lay by injecting 10 I.U. of oxytocin, intramuscularly, into the front limb (Ewert and Legler, 1978). Any recaptures were noted and all unmarked terrapin were tagged with a cable-tie tag labeled with a unique number, as well as an individually-coded PIT tag inserted sub-cutaneously on the rear left limb. The terrapins were then released as soon as possible near their location of capture.

In order to estimate the total number of adult females utilizing the nesting beach the data from 2011 were examined relative to data reported from 2006 to 2010 (Coleman *et* al., 2011). The Schnabel method (Schnabel, 1938) was used to determine the population size of nesting females using the following equation:

$$\frac{\sum(C_t * M_t)}{\sum(R_t) + 1}$$

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.

Upper and lower confidence limits of 95% were determined using the total number of recaptures and the methods described in Chapman and Overton (1966).

Depredated Nest Surveys.—The 1.8 km long Cedar Point Marsh nesting beach was surveyed for depredated nests approximately once a week for four nesting seasons (2008-2011; preliminary data from 2008-2010 listed in Coleman (2011) see appendices). Additionally, the northern-most portion of the nesting beach was periodically surveyed approximately once a month since it was accessible only by boat. The surveys consisted of looking for depredated nests, noting the location, and an approximate number of eggs (based on surrounding egg shells). Only digs with associated egg shells were counted as depredated nests.

Depredated nest locations were plotted onto Google Earth® for each year. The CPM beach was arbitrarily broken up into four equal sections and the number of nests in each section were quantified and compared.

Results

Nesting beach surveys at CPM were conducted over the 3 - 4 month nesting season for four consecutive years (2008-2011). Fresh nests were never detected during those surveys, although areas that looked like potential nests were often excavated. The shell-hash substrate of the beach made tracks of nesting turtles and signs of a nesting difficult, if not impossible, to identify. Additionally, freshly emergent nests were never observed. Nesting typically began in late April to early May and extended to early August (Figure 1). Depredated nests recorded during each of the nesting seasons are shown in Table 1. The mean number of depredated nests per season was 131± 24. The locations of nests are mapped on Figures 2 through 6.

The distribution and total number of depredated nests each year are summarized in Table 1. The nesting beach was broken into four sections each .275 miles long (Table 1; Figure 2). Thirteen nests from the 2008 season were excluded as accurate latitude and longitude measurements were not possible. The overall distribution of depredated nests throughout the years shows an uneven pattern along the entire nesting beach (Figure 2) (G-Test, $p<.05$).

A total of 13 females were captured on the nesting beach during the 2011 nesting season, 7 of those being recaptures marked in previous years. One terrapin, tag number 0171, has been captured every year after its initial capture in 2007. There were no terrapins both marked and recaptured during the 2011 nesting season. Using the Schnabel method, the estimate for adult female terrapins utilizing the nesting beach located on CPM was a total of 53 individuals with upper and lower confidence limits of 37 and 76, respectively. Assuming that the number of depredated nests reported above represents the majority of the nestings, the estimated total number of nesting females is consistent with the total number of depredated nests, assuming 2-3 clutches per female per season (Seigel, 1984; Roosenburg, 1991).

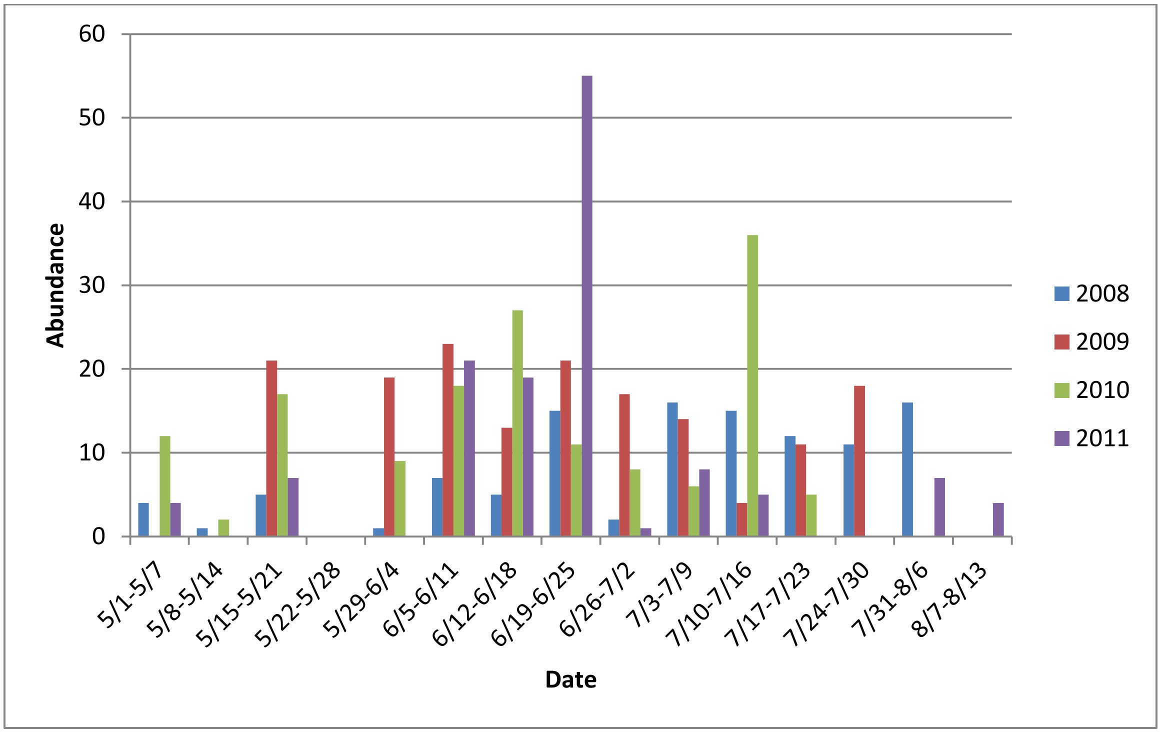

Figure 1. Temporal distribution of depredated nests over four nesting seasons (2008-2011).

Table 1. Yearly abundance and distribution of depredated nests. (See Figure 2 for area locations)

Area	Nesting Season			
	2008	2009	2010	2011
A	10	35	34	34
B	59	58	61	49
C	17	28	26	41
D	11	25	30	7
Total Nests	97	146	151	131

Figure 2. Distribution of depredated nests over four nesting seasons and assigned areas.

(Red=2008, blue=2009, green=2010, yellow=2011)

Figure 3. Depredated nest locations during the 2008 nesting season.

Figure 4. Depredated nest locations during the 2009 nesting season.

Figure 5. Depredated nest locations during the 2010 nesting season.

Figure 6. Depredated nest locations during the 2011 nesting season.

Discussion

The results indicate that the nesting at CPM beach for diamondback terrapins in Alabama starts in late April to early May and extends until early August. The period with the highest nesting abundance seems to occur in early June and continues to early July. Nesting in 2010, as well as 2011, appeared to occur in waves (Figure 1). It is unclear what may be causing the increase in nesting during certain times of the nesting season. However, anecdotal evidence suggests that an increase in nesting will follow a precipitation event. Increased capture of females in drift fences also seems to be related in some way to weather events, but more data must be gathered in order to fully understand the cues related to nesting.

Nesting was not distributed evenly across the nesting beach. Instead, there appear to be preferred nesting locations. It is plausible that certain characteristics of the beach make the areas more desirable for nesting such as the amount of vegetation, and accessibility of the beach from the marsh (e.g., close proximity to tidal channels). Although nesting is not uniformly distributed, it occurs over the entire length of the beach.

The nesting beach surveys were relatively consistent between years with approximately 131± 24 depredated nests per year. Assuming that diamondback terrapins lay 2-3 clutches per year (Seigel, 1984; Roosenburg, 1991), this represents the reproductive output of a minimum of approximately 44 to 65 adult females. However, if the depredated nests represent only a percentage of the total nesting a larger number of females would be predicted.

The mark-recapture data from adult nesting females for the 2011 season was analyzed relative to data from the four previous years (Coleman, 2011). Collectively these data predict that a total of 53 adult females utilize the CPM nesting beach (minimum 37, maximum 76, Chapman and Overton, 1966). These values are similar to those estimated from the depredated nest surveys, assuming that terrapins lay approximately 2-3 clutches a year in Alabama (Seigel, 1984; Roosenburg, 1991).

These data also allow the prediction of a depredation rate. A rate of depredation for the 2011 season can be estimated if it is assumed that each female lays three clutches per year for a total number of 159 nests, compared to the 131 depredated nests recorded. This predicts a depredation rate of approximately 82% of the total nests. However, if all females did not lay three clutches, the depredation rate would increase. In addition, if number of depredated nests was underestimated due to factors such as weather events removing evidence of depredation, the depredation rate would increase. Alternatively, if the abundance of females is underestimated then the depredation rate would decrease.

The high depredation rate predicted in this study is not unusual for diamondback terrapin nesting beaches. A review by Butler et al., (2004) reported that depredation rates ranged from 41 to 88% throughout the terrapins range. Roosenburg and Place (1995) reported 94% of nests were depredated in a sampled nesting area. The number of depredated nests recorded in the current study could be considered conservative, as it was not possible to survey some heavily vegetated areas that could have contained additional depredated nests. In addition, only digs with associated egg shells are counted as depredated nests. Digs without egg shells indeed may have been depredated nests that had any egg shell remains blown away, or as it has been suggested in some instances, that raccoons may consume the entire egg, including the shells (Feinburg and Burke, 2003).

Previous observations, including data with wildlife cameras, suggest that raccoons are the primary predator on this nesting beach (Wibbels, pers. comm.). Considering the relatively low numbers of female diamondback terrapins utilizing this beach, the number of nests laid could easily be depredated by a just a few predators. The results of the current study suggest, depredation of nests and potential hatchlings represents a major threat to the recovery of the CPM aggregation of diamondback terrapins. Management strategies addressing this threat could include head-start programs which circumvent early mortality and/or predator control programs. We are currently evaluating the effectiveness of a head-start program for reducing the high mortality of nests and hatchlings (Wibbels et al., 2009). Previous studies have also evaluated the effectiveness of predator control methods (Garmestani and Percival, 2005; Butler, XXX). However, these programs must be continued indefinitely; otherwise, predators quickly repopulate the area.

An additional threat which is limiting the recovery of the terrapin aggregation at CPM is incidental capture in crab traps. Previous studies at CPM, as well as numerous other locations throughout the range of terrapins, have shown that crab traps represent a major threat to terrapin populations (Roosenburg et al., 1997; Coleman et al., 2012). A potential method for alleviating this threat is the use of terrapin excluder devices (TEDs) on crab traps. These have been shown to be an effective method at significantly decreasing the capture of terrapins. For example, a study at CPM showed a 95% reduction in terrapin capture when TEDs were fitted to crab traps (Coleman et al., 2011).

The results of the current study extend those of previous studies indicating that the diamondback terrapin is a severely depleted species in Alabama. The results highlight one of the major threats to the population, depredation of nests. Further, the relatively low number of nesting females estimated in the current study may reflect a relatively high mortality of juveniles and adults, due to factors such as crab-trap induced mortality. The recovery of the diamondback terrapin in Alabama is dependent upon the development of a management strategy that effectively addresses these major threats.

Literature Cited

Brennessel, B. 2006. Diamonds in the Marsh. A Natural History of the Diamondback Terrapin. Hanover, NH: University Press of New England. 219 p.

Butler, J.A., C. Broadhurst, M. Green, Z. Mullin. 2004. Nesting, nest predation and hatchling emergence of the Carolina diamondback terraipn, *Malaclemys terrapin centrata*, in Northeastern Florida. American Midland Naturalist 152:145-155.

Carr, A. 1952. Handbook of Turtles: The Turtles of the United States, Canada, and Baja California. Ithaca, NY: Cornell University Press. 542 p.Tucker, A.D., N.N.

Chapman, D.G. and W.S. Overton. 1966. Estimating and testing differences between population levels by the Schnabel estimation method. Journal of Wildlife Management 30: 173-180.

Coleman, A. 2011. Biology and conservation of the diamondback terrapin, *Malaclemys terrapin pileata*, in Alabama. University of Alabama at Birmingham Graduate School.

Coleman, A. T. Wibbels, K. Marion, D. Nelson, J. Dindo. 2012. Effect of by-catch reduction devices (BRDS) on the capture of diamondback terrapins (*Malaclemys terrapin*) in crab pots in an Alabama salt marsh. The Journal of the Alabama Academy of Science 82:145-157

Davenport, J. 1992. The biology of the diamondback terrapin Malaclemys terrapin (Latreille). Testudo 3(4): pages unknown

Ewert, M.A. and J.M. Legler. 1978. Hormonal induction of oviposition in turtles. Herpetologists' League 34: 314-318.

Feinburg, J.A. and R.L. Burke. 2003. Nesting ecology and predation of diamondback terrapins, *Malaclemys terrapin*, at Gateway National Recreation Area, New York. Journal of Herpetology 37: 517-526.

Garmestani, A.S. and Percival, H.F. 2005. Raccoon removal reduces sea turtle nest depredation in the Ten Thousand Islands of Florida. Southeastern Naturalist 4: 469-472.

