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BIOLOGY, ECOLOGY, AND CONSERVATION OF HATCHLING AND POST-HATCHLING DIAMONDBACK TERRAPIN (*MALACLEMYS TERRAPIN PILEATA*)

by

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KEN MARION
DAVID NELSON

A THESIS

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

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2014

BIOLOGY, ECOLOGY, AND CONSERVATION OF HATCHLING AND POST-HATCHLING DIAMONDBACK TERRAPIN (*MALACLEMYS TERRAPIN PILEATA*)

TANDY LAURALIN DOLIN PETROV

BIOLOGY

ABSTRACT

The diamondback terrapin has been considered a keystone species along its range in the coastal, brackish estuaries bordering the Atlantic Coast and Gulf of Mexico. Historically, diamondback terrapin populations have been numerous; however, in Alabama; the population has dramatically declined over the past century due to a variety of threats. The threats in Alabama include predation by raccoons, incidental drowning in crab traps, as well as road mitigated mortality. Because of these threats, collaboration efforts between UAB, USA, DISL, and the Alabama Department of Conservation and Natural Resources, a head-start program has been initiated to mitigate the predation of hatchlings. As such, the diamondback terrapin head-start program provides a unique situation to evaluate the biology, ecology, and conservation of hatchling and post-hatchling diamondback terrapin. Studies involving the head-start program include evaluating maternal investment in eggs and offspring, growth patterns of offspring, evaluation of carapace shape as an accurate sexing method for immature terrapin, fitness assays, and feeding assays. By understanding the diamondback terrapin in the lab, we can better benefit their survival in the wild.

This thesis includes a series of studies related to the biology and conservation of the diamondback terrapin. Chapter 1 investigates maternal investment in offspring and the impact of incubation temperature on offspring relative to egg size, hatchling size, and growth. Chapter 2 evaluates a potential sexing technique for hatchling and immature terrapin based on carapace morphology, and Chapter 3 examines two aspects of fitness of head-start terrapin (righting response and ability to adapt to a live food source).

Keywords: conservation–head-start–maternal investment–fitness–righting response–morphometrics–terrapin

DEDICATION

To my parents, Mary Sue, Parry, and Jim Dolin, whose love and support have guided me through the early years of my life-long adventure. They always encouraged my unending demand to do everything and never wavered when I found yet another interesting critter to bring home.

To my husband, Stanislav, who without his love, support, and tireless evenings pouring over everything turtle, this thesis could not have been possible and who somehow finds a way to "STandy" by me in everything. It is because of him I was able to uptake my recent journey and to continue finding yet another interesting critter to bring home.

To the coast, where my heart fills at ease and my soul comes to rest, and to all the wonderful people living throughout its range, they show tenacity, resilience, and 'grits' can create a life that is richer than any unsurmountable trove of treasures.

ACKNOWLEDGEMENTS

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EVALUATION OF HEAD-STARTED DIAMONDBACK TERRAPIN, *MALACLEMYS TERRAPIN PILEATA*, FOR FITNESS AND ABILITY TO ADAPT TO A NATURAL PREY ITEM

The diamondback terrapin (*Malaclemys terrapin*) is one of the few reptiles to inhabit the coastal marshes of North America (Hart & Lee, 2006). Unlike other emydid turtles that only specialize in fresh water habitats, the diamondback terrapin is unusual in that it lives in these coastal, brackish water environments. Specifically, this species is known to inhabit salt marsh environments ranging from the northeast Atlantic coast of the U.S. to the western Gulf of Mexico in Texas (Hart & Lee, 2006). Throughout this range there have been 7 recognized subspecies (Brennessel, 2006). The subspecies that inhabits saltmarshes in Alabama is *Malaclemys terrapin pileata*, commonly known as the Mississippi diamondback terrapin or the 'Biloxi' terrapin (Carr, 1952). This subspecies has been reported to occur from the panhandle of Florida to the Louisiana-Texas border (Hart & Lee, 2006). In Alabama, aggregations have been reported from a variety of areas, including Dauphin Island Airport Marsh, Cat Island, Jemison Marsh, Point Aux Pines, Barton Island, and Cedar Point Marsh (Coleman, 2011).