Gustafson, D.J., Kilheffer, J., and Silliman, B.R. 2006. Relative effects of *Littoraria irrorata* and *Prokelisia marginata* on *Spartina alterniflora*. Estuaries and Coasts 29:639-644.

Jackson, C.G., Jr. and M.M. Jackson. 1970. Herpetofauna of Dauphin Island, Alabama. Quarterly Journal of Florida Academy of Sciences 33: 281-287.

Marion, K. and D. Nelson. 2004. Mississippi Diamondback Terrapin. In: R.E. Mirarchi, M.A. Bailey, T.M. Haggerty, and T.L. Best (Eds.). Alabama Wildlife, Volume Three: Imperiled Amphibians, Reptiles, Birds, and Mammals. Tuscaloosa, AL: University of Alabama Press, 225 pp.

Mirarchi, R.E., M.A. Bailey, T.M. Haggerty, and T.L. Best (Eds.). Alabama Wildlife, Volume Three: Imperiled Amphibians, Reptiles, Birds, and Mammals. Tuscaloosa, AL: University of Alabama Press, 225 pp.

New York Times article. 1881. A Mobile terrapin farm. Published February 4, 1881.

Roosenburg, W.M., and A.R. Place. 1995. Nest predation and hatchling sex ratio in the diamondback terrapin: Implications for management and conservation. Towards a Sustainable Coastal Watershed: The Chesapeake Experiment, Proceedings of a

Conference. Chesapeake Research Consortium Pub. No 149. Solomons, MD, Pp. 65-70.

Schnabel, Z.E. 1938. The estimation of total fish population of a lake. The American Mathematical Monthly 45: 348-352.

Seigel, R.A. 1984. Parameters of two populations of diamondback terrapins (*Malaclemys terrapin*) on the Atlantic coast of Florida. In: R.A. Seigel, L. E. Hunt, J. L. Knight, L. Malaret, and N. L. Zushlag (eds.) Vertebrate Ecology and Systematics - A Tribute to Henry S. Fitch. Museum of Natural History, University of Kansas, Lawrence, Kansas.

Silliman, B.R., van de Koppel, J., Bertness, M.D., Stanton, L.E., and Mendelssohn, I.A. 2005. Drought, snails, and large-scale die-off of southern U.S. salt marshes. Science 310:1803-1806.

Tucker, A.D., Fitzsimmons, N.N., Gibbons, J.W. 1995. Resource partitioning by the estuarine turtle *Malaclemys terrapin*: trophic, spatial and temporal foraging constraints. Herpetologica 51:167-181.

Wibbels, T., K. Marion, A. Coleman. 2009. The diamondback terrapin in Alabama: Causes for decline and strategy for recovery. Annual Report to the Alabama Department of Conservation and Natural Resources, State Wildlife Grant Program Grant. October 2009. 9 pp.

CHAPTER 2

EVALUATION OF THE MOVEMENTS OF ADULT FEMALE DIAMONDBACK TERRAPINS IN THE SALT MARSHES OF HERON BAY, ALABAMA

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In preparation for Journal of Herpetology

Format adapted for thesis

Abstract.—The diamondback terrapin, *Malaclemys terrapin*, was once an abundant species in the salt marshes of Alabama. Further, it was an important economic resource, and Alabama was home to one of the largest terrapin farms in the United States. A variety of threats have impacted this species and resulted in drastic declines over the past century. The diamondback terrapin is currently considered a "priority one species of highest conservation concern" in Alabama. Surveys during recent years indicate that the diamondback terrapin is currently represented by small aggregations in specific salt marshes along the Alabama coast. The current study evaluates the movements of adult female terrapins in the largest known aggregation in Alabama (i.e. Cedar Point Marsh). A total of 18 adult females were fitted with radio transmitters during the 2010 and 2011 nesting seasons and their movements subsequently monitored. The transmitters had a maximum range of approximately 1.0 km and a battery life of approximately one year. The results indicate that many of the females have relatively small home ranges (approximately 1.0 km or less), and remain resident in the Cedar Point Marsh directly adjacent to the nesting beach. Additionally, the results also indicate that some may migrate several kilometers across Heron Bay to nearby marshes. Collectively, the results verify the importance of the marshes encircling Heron Bay as critical habitat for adult female terrapins which nest on the Cedar Point Marsh nesting beach. This has significant implications for the ecology, conservation, and recovery of the diamondback terrapins in Alabama.

Key Words: Cedar Point Marsh; Conservation; Migration; Radio Telemetry; Threats

Introduction

Diamondback terrapins (*Malaclemys terrapin*) inhabit the brackish waters of estuaries, salt marshes, and bays from approximately from Cape Cod, MA to Corpus Christi, TX. They are an integral part of the salt marsh, and considered to be a keystone predator that helps to stabilize the salt marsh ecosystem (Tucker et al., 1995; Silliman et al., 2005; Gustafson et al. 2006). Of the seven recognized subspecies, the Mississippi diamondback terrapin (*M. t. pileata*) inhabits the salt marshes of Mississippi and Alabama.

Historical data indicate that terrapins were once very abundant in the salt marshes of Alabama (Carr, 1952; Jackson and Jackson, 1970; Nelson and Marion, 2004). During the late 1800s to early 1900s, it was an important economic resource in Alabama, and was exported heavily to the northeast for terrapin stew (New York Times, 1881; Brennessel, 2006). However, recent surveys indicate that this once abundant species is now depleted in the Alabama salt marshes, estuaries and bays (Nelson and Marion, 2004; Coleman et al., 2011). This decrease has occurred in spite of the abundance of salt marsh habitat in the coastal areas of Alabama and state protection of the species. The diamondback terrapin has declined in Alabama to the point that it is considered a “Priority One” species of “Highest Conservation Concern” (Mirarchi et al., 2004).

Surveys over the past few years indicate that the diamondback terrapin is represented by small aggregations in specific salt marshes along the coast of Alabama. By far, the largest aggregation identified to date is found in the Cedar Point Marsh (CPM), which is located north of Dauphin Island, approximately 53 females nest there on the adjacent beach annually (Coleman, 2011).

The post-nesting movements of females at Cedar Point are of ecological and conservational significance. Several studies involving other subspecies have shown that terrapins do show some home site fidelity, with ranges of movements varying from short distances of 1 km or less (Harden et al., 2007) to distances over 12 km (Spivey, 1998). This ability to travel relatively large distances leads to several important questions related to both the ecology and conservation of terrapins in Alabama. For example, do females that nest at Cedar Point reside in the adjacent marsh or do they travel to other marshes? Identification of critical habitat for these terrapins has significant implications for management strategies attempting to facilitate the recovery of the diamondback terrapin in Alabama.

Materials and Methods

Six 100-ft drift fences were utilized to catch females on the CPM nesting beach. Four pitfall traps were placed along each fence. Terrapins were captured between mid-May and mid-June. Eight adult female terrapins were tagged in 2010 and ten adult female terrapins were tagged in 2011. Ten I.U. of oxytocin was used to induce females to lay eggs in the laboratory (Ewert and Legler, 1978). The eggs were used to produce hatchlings for an ongoing conservation head-start program.

Prior to the attachment of a radio transmitter, the carapace was first sanded with 300 grit sanding paper to remove all loose material (e.g. algae, loose scutes). The carapace was then cleaned with 70% ethanol to remove all dust and any remaining loose debris and was allowed to dry. Gray Marine Tex® epoxy putty was mixed according to the manufacturer’s instructions and a base layer of putty (approximately 1-cm deep and the same dimensions of the transmitter) was formed on the highest point of the carapace just to the side of the keel. The transmitter (Model WL300-7PN, Hi-Tech Services, Camillus, NY) was then placed on top of the base layer with the antenna exiting the transmitter toward the terrapin's head and pushed down into the putty (Butler, 2002). A knife was then used to push epoxy around the sides of the transmitter to form a smooth transition from carapace to the top of the transmitter. The epoxy was allowed to harden and cure for 24 hours. The females were then released near their capture location.

Transmitter frequencies were monitored approximately one to two weeks from June through September either by walking the nesting beach or by boat in the main tidal creek channel of the marsh. After September transmitter frequencies were monitored approximately 1-3 times a month. Each transmitter had a unique frequency so that the locations of each individual could be monitored. Transmitters were located using an AVM (Colfax, California) receiver attached to a Yagi antenna. The location of each turtle was estimated by triangulation from at least two locations using a lensatic compass. We estimated a location accuracy of approximately 20 m to 50 m, depending on how close we could get to the transmitter using the tidal creek or the nesting beach. The location of each turtle was plotted using Google Earth®.

Results

2010 Nesting Season.— Of the eight terrapins equipped with radio transmitters in 2010, one transmitter quickly became detached from the terrapin and was located in the marsh. A second transmitter was removed from a terrapin due to a damaged antenna after it was captured on the nesting beach approximately 2 months after it was originally tagged. Of the remaining six terrapins, two were tracked for approximately two weeks; the remaining four were monitored in Cedar Point Marsh for 3- 4 months (Figure 1).

Figure 1 shows the movements of the turtles with release locations noted by stars and labeled with the transmitter number and associated release date. The location of the last reading is noted by a square with the transmitter number and associated date. Turtle 370 (indicated in light blue) continued to reside in the marsh for approximately three months after release, with its last known location on 9/24/10. After its initial move east into the marsh, it was consistently located in a relatively small area, moving only across the channel and back for the remainder of the time it was located. Turtle 450 (indicated by the red line) was located only two times after its release, with its second location being four months after release on 9/24/10. It had headed south approximately parallel to the nesting beach, stopping in a location with several small islands. Turtle 490 (indicated by the blue line) was located five times over the nesting season after its initial release, with the last location taken on 9/17/10. This turtle remained relatively close to the nesting beach. Turtle 572 (indicated by the yellow line) was also located five times over the nesting season with the last location noted on 9/24/10.

Figure 1. Locations of adult female terrapins in Cedar Point Marsh during the 2010 nesting season (May – September). (Turtle #, Date)

2011 Nesting Season.—A total of 10 adult female terrapins were tagged during the 2011 nesting season. Six terrapins were located at least once after their initial release. Turtles 711 (indicated by the red line), 730 (indicated by the green line), and 772 (indicated by the white line) were located 1 week after release, but were not detected again (Figure 2). Three terrapins were located consistently over relatively long periods of time: 3 to 7 months. Two terrapins, 650 (indicated by the yellow line) released on 5/18/11 and 690 (indicated by the blue line) released on 6/10/11, remained in cedar point marsh for the remainder of the season, with the last known locations taken on 12/8/11 and 9/14/11 respectively. Transmitter 791 (indicated by the pink line) released on 6/17/11 was not relocated until 8/2/11, where it was found to have moved approximately 2 km across Heron Bay into a marsh located on Mon Louis Island (Figure 3). This terrapin remained in this location for the remaining times it was located, with its final noted location on 8/19/11.

Figure 2. Close-up of the locations of adult female terrapins in Cedar Point Marsh (May – December). (Turtle#-date, turtles 730,650,711,690 were released on 6/10/11; turtles 772,791 were released on 6/17/11)

Figure 3. Locations of adult female terrapins in Cedar Point Marsh tracked in the 2011 nesting season. (Turtle #-date; turtles 730,650,711,690 were released on 6/10/11; turtles 772,791 were released on 6/17/11)

Discussion

2010 nesting season.—The results from the 2010 season suggest that the adult females remained resident in Cedar Point Marsh during and after the nesting season. Two terrapins were known to quickly have non-functioning transmitters, with one detached and one recovered with a damaged antennae. Of the six terrapins remaining, four were consistently tracked in the marsh adjacent to the nesting beach. This indicates high site fidelity, where terrapins stayed within approximately 1 km of the site at which they were caught and released; this finding is consistent with similar studies in subspecies populations located in other regions (Gibbons et al., 2001). The movements of the females often included an initial relatively long movement from their release location on the nesting beach. All four of these turtles remained in the marsh several months after the nesting season. The final locations noted for these females in 2010 occurred in mid to late September, following a period of relatively short movements. The fate of these turtles after late September as well as the fate of the two turtles that were not detected after the day of release is unclear. It is possible that the transmitters may have detached at some point while in the water, effectively blocking their signal from reaching the receiver. The antennas could also have been damaged, similar to that seen on one of the recovered turtle. Alternatively, it is possible that turtles migrated out of Cedar Point to other adjacent marshes. There are numerous marshes within the area that are well within the distances that terrapin are known to travel (Hurd et al., 1979; Spivey 1998). These marshes were not routinely surveyed during the 2010 nesting season, although the marshes immediately north and west of Cedar Point Marsh were occasionally surveyed for transmitter reception.

The lack of detecting turtles in CPM does not rule out the possibility that they could have been located there. The turtles could have been submerged during the time of the surveys (thereby extinguishing their transmission), or the transmitters could have become detached or the antennae damaged. It was verified that one transmitter became non-functional because of a damaged antenna within two months of release.

2011 Nesting Season.—The radio tracking survey performed in the 2011 was not as successful as the tracking in 2010. Four transmittered turtles were never detected after the day of release, and three turtles were tracked for only several weeks, but all three remained in the marsh for that period. Of the remaining three turtles, two remained resident in CPM during and after the nesting season. This reinforces the findings in the 2010 season that there is a resident population of nesting females in the marsh adjacent to the nesting beach. Of particular interest, the third turtle moved from CPM across Heron Bay to a marsh on Mon Louis Island. This marsh is approximately 2.8 km away from the release point. This is well within the distances that terrapins have been known to travel (Spivey, 1998; Gibbons et al., 2001; Butler, 2002). This verifies that the nesting beach at CPM may be utilized by females that reside (at least temporarily) in adjacent marshes in the Heron Bay area. A plausible reason for this occurrence is a lack of viable nesting beaches in other marshes. The Cedar Point Marsh nesting beach represents the largest, most suitable, and most stable nesting beach in the area. It is composed of a long oyster shell-hash beach that extends the entire length of the western border of CPM and is above the high tide line. This is in contrast to many of the other potential nesting beaches in adjacent marshes, which are very small and are often over washed at high tide. The marsh located on Mon Louis Island represents suitable habitat for the diamondback terrapin, but does not include high quality nesting sites on its eastern border.

The inability to detect some of the turtles in 2011 could be due to the same potential problems as indicated above. It is possible that the transmitters may have detached, or their antennas could have been damaged. Additionally, as indicated by turtle 791, these turtles could have migrated out of Cedar Point Marsh to other adjacent marshes, many of which are well within the distances that terrapin have been known to travel (Hurd et al., 1979; Spivey 1998). However, during the year, marshes immediately north and west of Cedar Point Marsh were occasionally surveyed and only turtle 791 was identified. As indicated above it is possible that the turtles could have been submerged during the time of the survey (thereby extinguishing their transmission).

Summary—The results of the current study indicate that many of the females nesting on the beach at CPM may reside in CPM itself. Furthermore, the results show one example of a turtle residing in an adjacent marsh utilizing CPM as a nesting beach. This suggests that CPM and its nesting beach represent critical habitat for diamondback terrapins in the Heron Bay area. Previous studies indicate that the nesting beach at CPM represents the most important and nesting beach identified to date for terrapins in Alabama (Coleman et al., 2007; Wibbels et al., 2009). Further, these previous studies also indicate that Cedar Point Marsh can represent a habitat utilized by all stages in the life history of the diamondback terrapin. This emphasizes the importance of CPM to the life history of the diamondback terrapin in the Heron Bay area. Therefore its preservation is of paramount importance to the recovery of the diamondback terrapin in Alabama. At the present time, the acquisition of Cedar Point Marsh for the Forever Wild Land Trust is under way; however, other threats still exist. The waters of Heron Bay are heavily impacted by the crab fishery and crab traps are occasionally used in the main channel of Cedar Point Marsh. The restriction of the crab fishery or the implementation of TEDs in CPM and Heron Bay would enhance the recovery of the diamondback terrapin in

Alabama. An additional threat which is impacting the nesting beach at CPM is the high depredation of nests by predators such as raccoons. Predation of nests and hatchlings has most likely increased over the past half century. Coastal development probably facilitates increased numbers of predators and easier access to the marsh. This threat is currently being addressed by an experimental head-start program that is attempting to circumvent the high mortality associated with nest depredation.

Literature Cited

Brennessel, B. 2006. Diamonds in the Marsh. A Natural History of the Diamondback Terrapin. Hanover, NH: University Press of New England. 219 p.

Butler, J.A. 2002. Population Ecology, Home Range, and Seasonal Movements of the Carolina Diamondback Terrapin, *Malaclemys terrapin centrata* in Northeastern Florida. Florida Fish and Wildlife Conservation Commission. Tallahassee, FL. 72 pp.

Carr, A.F. 1952. Handbook of Turtles: The Turtles of the United States, Canada, and Baja California. Ithaca, NY: Cornell University Press, 542 pp.

Coleman, A.T., T. Wibbels, K. Marion, D. Nelson, J. Borden, G. Langford and J. Dindo. 2007. Survey of Diamondback Terrapin, *Malaclemys terrapin*, populations in the salt marshes in Alabama. Abstract, Society for Integrative and Comparative Biology.

Gibbons, J.W., J.E. Lovich, A.D. Tucker, N.N. Fitzsimmons and J.L. Green. 2001. Demographic and Ecological Factors Affecting Conservation and Management of Diamondback Terrapins (*Malaclemys terrapin*) in South Carolina. Chelonian Conservation and Biology 4:66-74.

Gustafson, D.J., Kilheffer, J., and Silliman, B.R. 2006. Relative effects of *Littoraria irrorata* and *Prokelisia marginata* on *Spartina alterniflora*. Estuaries and Coasts 29:639-644.

Harden, L.A. N.A. Diluzio, J.W. Gibbons, and M.E. Dorcas. 2007. Spatial and thermal ecology of the diamondback terrapin (*Malacelmys terrapin)* in a South Carolina salt marsh. Journal of the North Carolina Academy of Sciences. 123:154-162.

Hurd, L.E., G.W. Smedes, and T.A. Dean. 1979. An Ecological Study of a Natural Population of Diamondback Terrapins (*Malaclemys t. terrapin*) in a Delaware Salt Marsh. Estuaries 2:28-33.

Jackson, C.G., Jr. and M.M. Jackson. 1970. Herpetofauna of Dauphin Island, Alabama. Quarterly Journal of the Florida Academy of Science 33: 281-287.

Marion, K. and D. Nelson. 2004. Mississippi Diamondback Terrapin. In: R.E. Mirarchi, M.A. Bailey, T.M. Haggerty, and T.L. Best (Eds.). Alabama Wildlife, Volume Three: Imperiled Amphibians, Reptiles, Birds, and Mammals. Tuscaloosa, AL: University of Alabama Press, 225 pp.

Mirarchi, R.E., M.A. Bailey, T.M. Haggerty, and T.L. Best (Eds.). Alabama Wildlife, Volume Three: Imperiled Amphibians, Reptiles, Birds, and Mammals. Tuscaloosa, AL: University of Alabama Press, 225 pp.

New York Times article. 1881. A Mobile terrapin farm. Published February 4, 1881.

Silliman, B.R., van de Koppel, J., Bertness, M.D., Stanton, L.E., and Mendelssohn, I.A. 2005. Drought, snails, and large-scale die-off of southern U.S. salt marshes. Science 310:1803-1806.

Spivey, P.B. 1998. Home range, habitat selection, and diet of the diamondback terrapin (*Malaclemys terrapin*) in a North Carolina estuary. Unpubl. M.S. Thesis, University of Georgia, Athens. 80 pp.

Tucker, A.D., N.N. Fitzsimmons, and J.W. Gibbons. 1995. Resource Partitioning by the Estuarine Turtle *Malaclemys terrapin*: Trophic, Spatial, and Temporal Foraging Constraints. Herpetologica 51: 167-181.

Wibbels, T., K. Marion, A. Coleman. 2009. The diamondback terrapin in Alabama: Causes for decline and strategy for recovery. Annual Report to the Alabama Department of Conservation and Natural Resources, State Wildlife Grant Program Grant. October 2009. 9 pp.

Chapter 3

EXPERIMENTAL EVALUATION OF HATCHLING SEX RATIOS PREDICTED FOR THE MAIN NESTING BEACH FOR THE DIAMONDBACK TERRAPIN, *MALACLEMYS TERRAPIN*, IN ALABAMA

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In preparation for Journal of Herpetology

Format adapted for thesis

Abstract—Cedar Point Marsh (CPM) has been identified as the location of the largest aggregation of diamondback terrapins (*Malaclemys terrapin*) in coastal Alabama. It includes a beach that supports the largest nesting aggregation of terrapins reported in the state. In the current study, temperature profiles were recorded for the nesting beach over the 2011 nesting season. The nesting season for diamondback terrapins in Alabama extends from early May through July. The results indicate that temperatures gradually increase during May and then stay relatively warm throughout the remainder of the nesting season, with the exception of weather events. The current study utilized a simple model for predicting sex ratios based on mean temperature of the nesting beach during the middle third of the incubation period. The results predict that the Cedar Point Marsh nesting beach produced a significantly female-biased hatchling sex ratio during the 2011 nesting season. Mixed sex ratios were predicted for nests laid at the start of the nesting season when temperatures were cooler. However, by mid- to late-May, significant female-biases or 100% female hatchling sex ratios would be predicted. Shaded areas (due to vegetation) and open areas were both relatively warm and predicted to produce significant female biases during the majority of the nesting season. An experimental approach was also adopted in which commercially-available turtle (*Trachemys scripta*) eggs were used as a surrogate for evaluating actual hatchling sex ratios produced on the CPM nesting beach. Eggs laid during two different time periods of the nesting season (i.e., early nesting season and mid-nesting season) were used in this study. The temperature-dependent sex determination in this species is similar to that of the diamondback terrapin. The results were consistent with our temperature-based prediction model in that all nests produced female biases or 100% females. Collectively, the results indicate that the nesting beach at Cedar Point Marsh was predicted to produce mostly female hatchlings during the 2011 nesting season. It is not clear if these data are representative of other years. For example, it is plausible that year to year variations in tropical weather systems could affect beach temperatures and thus hatchling sex ratios. It would of interest to evaluate if this hatchling bias is reflected in the overall population sex ratios in CPM. Female-biased sex ratios have frequently been reported in populations of turtles with temperature-dependent sex determination. From a conservation viewpoint, female biases could be advantageous in the recovery of a depleted population by enhancing future egg production.

Key Words: Cedar Point Marsh; Female bias; Natural sex ratios; Surrogate nest; Temperature profile

Introduction

The diamondback terrapin is known to exhibit temperature-dependent sex determination (TSD), where males are produced at lower temperatures (27 °C) and females are produced at higher temperatures (31 °C), with a pivotal temperature in the approximate range of 28.5 °C to 29.5 °C (Jeyasuria et al., 1994; Roosenburg and Place, 1995). In TSD, the temperature experienced during the temperature sensitive period (TSP) will irreversibly fix the sex of the individual. The TSP has been shown to occur during the middle third of incubation for the loggerhead (*Caretta caretta*), olive ridley (*Lepidochelys* olivacea), as well as some freshwater species (Yntema and Mrosovsky, 1982; Wibbels et al., 1994; Merchant-Larios et al., 1997). Therefore, nesting beach temperatures can be used as a predictor of hatchling sex ratios on a specific beach. Further, the chronology and abundance of nesting can be used together with temperature data to predict sex ratios produced during a nesting season. These data are of interest not only from an ecological standpoint, but may also be paramount in determining the best management strategy for aiding in the recovery of a depleted population.

It has been fairly common to use the mean temperatures experienced during the middle third of incubation as a good predictor of sex in hatchling sea turtles (Mrosovsky et al., 1984; Spotila et al., 1987; Hanson et al., 1998; Wibbels, 2003). This seems to work well in sea turtle nests because of the natural buffering effect of the changes in air temperature. There is very little fluctuation in temperature experienced in the nest chamber throughout the 24-hour day/night cycle in sea turtle nests, with the smallest amount of fluctuation at the lowest point of the nest (Hanson et al., 1998). In contrast, some nests laid at relatively shallow depths can experience large diurnal fluctuations.

Studies have shown that fluctuations as great as 10 °C can occur in the nests of the diamondback terrapin (Jeyasuria et al., 1994). It has been suggested that eggs experiencing large fluctuations in temperature during incubation and specifically during the thermo-sensitive period may stray from predicted sex ratios when using the mean temperature experienced as a predictor (Georges et al., 1994), if temperatures are near pivotal. In that case a constant temperature equivalent model can be used, which takes into account the large changes in temperature experienced in the nest chamber (Georges et al., 1994). Alternatively, if temperatures are well above or below pivotal, mean temperature represents an accurate predictor even in nests with large daily temperature fluctuation.

The most accurate method of evaluating hatchling sex ratios is through the verification of the sex of individual hatchlings. Hatchling turtles cannot be sexed by their external morphology, so the hatchling must be sacrificed in order to evaluate the morphology of the gonad (Yntema and Mrosovsky, 1982). However, this is not practical or acceptable when working with threatened or endangered species due to conservational considerations. Alternatively, the use of a "surrogate nest" of a different species with similar egg morphology and TSD may be an effective method for examining the actual sex ratios being produced on natural nesting beaches.