Ecology

Since the diamondback terrapin inhabits brackish waters, they are able to tolerate high levels of salinity that would leave their fresh water conspecifics dehydrated (Davenport, 1992). Like other emydids, and turtles in general, the terrapin has a lachrymal gland that is utilized to expel excess amounts of salts and other ions from its body in order to facilitate with overall homeostasis within the terrapins inter-cellular fluids (Davenport, 1992; Hart and Lee, 2006). These glands are not enough to completely regulate a terrapin's internal homeostasis, so the diamondback terrapin has instinctually adopted alternatives to lowering internal salt concentration and maintaining homeostasis of those inter-cellular fluids (Davenport, 1992). The alternatives to physiological regulation include the ability to drink a fine layer of fresh water off the top of the denser salt water, drink dew drops from leaves of marsh grasses, drink water from the scutes of fellow terrapin carapaces, in addition, the terrapin has a low skin permeability to salts and water (Davenport, 1992; Hart and Lee, 2006). These key adaptations allow for the survival of the terrapin in a brackish environment.

Terrapin will typically feed on a variety of salt marsh invertebrates including the periwinkle snail, *Littorina irrorata* (Silliman and Bertness, 2002; Davenport, 1992). Tucker *et al* (1995) observed that terrapins will also feed on small mollusks, crustaceans, and scavenge fish. The choice of prey has been strongly correlated to the terrapin's size, allowing only for a small overlap in diet between small and bigger terrapin, and playing a possible predatory role of the terrapin (Tucker *et al*, 1995). Dense populations of grazing snails have been shown to be detrimental to the diversity of the marsh grass community in salt marshes. In declining populations of terrapins, diversity of marsh grasses can decease, thus decreasing the stability of the ecosystem (Silliman *et al*., 2005; Gustafson *et al.*, 2006). Because terrapin feed on these grazing snails, it has been hypothesized that the terrapin represent a keystone species that helps maintain the stability and diversity of the marsh ecosystem (Tucker *et al*, 1995).

Diamondback terrapin exhibit sexual dimorphism as adults. It can typically take a female terrapin twice as long to mature as it does for male terrapin, approximately six years and three years, respectively (Davenport, 1992). Once adults, the female dwarfs the male and their home ranges begin to diverge. Males, as well as juveniles, tend to linger closer to shallow shores while females, being larger, will occasionally venture out into the open ocean (Davenport, 1992). As hatchlings, many theories are associated with their life history. Of these theories, some suggest that diamondback terrapin hatchlings will stay hidden under the marsh selectively hiding in open pockets of shell hash and other debris (Burger, 1976).

After brumation, males will typically wait in the open marsh waters for the females in order to mate. Mating occurs typically in early May to early June and the eggs laid are usually in clutches of 5-12 eggs (Davenport, 1992; Hart and Lee, 2006). Normally, eggs incubate 40-60 days, with the first egg hatchings coming from warmer temperatures (Brennessel, 2006). Terrapins also exhibit temperature-dependent sex determination (TSD) where the temperature of the eggs' surroundings influences the outcome of the terrapin's sex (Jeyasuria and Place, 1997). Typically, females hatch at warmer temperatures (~31°C) and males hatch at cooler temperatures (~26°C). However, without knowing the exact temperature at which an embryo was incubated, sex is not obvious until adulthood, when secondary sexual characteristics become obvious (Coleman, 2011). Currently, the only non-lethal methods known for sexing sub-adults is through the direct observation of gonads via laparoscopy or dissection.

Adult male and female terrapin display unique sexual dimorphic characteristics which include tail length, overall body size, and head width. Female terrapins are larger than males, and they have larger more robust heads and jaws (Davenport, 1992; Roosenburg *et al*., 1999; Coleman, 2011). This illustrates how diet can be selective for either sex with larger females preying upon larger marsh invertebrates and males preying upon smaller invertebrates and soft tissue organisms (Tucket *et al*, 1995). On the other hand, males have larger more robust tails than females. This is thought to allow for easier copulation in the marsh channels (Brennessel, 2006).