The diamondback terrapin is considered a "priority one species of highest conservation concern" in Alabama (Mirarchi et al., 2004). Therefore, its reproductive ecology is of conservational interest. For example, population sex ratios can significantly affect the recovery of depleted species (Wibbels, 2003). The current study uses two approaches to predict and evaluate hatchling sex ratios produced on the most important nesting beach for diamondback terrapins in Alabama. The first approach utilizes nesting beach temperatures to predict hatchling sex ratios at various times during the nesting season. The second approach directly examines hatchling sex ratios utilizing a surrogate species, the red-eared slider (*Trechemys scripta*). This turtle represents an optimal surrogate for studying temperature-dependent sex determination in the diamondback terrapin for several reasons. Its eggs are commercially available in large numbers due to its popularity in the pet trade. The size and shape of the eggs of this species of turtle are also very similar to that of the diamondback terrapin. TSD in this species also has similar characteristics to that of the diamondback terrapin. In particular, it has a similar pivotal temperature of 29.2-29.4 °C (Etchberger et al., 1991; Wibbels and Crews, 1994, 1995) compared to 28.5-29.5 °C found to produced mixed sexes in *M. terrapin* (Roosenburg and Place, 1995). It also has a similar "transitional range of temperatures," which is the range in which mixed sex ratios are produced. The nesting season of *T. scripta* also occurs at a similar time as *M. terrapin*, making the timing of experiments under natural conditions plausible. Thus, *T. scripta* represents an advantageous model for examining hatchling sex ratio production on natural nesting beaches for diamondback terrapins.

Materials and Methods

Evaluation of beach temperatures—The study site was located in Cedar Point Marsh (CPM) which is located just north of Dauphin Island, Alabama, in the Heron Bay portion of the Mississippi Sound. A shell-hash beach forms the western border of CPM. This beach represents the most important nesting site to date for diamondback terrapins in Alabama. To evaluate nesting beach temperatures, a total of 23 dataloggers were used to record temperatures on the CPM nesting beach. The data loggers were buried in areas where nesting had been previously documented. These data loggers are accurate to approximately 0.3-0.4 °C and were programmed to record temperatures on an hourly basis. Data loggers were downloaded with the HOBO software and exported into Excel files for data analysis.

A simple model for predicting hatchling sex ratios was used in the current study based on mean temperatures during the middle third of incubation. This model was used to generate mean middle third temperatures for each lay date of the nesting season. Mean middle third temperatures were calculated using the hourly recordings from the data loggers. The length of the middle third was based on an mean total incubation period of 48 days, which is typical for nests incubated at temperatures similar to those on the CPM nesting beach. The results were examined relative to the chronology and abundance of diamondback terrapins nesting at CPM.

In addition to temperature data, meteorological data were gathered from the Mobile Bay National Estuary Program Environmental Monitoring database. The weather station is located on Cedar Point Fishing Pier, approximately 2 km from the center of Cedar Point Marsh. Meteorological data were downloaded for each month and the precipitation data were taken and aligned with the data gathered by the data logger.

Experimental surrogate model—Due to its conservation status, it was not plausible to sacrifice hatchling terrapins for verification of actual hatchling sex ratios produced on the CPM nesting beach. Therefore, eggs from a surrogate species, the red-eared slider, were utilized to directly evaluate hatchling sex ratio production.

Red-ear slider eggs were obtained from the Klibert Turtle Farm (Hammond, Louisiana) and transported to Cedar Point Marsh. Eggs were obtained at two different time periods during the nesting season (May 18 and June 23). Eggs were assigned to one of three groups based on beach vegetation: i.e., heavy shading, partial shading, or no shading areas. “Heavy shading” was defined by an area around the nest surrounded on all sides by vegetation shading the nest from direct sunlight. “Partial shading” was defined by an area around the nest with vegetation present on only one side of the nest, leaving the nest exposed to direct sunlight for half of the day. “No shading” was defined by an area around the nest devoid of vegetation, subjecting the nest to direct sunlight for the entire day. Only eggs showing signs of chalking were assigned to each nest to avoid using eggs that would not develop.

Nests were distributed across the nesting beach in locations identified as the heavy nesting areas by previous depredation surveys (Coleman, 2011). For each nest a hole was dug approximately 18 cm deep, determined to be the depth at which hatching success rate for the diamondback terrapin was greatest (Burger, 1976). A data logger was placed at the bottom of each nest. Ten eggs were placed in each nest. Each nest was then covered with the sand/shell-hash. To prevent depredation, a 36" X 36" square of hardware cloth with a mesh size of .5" was centered over the nest; tent stakes were used on each corner to hold the hardware cloth in place. The exposed edges of the hardware cloth were then covered with a thin layer of sand/shell hash. A wooden stake was then placed near one of the corners of the hardware cloth at each nest to allow for quick identification of a nest in the event of the hardware cloth becoming no longer visible. The latitude and longitude of each nest was also recorded to aid in relocating the nests.

The nests were then left to incubate at Cedar Point for at least 35 days to ensure they had completed their thermo-sensitive periods. The nests were checked each week after the experiment was set up to look for any evidence of depredation. Any attempts to dig under the hardware cloth by predators were noted and any disturbed areas were subsequently covered with sand to return the site to its pre-disturbed state.

Toward the end of the incubation period, nests were excavated (after the middle third of incubation) and the eggs were removed and transported to laboratory incubators. The data logger was left at the bottom of each nest and a new clutch of eggs was placed into each nest and covered with the same procedure as the first set. Eight eggs per nest were used for the second trial, with the last nest holding the 20 remaining eggs. One nest had been infiltrated by ants and a new nest was dug approximately 2 m away from the original nest. After allowing the second groups of eggs to develop through their thermo-sensitive periods, they were carefully excavated and placed into divided containers for transport back to the lab for identification. The data loggers were also recovered and were downloaded for analysis.

Embryos from eggs were dissected and the external morphology of the gonad and oviduct were evaluated to determine sex. The sex of each turtle was independently determined by two individuals to ensure an accurate identification.

Results

Mean daily temperatures generally increased during the early portion of the nesting season and then stayed relatively high, with fluctuations relating to weather systems moving through the area (Figure 1). The mean temperatures during the middle third of incubation are plotted relative to the mean of precipitation during the middle third of the incubation period on Figure 2.

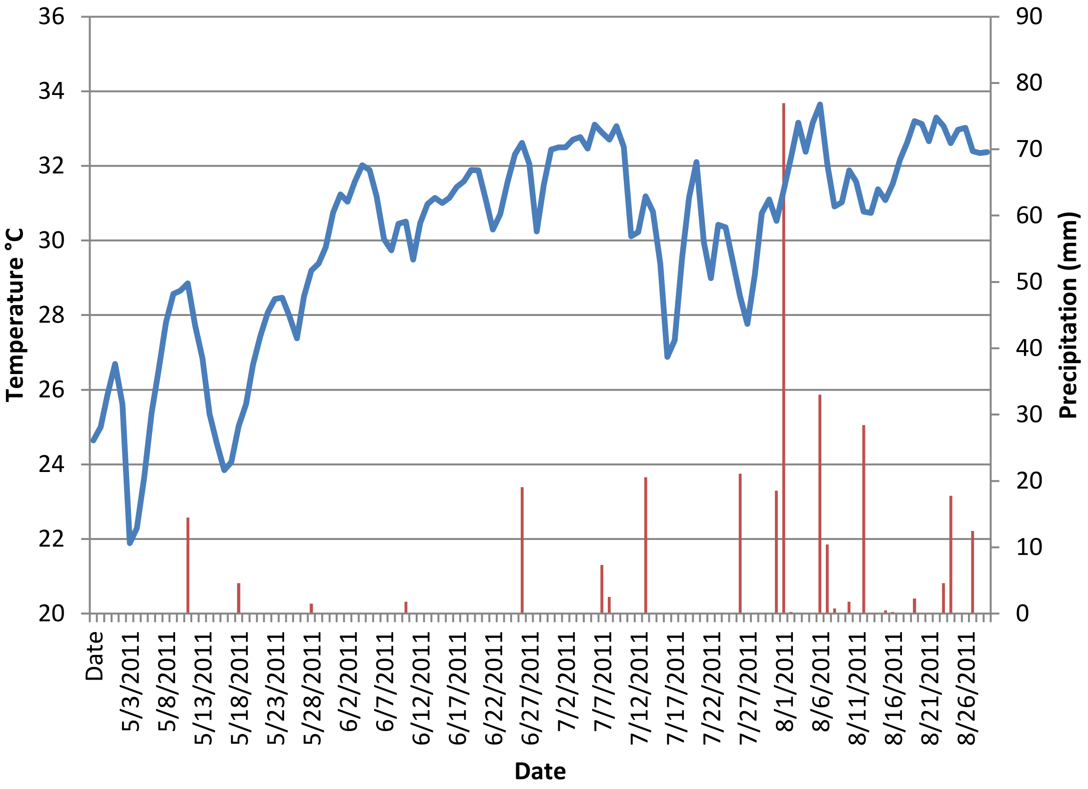

Figure 1. Mean daily temperatures and precipitation in Cedar Point Marsh, AL during the 2011 nesting season. (Blue indicates mean daily temperature, red indicates depredated nest abundance)

Figure 2 shows that by early May the temperatures experienced during the middle third of incubation quickly rose above the range of mixed sex producing temperatures and continued to increase until there was a relatively large rain event in the second week of June. During that time temperatures decreased but never below the range that produces 100% females. Based on depredated nest abundance (Figure 5), nesting begins in late April to early June and continues through the end of July, with the highest level of nesting occurring from early June to early July. Although male-biased or mixed sex ratios could be predicted for the beginning of the nesting season, the model predicts that the majority of the nesting season would produce 100% females. The periods showing the highest abundance of depredated nests, and therefore nesting, experienced temperatures that would have produced all females. Only nests laid at the start of the nesting season (i.e. early May or before) would have experienced mean middle third temperatures that would be predicted to produce male-biased or mixed sex ratios.

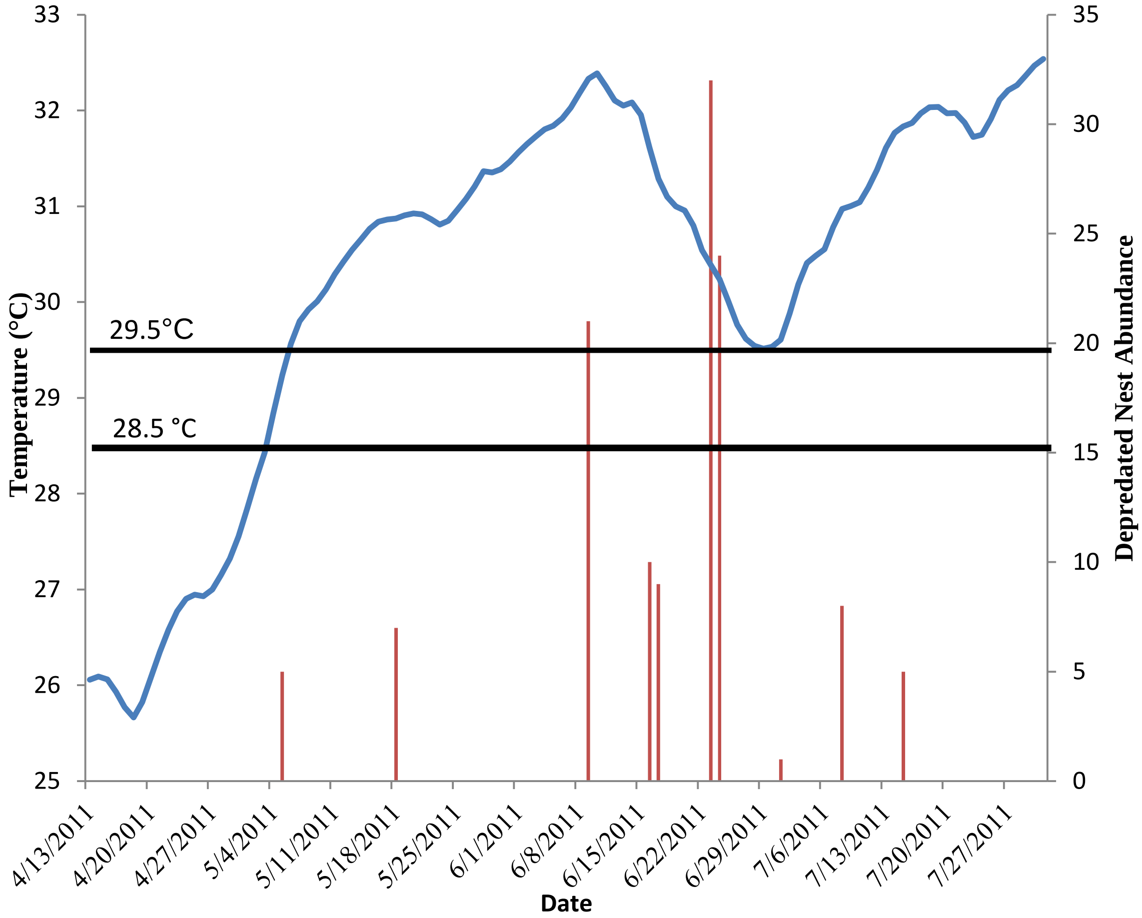

Figure 2. Mean Middle Third Temperatures and Depredated Nest Abundance. (Blue indicates mean middle third temperatures, red indicates depredated nest abundance, 28.5 °C- 29.5 °C is the range where mixed sex ratios are produced (Roosenburg and Place, 1995))