Temperature-Dependent Sex Determination

A variety of sex determination systems have been identified in turtles, ranging from genetic sex determination or GSD, including both male and female heterogametic systems to temperature-dependent sex determination systems, or TSD (Janzen and Krenz, 2004). The majority of turtles, including the diamondback terrapin, possess temperature-dependent sex determination. There are multiple patterns of TSD in turtles, and the diamondback terrapin has a M:F pattern in which cooler incubation temperatures produce males and warmer incubation temperatures produce females (Jeyasuri and Place, 1997). In the diamondback terrapin, temperatures of approximately 28°C and below produce males, whereas temperatures of 30°C or above produce females. Incubation temperature appears to affect sex determination during the approximate middle third of the incubation period (Wibbels, et al., 1991). It has been hypothesized that female-producing temperatures enhance aromatase activity in the gonad, resulting in the production of estrogen, which stimulates ovarian differentiation (Pieau et al., 1998).

TSD has a variety of implications in regards to the ecology, evolution, and conservation of the diamondback terrapin. For example, TSD can produce a wide variety of sex ratios, and those sex ratios can significantly affect the reproductive ecology and reproductive output of a population (Wibbels, 2003; Coyne and Landry, 2007). In regards to evolution, a variety of hypotheses have been suggested to account for the possible advantages of this type of sex determination (Shine, 1999; Girondot, 1999). Currently the most accepted is the “differential fitness hypothesis” (Shine 1999) in which the sex of offspring varies with the environment in order to enhance fitness. This model illustrates that the certain environmental conditions may favor one sex over the other.

Finally, TSD has significant implication for conservation since the sex ratio within an endangered population can affect its rate of recovery (Coyne and Landry, 2007). Therefore, it may be advantageous to produce specific sex ratios in a conservation program in order to optimize the recovery of the species (Wibbels, 2003).

Conservation Status of Diamondback Terrapin in Alabama

Historically, terrapin were abundant in the salt marshes of Alabama and across their range. In the 1800's a culinary fad of consuming terrapin as a delicacy initiated terrapin trade and farming throughout the southeastern U.S. (Brennessel, 2006; Hart and Lee, 2006; Schaffer *et al.*, 2008). Alabama had possibly the largest terrapin farm in the U.S. during the late 1800's. It was operated by Nathan Dorlan and was located north of Dauphin Island near Cedar Point. The farm contained approximately 20,000 terrapins at its peak, and was supplemented by approximately 8,000 terrapins per year that Nathan Dorlan would purchase from locals. During a normal harvesting season, 12,000 terrapins would be exported from the farm to New England. Terrapin stew remained popular and prominent with the wealthy until prohibition, when one of the major ingredients, sherry, was unobtainable (Brennessel, 2006; Hart and Lee, 2006; Schaffer *et al.*, 2008). During the mid- 1900's, crab traps were implemented in the blue crab (*Callinectes sapidus*) fishery along the coast of Alabama (Coleman, 2011; Kennedy *et al*, 2007). With the implementation of crab traps along the Gulf and Atlantic coasts, terrapin populations decreased due to terrapins drowning in the crab traps (Roosenburg et al, 1997; Wood, 1997). In addition to the impact of actively-fished crab traps, many drowning incidents may also have occurred in "ghost" or derelict crab traps (Coleman, 2011; Roosenburg *et* *al*, 1995). Although terrapins were once numerous in the salt marshes of Alabama, presently they are scarce (Coleman, 2011).

As indicted above, in Alabama, terrapin aggregations have been reported from a variety of areas including Dauphin Island Airport Marsh, Cat Island, Jemison Marsh, Point Aux Pines, Barton Island, and Cedar Point Marsh (Coleman, 2011). The largest aggregation of diamondback terrapin has been reported to inhabit Cedar Point Marsh, which is located immediately north of Dauphin Island, AL (Coleman, 2011). Cedar Point Marsh is bordered to the west by an extensive shell hash beach that has been reported to support the largest nesting aggregations of female diamondback terrapin in Alabama (Coleman, 2011). The Cedar Point Marsh ecosystem is dominated by the marsh grass species *Juncus* and *Spartina* and has abundant food sources for terrapins, including *Littorina irrorata* (A. D. Tucker, N. N. FitzSimmons, & J. W. Gibbons, 1995) and a variety of other invertebrates that terrapin have been reported to feed upon (Anton D Tucker, Nancy N FitzSimmons, & J Whitfield Gibbons, 1995). Cedar Point Marsh also has a central channel that runs through the majority of the marsh. Since the channel is relatively shallow, it is rarely fished by the blue crab fishery. As such, Cedar Point Marsh has a variety of characteristics that may account for the relative abundance of terrapins that are found in that location. Although Cedar Point Marsh has the largest aggregation of terrapins reported in Alabama, there appears to be less than 400 individuals (Coleman, 2011).