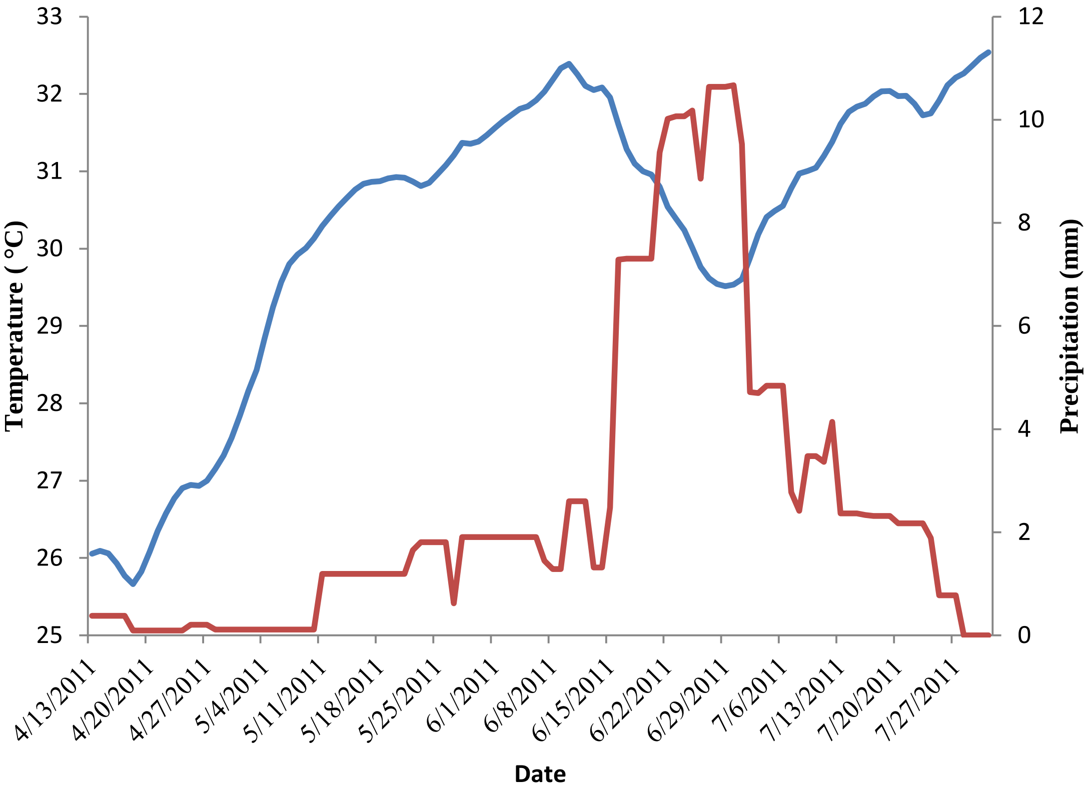

Figure 3. Mean middle third Temperatures and mean Precipitation. (Blue indicates mean middle third temperatures, red indicates mean precipitation)

The sex ratios produced from both sets of surrogate nests (laid on May 18 and June 23 respectively) are shown in Tables 1, 2. The results show that there was a significant female bias for both the group laid on May 18, (P=2.36459E-11), and for the group laid on June 23, (P=1.1212E-19). In fact, all 28 nests produced female biases or 100% females. Only three of these nests produced any males. The mean temperatures experienced during the middle third of incubation for all nests in both groups were at or above temperatures that produce mixed sex ratios. Of the 150 embryos, only 6 were male. Three males were produced in the May 18 group and three in the June 23 group. Ants were found to have infiltrated one nest, and two nests showed no development of embryos in any egg within the nest (Table 1). Individuals labeled unknown either had their gonads inadvertently discarded during the dissecting process or had undeveloped embryos.

Table 1. Sex Identification for Nests Laid in May. (Ants= all eggs dead, nest covered in ants; NO DEV= no development occurred in nest)

Date "laid"	**Cover type**	**Avg Mid 1/3 °C**	**# of eggs**	**Females**	**Males**	**Unkown**
5/18/2011	Open	30.8106224	10	1	0	
5/18/2011	Open	30.63867188	10	Ants		
5/18/2011	Open	30.09920052	10	5	0	
5/18/2011	Open	29.93034375	10	4	0	
5/18/2011	Open	30.33317969	10	4	0	1
5/18/2011	Partial	29.75019531	10	4	0	
5/18/2011	Partial	31.38124479	10	7	0	
5/18/2011	Partial	32.14226563	10	7	1	1
5/18/2011	Partial	31.73820052	16	NO DEV		
5/18/2011	Heavy	31.25559115	10	9	0	
5/18/2011	Heavy	31.31589323	10	5	2	
5/18/2011	Heavy	30.8260625	10	1	0	
5/18/2011	Heavy	30.07580469	10	6	0	
5/18/2011	Heavy	30.91958333	10	NO DEV		
Total			146	53	3	

Table 2. Sex Identification for Nests Laid in June. (Ants= all eggs dead, nest covered in ants; NO DEV= no development occurred in nest)

Date "laid"	**Cover type**	**Avg. Mid 1/3 °C**	**# of eggs**	**Females**	**Males**	**Unkown**
6/23/2011	Open	29.77605714	8	6	0	
6/23/2011	Open	30.11769091	20	20	0	
6/23/2011	Open	30.17401818	8	8	0	
6/23/2011	Open	30.33555844	8	7	0	1
6/23/2011	Open	30.85	8	Ants		
6/23/2011	Partial	29.70791688	8	8	0	
6/23/2011	Partial	30.62119221	8	8	0	
6/23/2011	Partial	31.31800519	8	NO DEV.		
6/23/2011	Partial	31.38713247	8	4	3	1
6/23/2011	Heavy	30.29755584	8	3	0	
6/23/2011	Heavy	30.38261818	8	7	0	1
6/23/2011	Heavy	30.51605195	8	8	0	
6/23/2011	Heavy	30.80836104	8	8	0	
6/23/2011	Heavy	30.96863636	8	4	0	
Total			124	91	3	

Discussion

The Cedar Point Marsh nesting beach supports the largest nesting aggregation of diamondback terrapins identified to date in Alabama. The current study utilized a dual approach to predict hatchling sex ratios produced from this nesting beach. Temperature data at nest depth indicated that beach temperatures rose early in the nesting season and remained relatively high throughout the remainder of the nesting season. Interestingly, even nests placed in shaded areas appeared relatively warm and at times were as warm, or warmer, than nests on the open beach. The relatively warm nature of the shaded areas could relate to factors such as beach height or distance from water table at different locations on the beach.

Based on mean middle third temperatures, nests laid at the start of the nesting season would be predicted to produce male biases or mixed sex ratios. Whereas, nests laid during the majority of the nesting season would be predicted to produce 100% females. Although some nesting does occur in late April and early May, most nesting occurs from mid-May through early July, corresponding to the production of 100% females. In fact, based on this model, nests laid after May 6 (Figure 2) would be predicted to produce all females.

The results from the experiment using surrogate nests also suggested that a strong female-biased hatchling sex ratio was produced. All 28 nests from two different time periods during the nesting season produced female biases or 100% females. Although this was a surrogate species, its TSD has similar characteristics to that of the diamondback terrapin, including similar pivotal temperature and transitional range of temperature

(Etchberger et al., 1991; Roosenburg and Place, 1995; Jeyasuria and Place, 1997; Dodd et al., 2005)

Considering the relatively warm temperatures during the nesting season at CPM nesting beach, the mean temperature during the middle third of incubation appears to be an accurate and practical model for predicting hatchling sex ratios produced during the majority of the nesting season. This was supported by nest data from the surrogate nest study. It would be of interest in future studies to examine large numbers of nests laid at the start of nesting season when nest temperatures would be predicted to produce mixed hatchling sex ratios. This would provide an optimal experimental design for comparing the accuracy of the mean temperature during the middle-third model to the constant temperature equivalent model described by Georges *et al.* (1994). Regardless, both the temperature data and the surrogate study indicate that strong female biases were evident for the 2011 nesting season at CPM nesting beach.

The existence of a female-biased sex ratio has implications for the ecology, evolution, and conservation of the diamondback terrapin in Alabama. From a practical viewpoint, the nesting beach at CPM is a relatively elevated, with limited amounts of vegetation. The height of the CPM nesting beach may enhance the survival of nests since they are not regularly exposed to tidal flooding. However, the height and limited vegetation there may contribute to the relatively warm beach temperatures recorded during the 2011 nesting season. For example, a lower beach would be closer to the water table and its temperature could be buffered by its close proximity to the water.

It is not clear if the female bias predicted for 2011 can be extrapolated to other years. However, if the topography and vegetation of the beach, as well as the seasonal weather patterns remain relatively constant, it is plausible that female biases could predominate in other years. The female bias predicted in the current study could represent an important aspect of the reproductive ecology of the diamondback terrapin. A variety of sex ratios have been reported for turtle species with TSD (Wibbels, 2003). Interestingly, female-biased sex ratios appear to be more prevalent than male-biased sex ratios. A variety of hypotheses have been proposed to address the evolutionary implication of why biased sex ratios may exist in TSD species (Girondot et al., 1998; Shine, 1999). The most accepted hypothesis relates to sex-specific differential fitness in which it may be advantageous to produce a specific sex relative to factors such as environmental conditions.

From a conservation viewpoint, a female-biased sex ratio could enhance the recovery of this species. The diamondback terrapin in Alabama is currently considered a "priority one species of highest conservational concern", and the nesting beach at CPM appears to be the most important nesting beach for the diamondback terrapin in Alabama. Previous studies have suggested that the production of female biased sex ratios could accelerate the recovery of a depleted turtle species (Wibbels, 2007). Assuming that males do not become a limiting factor in the diamondback terrapin's reproductive ecology, the production of a female-biased hatchling sex ratio could be considered advantageous, and therefore incorporated into the management strategy for the diamondback terrapin in Alabama. Future studies examining population sex ratios would provide insight on whether female-biased sex ratios are common in the population and if they persist through all life stages. Considering that this species is the subject of an experimental head-start program, it may be beneficial to produce a female biased sex ratio as part of the management strategy for this depleted species.

From a management viewpoint, the current study also addressed the potential protection of nests on the natural nesting beach. In the surrogate study, hardware cloth was used to cover the nest to prevent depredation. Although depredation of terrapin nests appears to be very high on this nesting beach, the hardware cloth was 100% effective in the protection of nests. While on the CPM nesting beach the shell-hash substrate makes identification of freshly laid nests extremely difficult, it is plausible that this strategy could be used to protect natural nests *in situ*, thus enhancing nest survival and hatchling production on other beaches where nest identification is possible.

Literature Cited

Burger, J. 1976. Temperature relationships in nests of the northern diamondback terrapin, *Malaclemys terrapin terrapin*. Herpetologica 32:412-418.

Coleman, A. 2011. Biology and conservation of the diamondback terrapin, *Malaclemys terrapin pileata*, in Alabama. University of Alabama at Birmingham Graduate School.

Etchberger, C.R., J.B. Phillips, M.A. Ewert, C.E. Nelson, and H.D. Prange. 1991. Effects of oxygen concentration and clutch on sex determination and physiology in red-eared slider turtles (*Trachemys scripta*). Journal of Experimental Zoology 258: 394-403.

Ewert M.A. 1985. Embryology of turtles. Pp. 75–267 in C. Gans, F. Billett, and P.F.A. Madereson, (Eds.), Biology of the Reptilia. Wiley, New York.

Dodd, K.L., C. Murdock, T. Wibbels. 2006. Interclutch variation in sex ratios produced at pivotal temperature in the red-eared slider, a turtle with temperature-dependent sex determination. Journal of Herpetology 40:544-549.

Georges, A., C.J. Limpus, and R. Stoutjesdijk. 1994. Hatchling sex in the marine turtle *Caretta caretta* is determined by proportion of development at a temperature, not daily duration of exposure. Journal of Experimental Zoology 270: 432-444.

Girondot, M., H. Fouillet, and C. Pieau. 1998. Feminizing turtle embryos as a conservation tool. Conservation Biology 12:353-362.

Hanson, J., T. Wibbels, and R.E. Martin. 1998. Predicted female bias in sex ratios of hatchling loggerhead sea turtles from a Florida nesting beach. Canadian Journal of Zoology 76: 1850-1861.

Jeyasuria, P., and A. R. Place. 1997. Temperature dependent aromatase expression in developing diamondback terrapin (*Malaclemys terrapin*) embryos. Journal of Steroid Biochemistry and Molecular Biology 61:415-425

Jeyasuria, P., W.M. Roosenburg, and A.R. Place. 1994. Role of P450 aromatase in sex determination of the diamondback terrapin, *Malaclemys terrapin*. Journal of Experimental Zoology 270:95-111.

Merchant-Larios, H., S. Ruiz-Ramirez, N. Moreno-Mendoza, and A. Marmolejo-Valencia. 1997. Correlation among thermosensitive period, estradiol response, and gonad differentiation in the sea turtle *Lepidochelys olivacea*. General and Copmarative Endocrinology 107:373-385.

Mirarchi, R.E., M.A. Bailey, T.M. Haggerty, and T.L. Best (Eds.). Alabama Wildlife, Volume Three: Imperiled Amphibians, Reptiles, Birds, and Mammals. Tuscaloosa, AL: University of Alabama Press, 225 pp.

Mrosovsky, N., S.R. Hopkins-Murphy, and J.I. Richardson. 1984. Sex ratio of sea turtles: seasonal changes. Science 225:739-741

Roosenburg, W.M. and A.R. Place. 1995. Nest predation and hatchling sex ratio in the diamondback terrapin: Implications for management and conservation. Towards a Sustainable Coastal Watershed: The Chesapeake Experiment, Proceedings of a Conference. Chesapeake Research Consortium Pub. No 149. Solomons, MD. pp. 65-70.

Shine, R. 1999. Why is sex determined by nest temperature in many reptiles?. Trends in Ecology & Evolution 14:186-189.

Spotila, J.R., E.A. Standora, S.J. Morreale, and G.J. Ruiz. 1987. Temperature dependent sex determination in the green turtle (*Chelonia mydas*): effects on the Sex ratio on a natural nesting beach. Herpetologica 43:74-81.

Wibbels, T., J.J. Bull, and D. Crews. 1994. Temperature-dependent sex determination: A mechanistic approach. Journal of Experimental Zoology 270: 71-78.

Wibbels T., and D. Crews. 1994. Putative aromatase inhibitor induces male sex determination in a female unisexual lizard and in a turtle with TSD. Journal of Endocrinology 141: 295-299.

Wibbels T., and D. Crews. 1995. Steroid-induced sex determination at intermediate incubation temperatures in a turtle with TSD. General and Comparative Endocrinology 100: 53-60.

Wibbels, T. 2003. Critical approaches to sex determination in sea turtles. Pp. 103-134 in P.J. Lutz, J.A. Musick, and J. Wyneken, (Eds.), The Biology of Sea Turtles, Vol. 2. CRC Press, USA.

Wibbels, T. 2007. Sex determination and sex ratios in ridley turtles. Pp. 167-189 in P.T. Plotkin. (Ed.), Biology and Conservation of Ridley Sea Turtles. Johns Hopkins Univ. Press, USA.

Yntema, C.L., and N. Mrosovsky. 1982. Critical periods and pivotal temperatures for sexual differentiation in loggerhead turtles. Canadian Journal of Zoology 60: 1012-1016.

CHAPTER 4

EVALUATION OF A SEXING TECHNIQUE FOR JUVENILE DIAMONDBACK TERRAPINS USING TESTOSTERONE RIA

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In preparation for Journal of Herpetology

Format adapted for thesis

Abstract—Sex ratios have significant implications for the ecology, evolution, and conservation of turtles with temperature-dependent sex determination. The diamondback terrapin in Alabama is a depleted species that is currently the subject of a recovery program. Population sex ratios are of particular interest because they can affect the recovery rate of a depleted species. Unfortunately, sexually-dimorphic characteristics have not been identified for accurately sexing juvenile diamondback terrapins. Therefore, the purpose of the current study was to develop an accurate and non-lethal sexing technique for juvenile diamondback terrapins. This study utilized a radioimmunoassay to examine serum testosterone levels in captive-reared terrapins that were hatched from eggs incubated in the laboratory at known male-producing or female-producing temperatures. The results indicate that testosterone can be used as an accurate predictor of sex in juvenile terrapins. This technique can potentially be used to evaluate sex ratios in naturally-occurring diamondback terrapin populations.

Key Words: Serum testosterone; Radioimmunoassay

Introduction

The ability to accurately identify the sex of individuals in a population can be very important when trying to understand the ecology and population dynamics of a species. An understanding of population sex ratios is a perquisite for the development of specific conservation strategies to enhance the recovery of species in decline. There are a several methods that can be used to accurately identify the sex of an individual. However, not all methods can be applied across all species and all life stages.

Diamondback terrapins (*Malaclemys terrapin*) display sexual dimorphism where the female is up to four times as large in weight as the males (Hart and Lee, 2006). Many turtle species, including the diamondback terrapin, require a relatively long period of time to reach sexual maturity. It may take at least six years for a female to become reproductively mature (Davenport, 1992). While adults show a distinct sexual dimorphism (e.g., larger tails in males, and large heads in females), sexually dimorphic characteristics have not been identified in juvenile terrapins. Therefore, other methods besides external morphology must be used to accurately sex individuals who have not yet progressed through the pubescent stage.

The definitive method of determining the sex of an individual turtle is by the examination of the gonads (Yntema and Mrosovsky, 1982; Merchant-larios, 1997; Wibbels, 2003). Unfortunately, this requires the dissection of the hatchling which is not practical when working with depleted species of conservational interest.

Laparoscopy has been used as a non-lethal method for accurately identifying the sex of juvenile sea turtles, but it involves an invasive procedure that requires surgical training in which the gonads are directly observed using a laparoscope (Owens, 1999; Wibbels et al., 2000). When done properly, this method represents an accurate method of sexing juvenile turtles (Wibbels et al., 2000).

Examination of blood testosterone levels has been shown to be an accurate and practical method of evaluating the sex of a relatively large number of juvenile sea turtles. Wibbels *et al*. (2000) reviewed several studies that showed that serum testosterone levels differ between sexes of juvenile sea turtles, where male serum testosterone levels are significantly higher than those of females. This method of sexing has several advantages over other methods. The amount of field equipment needed is very minimal, requiring only blood colleting supplies. Testosterone is also relatively stable, and frozen serum samples can be stored for prolonged periods of time. This method is also capable of testing a high volume of samples, making it more effective when sexing large numbers of individuals (Wibbels et al., 2000).

Although the testosterone sexing technique has been validated in several sea turtle species, this method has not been evaluated in the diamondback terrapin. The current study examines serum testosterone levels in captive-reared terrapins that were hatched from eggs incubated in the laboratory at known male-producing or female-producing temperatures.

Materials and Methods

Nesting female diamondback terrapins were captured at Cedar Point Marsh, Alabama, using drift fences and pitfall traps during the 2009 and 2010 nesting season. Turtles were induced to lay eggs in captivity by injecting 10 I.U. of oxytocin into the front limb (Ewert and Legler, 1978). Eggs were incubated at male-producing temperatures (i.e. 26 °C) or female-producing temperatures (i.e. 31 °C) (Jeyasuria and Place, 1997). Terrapins were reared for approximately 2 years as part of a head-start research program. Prior to their release, tail length was measured by measuring the distance from the plastron to the middle of the cloacal opening.

Blood samples were obtained prior to the release of the head-started turtles into Cedar Point Marsh. To draw blood, a towel was used to hold the head of the terrapin in the retracted position. A 5/8” 26-gauge needle with a 1-ml syringe was inserted just under the carapace centered with the nuchal scute at a 45° angle with the needle pointed up towards the carapace (Wibbels et al., 1998). Approximately 0.7 ml of blood was drawn and then deposited into a 1.5ml centrifuge tube. The blood was then spun in a centrifuge and the serum transferred to a clean 1.5 ml centrifuge tube. These samples were immediately placed on ice and were then transferred into a -20 °C freezer.

Radioimmunoassay.—To predict the sex of the juvenile turtles, a testosterone RIA, similar to that described by Geis *et al*. (2005) and Witzell *et al*. (2005), was utilized to measure circulating testosterone levels (Owens et al., 1978; Wibbels et al., 2000). Approximately 200-µl aliquots of each sample were assayed in duplicate. Steriod hormones were extracted from each aliquot using 3 ml of anhydrous diethyl ether. The samples were then dried under nitrogen gas and resuspended in 500 µl of Tris-gel buffer. To assess extraction efficiencies, approximately 1000 counts per minute (cpm) of tritiated testosterone (Perkin Elmer, Waltham, MA) were added to each aliquot prior to extraction. From each resuspended sample, 200 µl were assayed by adding 100 µl of testosterone antisera (Fitzgerald International, Acton, MA) and approximately 10,000 cpm of tritiated testosterone in a 100-µl volume. The samples were allowed to incubate for a minimum of 12 h at 4 ºC. After incubation, 3 ml of a dextran-coated charcoal suspension were added to each assay tube. The assay tubes were then incubated for 15 min, and centrifuged for 15 min to separate bound and unbound fractions. The bound fraction was poured into polyethylene scintillation vials and 3 ml of scintillation cocktail (Scintiverse, Fisher Scientific, GA) were added to each vial. The vials were counted on a beta counter. To generate a standard curve, serial dilutions of testosterone standard were included with each assay and ranged from 15.625 to 2000 pg/ml. A minimum of two control samples were run in each assay to generate intra- and interassay coefficients of variation. Five assays were conducted to complete the evaluation of all samples in duplicate. Mean assay sensitivity was 36.32 pg ± 5.44. Intra- and interassay coefficients of variation were calculated to be 5.2% and 11.1% respectively.

Results

A total of 77 serum samples were run in duplicates to determine the testosterone concentration of captive-reared terrapins. Serum testosterone concentration levels ranged from approximately 294 pg/ml to approximately 7608 pg/ml. Testosterone levels relative to the predicted sex based on incubation temperature of the egg are shown in Figure 1. The data show a bimodal distribution in which the predicted males have significantly higher testosterone than the predicted females (t-test, $P < 0.05$). Although the majority of males and females appeared to separate out into two distinct groups, there was some overlap between predicted males and females. However, there was one distinct male outlier with a very low level of testosterone and one distinct female outlier with a very high level (Figure 1).

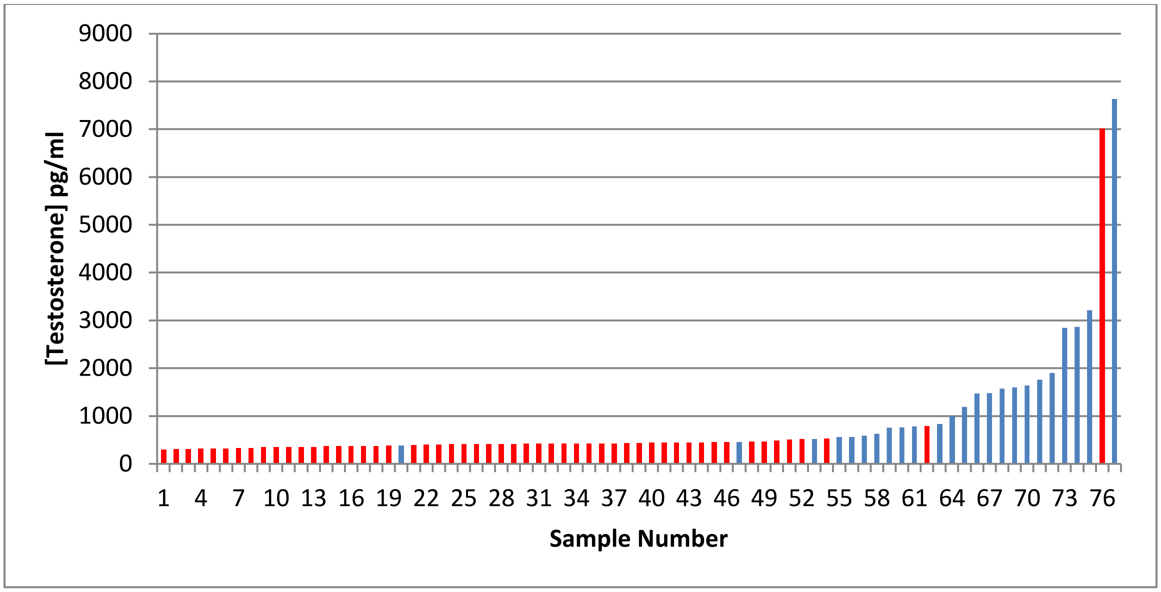


Figure 1. Concentrations of Testosterone in Blood Serum Samples. (Red bars indicate individuals incubated at 31 °C, blue bars indicated incubation at 26 °C)

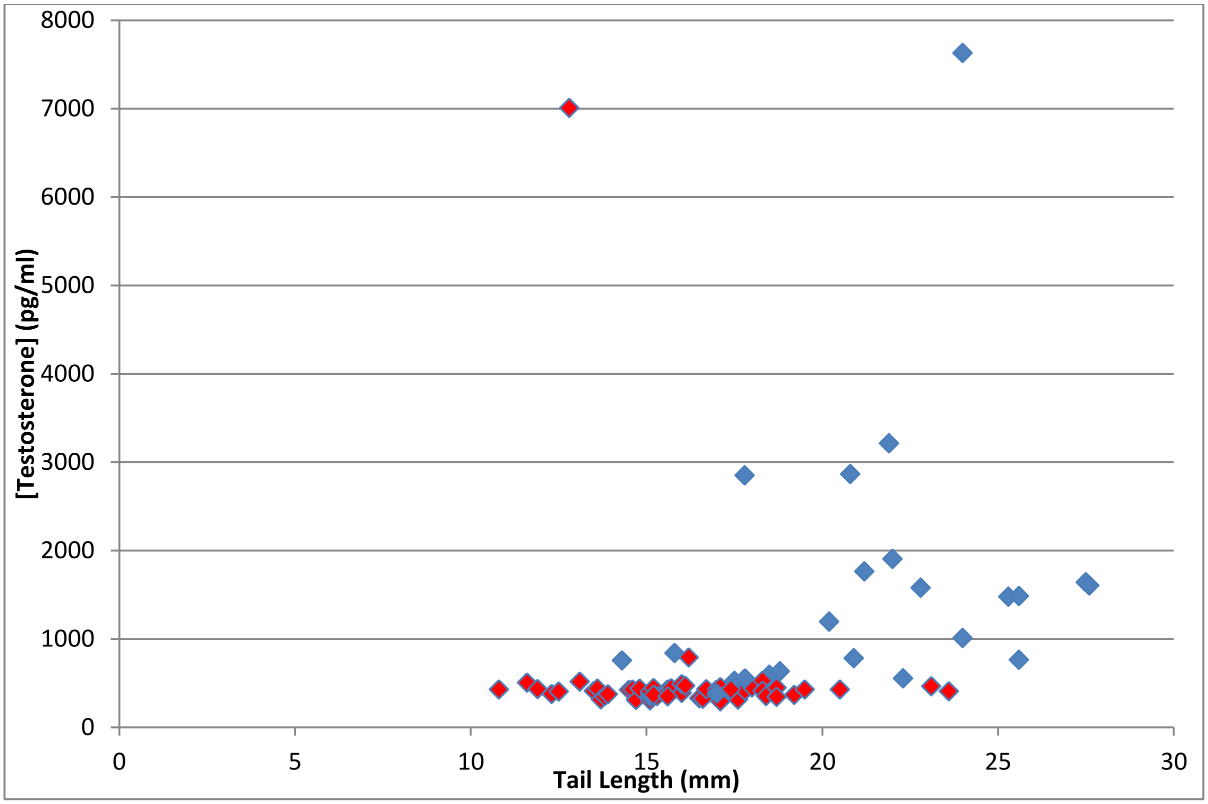


Figure 2. Tail length vs testosterone concentration (Red represents females, Blue represents males).