Major Threats Impacting the Diamondback Terrapin in Alabama

A variety of threats have contributed to the decline of the diamondback terrapin in Alabama. These threats include the impact of the crab fishery and its dramatic increase in Alabama over the past 70 years. Additionally, predators (particularly raccoons) have a significant impact on nests in Alabama. It is plausible that the impact of predators has increased due to increased predator abundance associated with coastal development. Further, as the number of terrapin decline, there are fewer nests, so the percentage of eggs taken by predators, such as raccoons, increases due to the lack of predator satiation (Roosenburg *et al.*, 1997; Wood, 1997; Roosenburg and Green, 2000). The increase in coastal development can also adversely affect the amount and health of salt marsh wetlands. Further, coastal development can include new roads, causeways, and bridges which can potentially increase predator access to important nesting areas, and can often result in road/automobile-induced mortality of terrapin (Wood and Herlands, 1997; Szerlag and McRoberts, 2006). Collectively, these threats have resulted in drastic declines in terrapin populations in a variety of locations throughout the salt marsh wetlands of U.S. coastal waters, including Alabama.

Conservation of the Diamondback Terrapin in Alabama

Over the past decade, a collaboration between the University of Alabama at Birmingham (UAB), University of South Alabama (USA), Dauphin Island Sea Lab (DISL), and the Alabama Department of Conservation and Natural Resources has resulted in a wide variety of surveys documenting the location and abundance of terrapin and terrapin nesting in Alabama (Coleman, 2011). Further, this collaborative project includes specific activities which are targeted at addressing major threats impacting terrapin in Alabama. Specifically, this project is attempting to mitigate the impact of predators on nests by capturing females as they are searching for a nesting location. These females are induced to lay eggs in the lab, and the eggs are then incubated and hatched in the laboratory, thus circumventing the high level of nest predation which has been documented on the Cedar Point Marsh nesting beach (Coleman, 2011). The females are captured in pitfall traps associated with drift fences along the shell hash beaches at Cedar Point Marsh. As nesting females come to shore, they come upon the drift fence and will walk along it until they fall into the pit trap. Once the nesting terrapin is captured, the terrapin can then be transported to UAB in order to lay eggs, and then they are tagged and released near the original capture location.

In addition to decreasing nest predation, an experimental head-start program is being conducted in an attempt to circumvent mortality associated with early life history stages. The eggs obtained from the captured females are incubated either at 26°C (male-producing) or 31°C (female-producing). Once eggs have hatched, the hatchlings are raised in the lab until they reach a minimum mass of 100 to 200 grams, which typically takes approximately 2 years. Prior to release, all head-start terrapin are tagged with shell tags and permanent PIT tags.

Overview of Thesis Research

The proposed research focuses on a number of subjects related to the biology and conservation of the diamondback terrapin. Each of proposed studies provides insight on specific aspects of the ecology, evolution, and conservation of this species. The availability of eggs and hatchlings from the diamondback terrapin head-start program at UAB provides the opportunity to study a variety of aspects regarding the biology of this species. The study described in the Chapter 1 focuses on maternal investment in eggs and hatchling terrapins. It specifically addresses the “optimal egg size theory” (OES) (Smith and Fretwell 1974) and “developmental plasticity theory” (Roosenburg and Dunham, 1997). This study also evaluates terrapin growth rates relative to clutch, egg size, hatchling size, and incubation temperature/sex. The results provide insight for the ecological and evolutionary aspects of diamondback terrapin reproduction regarding female investment in offspring Chapter 2 evaluates the morphology the carapace as a potential method for sexing juvenile terrapins. It specifically evaluates previous methodology used to characterize male versus female turtles. Chapter 3 addresses applied aspects of terrapin biology and conservation related to the head-start project. Several studies are included in that chapter. The first study evaluates fitness in hatchling and post hatchling terrapins relative to egg size, age, sex, and clutch/mother. A second study evaluates the ability of head-start terrapins to adapt to a natural food source after being captive reared for up to 2 years. Collectively, the research provides a better understanding of the ecology, evolution, and conservation of the diamondback terrapin in Alabama.