The sex predicted by incubation temperature was compared to tail length and a significant difference was detected (t-test, $p < 0.05$). However, the ranges overlapped to a large extent (Figure 2). The tail length of individual terrapins was compared to testosterone levels. Regression analysis indicated a significant relationship between testosterone level and tail length ($p < 0.05$)

Four of the 77 samples tested showed testosterone concentrations that were inconsistent with the known sex; that is, the sex determined by examining incubation temperature was male, while the testosterone concentrations indicated a female or vice versa. The head width vs carapace length was compared in those samples and one outlier was found to show a female head width to carapace length ratio, as well as a testosterone concentration consistent with female levels.

Discussion

The results indicate that in juvenile terrapins, males have significantly higher serum testosterone levels in comparison to females**.** Although there was some overlap between predicted males and females, there was a distinct bimodal distribution. However, there were two individuals that were atypical; a predicted male with relatively low levels of testosterone and a predicted female with relatively high levels. The reason for this inconsistency in not clear; the predicted sex of these individuals was based on incubation temperature, which should be a reliable indicator (Jeyasuria and Place, 1997). However, it is plausible that the sex of these turtles was misidentified. During the two years of aquaculture, there were approximately five instances in which two terrapins escaped from their tanks during the same time period and were reassigned to the closest tank by animal resource personnel. Therefore, it is possible that the predicted sex (based on incubation temperature) of a few of the terrapins used in the current study was incorrect. Since these head-start terrapins were released into the wild, it is not possible at this time to verify sex.

Regardless, the results show significant differences in the serum testosterone concentrations between predicted males and females. Furthermore, the characteristics of the bimodal distribution suggest that testosterone could be an accurate predictor of sex in juvenile terrapins. For example, if 528 pg/ml was used as an approximate cutoff between males and females, it would be an accurate predictor for 94.4% of the females in the study and 91.3% of the males in the study (Figure 1). Thus the results suggest that serum testosterone level could prove to be a useful technique for predicting populations’ sex ratios of juvenile terrapins. Such a technique would be a valuable tool for facilitating the evaluation of population sex ratios in the wild. Serum testosterone has been shown to be an accurate indicator of sex in juvenile sea turtles, and it has been frequently used to estimate juvenile sex ratios in sea turtle populations (Wibbels et al., 2000).

Considering that head-start terrapins are being produced yearly as part of an ongoing conservation program in Alabama, they could be used to further refine the male and female ranges of serum testosterone levels and the extent to which the ranges overlap. Due to improvements in 2011, there has not been an incident in which two terrapins have escaped simultaneously. Further, it would be of particular interest to use individuals in which the sex was verified by laparoscopy.

The results also suggest that testosterone levels in male juvenile terrapins could be affecting tail length, a known secondary sexual characteristic in all turtles, including terrapins. However, the tail length ranges of the predicted males and females overlapped to a great extent; thus, tail length may not be a practical method for predicting the sex of juvenile turtles.

Literature Cited

Davenport, J. 1992. The biology of the diamondback terrapin Malaclemys terrapin (Latreille). Testudo 3: pages unknown

Ewert, M.A., and J.M. Legler. 1978. Hormonal induction of oviposition in turtles. Herpetologists' League 34: 314-318.

Geis, A.A., W.J. Barichivich, T. Wibbels, M. Coyne, A.M. Landry Jr., and D. Owens. 2005. Predicted sex ratio of juvenile kemp's ridley sea turtles captured near Steinhatchee, Florida. Copeia 2005:393-398.

Hart, K.M., and D.S. Lee. The diamondback terrapin: The biology, ecology, cultural history, and conservation status of an obligate estuarine turtle. Studies in Avian Biology 32:206-213.

Jeyasuria, P., and A. R. Place. 1997. Temperature-dependent aromatase expression in developing diamondback terrapin (*Malaclemys terrapin*) embryos. Journal of Steroid Biochemistry and Molecular Biology 61:415-425.

Merchant-Larios, H., S. Ruiz-Ramirez, N. Moreno-Mendoza, and A. Marmolejo-Valencia. 1997. Correlation among thermosensitive period, estradiol response, and gonad differentiation in the sea turtle *Lepidochelys olivacea*. General and Copmarative Endocrinology 107:373-385.

Owens, D.W., J.R. Hendrickson, V. Lance, and I.P. Callard. 1978. A technique for determining sex of immature *Chelonian mydas* using radioimmunoassay. Herpetoligica 34:270-273.

Owens, D.W. 1999. Reproductive cycles and endocrinology. Pp.119-123 in K.L. Eckert, K.A. Bjorndal, F.A. Abreu-Grobois, M. Donnelly (Eds.), Reseach and

Management Techniques for the Conservation of Sea Turtles. IUCN/SSC Marine Turtle Specialist Group Pub. No. 4

Wibbels, T., J. Hanson, G. Balazs, Z.-M. Hillis-Starr, B. Philips. 1998. Blood sampling techniquesfor hatchling cheloniid sea turtles. Herpetol. Rev. 29:218–220.

Wibbels, T., D.M. Owens, and C.J. Limpus. 2000. Sexing juvenile sea turtles: is there an accurate and practical method?. Chelonian Conservation and Biology 3:756-761.

Wibbels, T. 2003. Critical approaches to sex determination in sea turtles. Pp. 103-134 in P.J. Lutz, J.A. Musick, and J. Wyneken, (Eds.), The Biology of Sea Turtles, Vol. 2. CRC Press, USA.

Witzell, W.N., A.A. Geis, J.R. Schmid, and T. Wibbels. 2005. Sex ratio of immature Kemp’s ridley turtles (*Lepidochelys kempi*) from Gullivan Bay, Ten Thousand Islands, south-west Florida. Journal of the Marine Biological Association of theUnited Kingdom 85:205-208

Yntema, C.L., and N. Mrosovsky. 1982. Critical periods and pivotal temperatures for sexual differentiation in loggerhead turtles. Canadian Journal of Zoology 60: 1012-1016.

FINAL DISCUSSION

The results presented in this thesis addressed various aspects of the reproductive ecology of the terrapin which are prerequisites for developing an effective management strategy to enhance the recovery of the diamondback terrapin in Alabama.

In chapter 1, the nesting characteristics and abundance of female terrapins utilizing the CPM nesting beach was examined. The results indicate that a mean of 131 ± 24 nests were depredated during the 2008-2011 nesting seasons. Although nesting beach surveys indicated that the entire length of the CPM beach was utilized for nesting, it was not distributed uniformly across the beach. Instead, depredated nests seemed to be concentrated in specific areas related to factors such as vegetation and proximity to tidal creeks. Assuming females lay 2-3 clutches (Seigel, 1984; Roosenburg, 1991), the depredated nest abundance represents the reproductive output of approximately 44-65 individuals. The abundance of adult females was estimated to be 53 utilizing the Schnabel method and mark-recapture data from 2006-2011. Collectively, the results suggest that the majority of the nests laid on the CPM nesting beach are depredated, and highlights the importance of incorporating the mitigation of nest and hatchling predation in an effective management strategy for this population. Additionally, the low female abundance estimated suggests that few individuals reach maturity and thus, high mortality rates in the wild pose a major threat to the recovery of the terrapin population in Alabama.

In chapter 2, the inter-nesting and post-nesting movements of adult females over two years were examined. A total of 18 terrapins were fitted with transmitters over the 2010 and 2011 nesting seasons. In 2010, four turtles were tracked for relatively long periods of time (3-4 months). These turtles remained in CPM adjacent to the CPM nesting beach on which they had been captured. Two turtles were known to have malfunctioning transmitters (one transmitter became detached and one was recover 2 months after release with a broken antenna). The remaining two terrapin in 2010 were never located after the initial release date. Tracking during the 2011 nesting season was not as successful as in 2010. Ten transmitters were deployed and only three were tracked for relatively long periods of time. Two of these turtles remained in CPM adjacent to the nesting beach and one was located across Heron Bay in a marsh on Mon Louis Island. Three turtles were located one week after their initial release date and four turtles were never located after their initial release date. The fates of the undetected turtles are unclear. One explanation is that these turtles may have moved outside of the range of the receiver to adjacent marshes. Alternatively, they may have remained in CPM either eluding detection by remaining underwater during surveys (thus extinguishing their transmission), or the transmitter could have become defective (e.g., damaged antenna).

Regardless, the results from both the 2010 and 2011 indicate that CPM, as well as adjacent marshes in the Heron Bay area serve as critical habitat for the diamondback terrapin in Alabama. In addition, the study highlights the importance of the CPM nesting beach for not only terrapins residing in CPM, but also for terrapins residing in marshes in the Heron Bay area. Although, the purchase and protection of CPM is currently in progress, crab trap mortality may still pose a large threat to terrapins moving across the bay (which is heavily fished) to adjacent marshes.

In chapter 3, hatchling sex ratios were predicted for the 2011 nesting seasons using a dual approach consisting of a mean temperature-based model, as well as the direct sexing of a surrogate species incubated on the CPM nesting beach. The temperature-based model indicated that the CPM nesting beach is relatively warm and produces 100% females for the majority of the 2011 nesting season. Only nests laid at the start of the nesting season were predicted to produce mixed hatchling sex ratios. These findings were supported by the experimental surrogate egg study. A strong female-biased hatchling sex ratio was indicated when surrogate eggs were incubated under natural conditions on the CPM nesting beach. It may be possible to use this model to predict future and past hatchling sex ratios as long as topography, vegetation, and weather events remain relatively constant. The existence of a female-biased hatchling sex ratio has been shown to be fairly common in species exhibiting temperature-dependent sex determination (Wibbels, 2003). A female-biased sex ratio has been shown to be beneficial in accelerating the recovery of a depleted population (Wibbels, 2007). It would be of interest to examine sex ratios in the population to identify if this strong female-bias persists throughout all life stages.

In chapter 4, radioimmunoassay of serum testosterone levels was evaluated as a non-lethal juvenile sexing technique. Juveniles are not subject to sex specific migration and thus an accurate way of sampling to examine sex ratios in the population. Identification of juvenile sex is not possible through examination of external morphology, and current sexing techniques (direct examination of the gonad through either laparoscopy or histology) are highly invasive, and in some cases lethal. These methods of sexing are not practical when working with threated or endangered species. Serum testosterone levels have been shown to be an effective method of sexing juvenile sea turtles (Wibbels et al., 2000). The results indicated that radioimmunoassay of serum testosterone levels in juvenile terrapins is a practical sexing method. An understanding of the sex ratios found at all life stages of a population can provide insight into sex specific mortality, and can provide important information for enhancing the management strategy of the diamondback terrapin in Alabama.

Understanding the reproductive ecology of the diamondback terrapin is a prerequisite to creating an effective management strategy for the recovery of the terrapin in Alabama. Collectively the results from the current thesis indicate that the diamondback terrapin in Alabama is severely depleted and the recovery of this species relies on a comprehensive management strategy that addresses not only the threats facing the species but also how those threats interact with the terrapins at various life stages . Two major threats affecting the population in Alabama are nest and hatchling depredation, and crab trap-induced mortality. Currently, an experimental head-start program is being evaluated in an attempt to circumvent the high nest and hatchling mortality. Eggs are incubated in laboratory incubators and terrapins are reared for approximately 2 years before release. It is important that mortality at all life stages is addressed to create an effective management strategy. Therefore, the mitigation of crab trap induced mortality is paramount to the recovery of the population in Alabama. TEDs have been shown to reduce the incidental catch of terrapins by 95% (Coleman et al., 2012).

Collectively, the data presented in this thesis regarding adult female abundance, adult female critical habitat, hatchling sex ratios, and population sex ratio methodology provide baseline that highlight the need for conservation measures and provide information required for the development of an effective management program for the recovery of the diamondback terrapin in Alabama.

GENERAL REFERENCES

Brennessel, B. 2006. Diamonds in the Marsh. A Natural History of the Diamondback Terrapin. Hanover, NH: University Press of New England. 219 p.

Burger, J. 1976. Behavior of hatchling diamondback terrapin (Malaclemys terrapin) 9n the field. Copeia 1976:742-748.

Burger, J. 1977. Determinants of hatching success in the diamondback terrapin, *Malaclemys terrapin.* American Midland Naturalist 97:444-464.

Butler, J.A., C. Broadhurst, M. Green, and Z. Mullin. 2004. Nesting, nest predation and hatchling emergence of the Carolina diamondback terrapin, *Malaclemys terrapin centrata*, in Northeastern Florida. American Midland Naturalist 152: 145-155.

Carr, A. 1952. Handbook of Turtles: The Turtles of the United States, Canada, and Baja California. Ithaca, NY: Cornell University Press. 542 p.

Cecala, K. K., J. W. Gibbons, and M. E. Dorcas. 2008. Ecological effects of major injuries in diamondback terrapins: implications for conservation and management. Aquatic Conservation: Marine and Freshwater Ecosystems DOI: 10.1002/aqc.

Coleman, A. 2011. Biology and conservation of the diamondback terrapin, *Malaclemys terrapin pileata*, in Alabama. Dissertation. University of Alabama at Birmingham Graduate School.

Davenport, J. 1992. The biology of the diamondback terrapin Malaclemys terrapin (Latreille). Testudo 3(4): pages unknown

Davenport, J. and J. F. Ward. 1993. The effects of salinity and temperature on appetite in the diamondback terrapin, *Malaclemys terrapin* (Latreille). Herpetological Journal 3:95-98.

Feinburg, J.A. and R.L. Burke. 2003. Nesting ecology and predation of diamondback terrapins, *Malaclemys terrapin*, at Gateway National Recreation Area, New York. Journal of Herpetology 37: 517-526.

Gibbons, J.W., J.E. Lovich, A.D. Tucker, N.N. Fitzsimmons, and J.L. Greene. 2001. Demographic and ecological factors affecting conservation and management of the diamondback terrapin (*Malaclemys terrapin*) in South Carolina. Chelonian Conservation and Biology 4: 66-74.

Grosse, A.M., van Dijk, J.D., Holcomb, K.L., and Maerz, J.C. 2009. Diamondback terrapin mortality in crab pots in a Georgia tidal marsh. Chelonian Conservation and Biology. 8:98-100.

Gustafson, D.J., Kilheffer, J., and Silliman, B.R. 2006. Relative effects of *Littoraria irrorata* and *Prokelisia marginata* on *Spartina alterniflora*. Estuaries and Coasts 29:639-644.

Hart, K. M., and D. S. Lee. 2006. The Diamondback terrapin: the biology, ecology, cultural history, and conservation status of an obligate estuarine turtle. Studies in Avian Biology 32:206-213.

Jeyasuria, P., W.M. Roosenburg, and A.R. Place. 1994. Role of P450 aromatase in sex determination of the diamondback terrapin, *Malaclemys terrapin*. Journal of Experimental Zoology 270:95-111.

Lovich, J.E., A.D. Tucker, D.E. Kling, J.W. Gibbons, and T.D. Zimmerman. 1991. Hatchling behavior of diamondback terrapins (*Malaclemys terrapin*) released in a South Carolina salt marsh. Herpetological 22: 81-83.

Mann, T.M. 1995. Population surveys for diamondback terrapins (*Malaclemys terrapin*) and Gulf salt marsh snakes (*Nerodia clarkii clarkii*) in Mississippi. Museum Technical Report No. 37, Mississippi Museum of Natural Sciences. Jackson, MS. 75 p.

Marion, K. and D. Nelson. 2004. Mississippi Diamondback Terrapin. In: R.E. Mirarchi, M.A. Bailey, T.M. Haggerty, and T.L. Best (Eds.). Alabama Wildlife, Volume Three: Imperiled Amphibians, Reptiles, Birds, and Mammals. Tuscaloosa, AL: University of Alabama Press, 225 pp.

New York Times article. 1881. A Mobile terrapin farm. Published February 4, 1881.

Roosenburg, W.M. 1990. The diamondback terrapin: Population dynamics, habitat requirements, and opportunities for conservation. Proceedings from: New Perspectives in the Chesapeake System: A Research and Management Partnership. Chesapeake Research Consortium Publication 137: 227-234.

Roosenburg, W. M. 1994. Nesting habitat requirements of the diamondback terrapin: a geographic comparison. Wetlands Journal 6(2):9-12.

Roosenburg, W.M. and A.R. Place. 1995. Nest predation and hatchling sex ratio in the diamondback terrapin: Implications for management and conservation. Towards a Sustainable Coastal Watershed: The Chesapeake Experiment, Proceedings of a Conference. Chesapeake Research Consortium Pub. No 149. Solomons, MD. pp. 65-70.

Roosenburg, W.M., W. Cresko, M. Modesitte, and M.B. Robbins. 1997. Diamondback terrapin (*Malaclemys terrapin*) mortality in crab pots. Conservation Biology 11: 1166-1172.

Roosenburg, W.M., K.L. Haley, and S. McGuire. 1999. Habitat selection and movements of diamondback terrapins, *Malaclemys terrapin*, in a Maryland estuary. Chelonian Conservation and Biology 3: 425-429.

Silliman, B.R., van de Koppel, J., Bertness, M.D., Stanton, L.E., and Mendelssohn, I.A. 2005. Drought, snails, and large-scale die-off of southern U.S. salt marshes. Science 310:1803-1806.

Seigel, R.A.1980. Predation by raccoons on diamondback terrapins, *Malaclemys terrapin tequesta.* Journal of Herpetology 14:87-89.

Seigel, R.A. 1984. Parameters of two populations of diamondback terrapins (*Malaclemys terrapin*) on the Atlantic coast of Florida. In: R.A. Seigel, L. E. Hunt, J. L. Knight, L. Malaret, and N. L. Zushlag (eds.) Vertebrate Ecology and Systematics - A Tribute to Henry S. Fitch. Museum of Natural History, University of Kansas, Lawrence, Kansas.

Tucker, A.D., N.N. Fitzsimmons, and J.W. Gibbons. 1995. Resource partitioning by the estuarine turtle *Malaclemys terrapin*: Trophic, spatial, and temporal foraging constraints. Herpetologica 51: 167-181.

Wibbels, T., D.M. Owens, and C.J. Limpus. 2000. Sexing juvenile sea turtles: is there an accurate and practical method?. Chelonian Conservation and Biology 3:756-761.

Wibbels, T. 2003. Critical approaches to sex determination in sea turtles. Pp. 103-134 in P.J. Lutz, J.A. Musick, and J. Wyneken, (Eds.), The Biology of Sea Turtles, Vol. 2. CRC Press, USA.

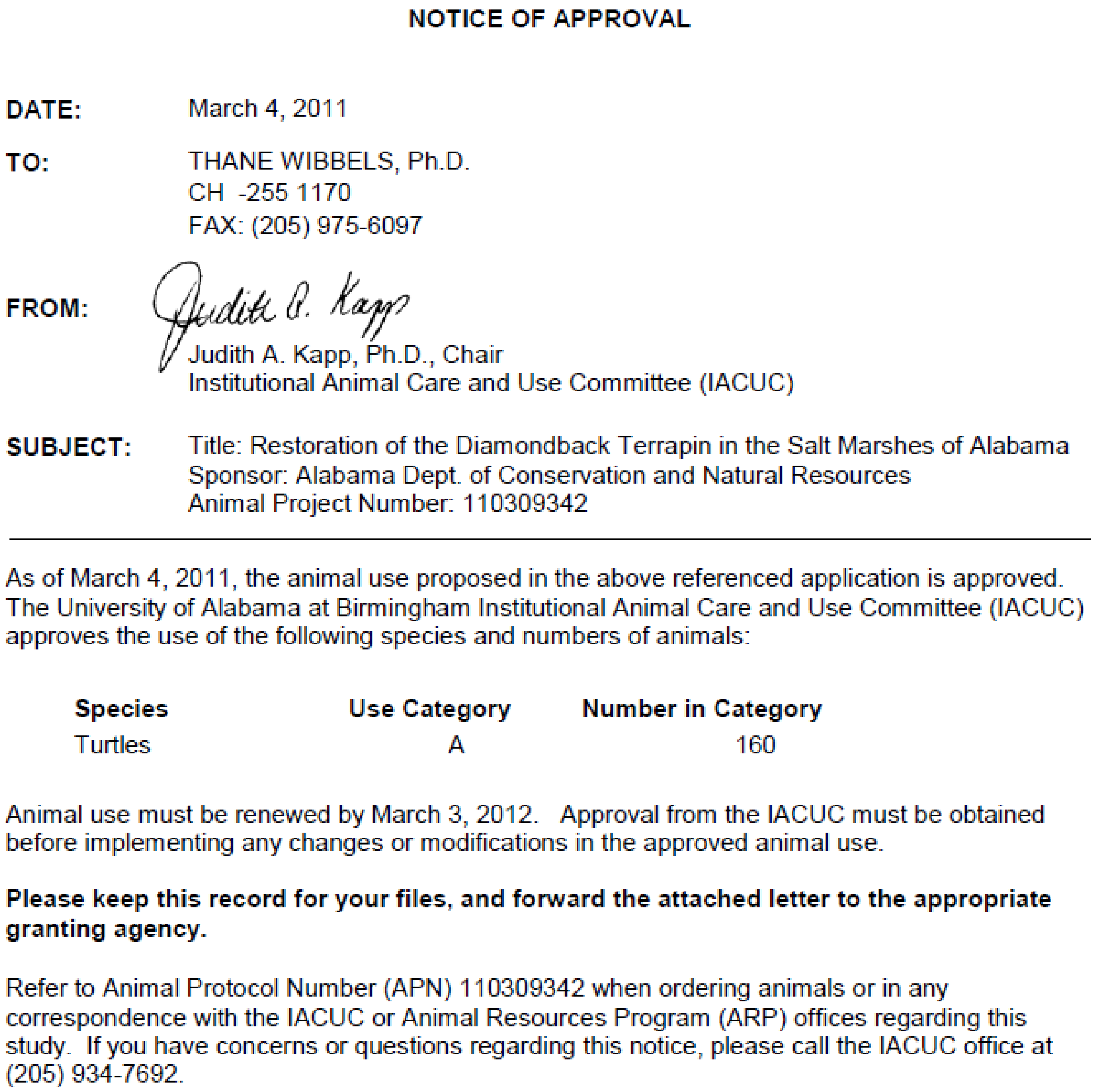
Wibbels, T. 2007. Sex determination and sex ratios in ridley turtles. Pp. 167-189 in P.T. Plotkin. (Ed.), Biology and Conservation of Ridley Sea Turtles. Johns Hopkins Univ. Press, USA.

Wood, R. C. 1997. The impact of commercial crab traps on northern diamondback terrapins, *Malaclemys terrapin terrapin*. Pages 21-27. In J. Van Abbema editor. Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles--An International Conference. New York Turtle and Tortoise Society, New York, USA.

Wood, R.C. and R. Herlands. 1997. Turtles and tires: The impact of roadkills on Northern Diamondback Terrapin, *Malaclemys terrapin terrapin*, populations on the Cape May Peninsula, Southern New Jersey, USA. Proceedings from: Conservation, Restoration, and Management of Tortoises and Turtles-An International Conference 46-53.

APPENDIX A

Institutional Animal Care and Use Committee (IACUC)



NOTICE OF APPROVAL

DATE: March 4, 2011

TO: THANE WIBBELS, Ph.D.
CH -255 1170
FAX: (205) 975-6097

FROM: Judith A. Kapp
Judith A. Kapp, Ph.D., Chair
Institutional Animal Care and Use Committee (IACUC)

SUBJECT: Title: Restoration of the Diamondback Terrapin in the Salt Marshes of Alabama
Sponsor: Alabama Dept. of Conservation and Natural Resources
Animal Project Number: 110309342

As of March 4, 2011, the animal use proposed in the above referenced application is approved. The University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) approves the use of the following species and numbers of animals:

Species	**Use Category**	**Number in Category**
Turtles	A	160

Animal use must be renewed by March 3, 2012. Approval from the IACUC must be obtained before implementing any changes or modifications in the approved animal use.

Please keep this record for your files, and forward the attached letter to the appropriate granting agency.

Refer to Animal Protocol Number (APN) 110309342 when ordering animals or in any correspondence with the IACUC or Animal Resources Program (ARP) offices regarding this study. If you have concerns or questions regarding this notice, please call the IACUC office at (205) 934-7692.