CHAPTER 1

EVALUATION OF MATERNAL INVESTMENT IN OFFSPRING OF THE DIAMONDBACK TERRAPIN (*MALACLEMYS TERRRAPIN PILEATA*): EGG SIZE AND GROWTH

by

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ABSTRACT

Maternal investment and hatchling growth were investigated in the diamondback terrapin. Adult female terrapins were captured on a nesting beach at Cedar Point Marsh, Alabama. Females were induced to lay their eggs in the laboratory. The number of eggs per clutch, size of eggs and hatchlings, and growth rates of hatchlings and post-hatchlings were examined in 11-19 clutches per nesting season over four years of study. The number of eggs per clutch did not vary significantly between females. The size of eggs and hatchlings varied significantly by clutch, with larger females tending to produce larger eggs. The results are not consistent with the optimal egg size theory, but in contrast appear more consistent with the developmental plasticity hypothesis. The results also indicated clutch-specific and sex-specific growth patterns in the hatchling and post-hatchling terrapins. The results of this study provide insight on the strategy for maternal investment and its potential long term effects on the fitness and growth of the diamondback terrapin.

Keywords: diamondback terrapin–optimal egg size theory–development plasticity–maternal investment

INTRODUCTION

A variety of theories have been proposed regarding strategies for maternal investment in offspring. For example, in the case of oviparous reptiles, the number of eggs per clutch and egg size can vary to potentially enhance reproductive success by maximizing hatchling fitness. Smith and Fretwell (1974) proposed the “optimal egg size theory” (OES) in an effort to describe female investment in offspring. The theory proposes that the production of hatchlings is limited by resources, so females will produce hatchlings of a minimum size, and will increase the number of hatchlings, rather than the size of the hatchlings, in accordance with the availability of resources. For example, Congdon *et al* (1983) observed that in chicken turtles (*Deiroochelys reticularia*) from South Carolina that there was a positive relationship between egg size and adult female size. They also noted that there was a weak relationship between clutch size and female size. OES theory was not detected in this study, as had been predicted. Instead a negative relationship was observed between clutch size and egg size (Congdon *et al*, 1983). However, in 1985, Congdon and Gibbons studied 12 species of turtles and generally found a positive correlation between adult female body size and clutch size. However, in the box turtle (*Terrapene carolina*) and the chicken turtle, Congdon and Gibbons did not observe this positive correlation. In three of the turtles examined; chicken turtles, mud turtles (*Kinosternon subrubrum*), and red-eared sliders (*Trachemys scripta*), they observed that egg size increased with adult female size. As in the previous study, Congdon *et al*’s, a negative relationship was not observed. Two years later, in 1987, Congdon and Gibbons, argued that pelvic girdle size restricted the size of eggs in smaller turtle species, thus restricting them to smaller clutch sizes. This could explain why in small species of turtles OES was not observed.

OES obtained supporting evidence in a study by Bjorndal and Carr (1989) in a population of green sea turtles (*Chelonia* mydas) inhabiting Tortugeura, Costa Rica. They found that the average egg size was 44.4 mm, OES predicted an egg size of 45mm. This agreed with the OES theory for egg size. However, a non-significant relationship existed between female body size and egg size. Bjorndal and Carr (1989) did observe that female carapace length accounted for more variation in the size of the clutch than the size of the eggs.

In addition to the OES theory, the “developmental plasticity” hypothesis has been suggested as an alternative strategy. This proposes that females would vary the size of their offspring (or eggs) depending on their specific status and environmental conditions in an effort to maximize survival of offspring (Capinera, 1979; Kaplan, 1980; Kaplan and Cooper, 1984). Developmental plasticity was proposed to account for turtles not following the optimal egg size strategy, but instead opting for a varying maternal investment strategy. Roosenburg and Dunham (1997) used a diamondback terrapin population from the Chesapeake Bay area to understand alternative allocation strategies and to test both the developmental plasticity hypothesis and OES. They found that egg and clutch size varied considerably within females from year to year and that a normal pattern could not be distinguished. They also found that egg sizes within clutches were similar; however, the egg mass, length, and width, as well as hatchling mass, length, and width varied significantly between clutches. They suggested that the environment affected females differently and that females might vary their strategy for maternal investment. Further, the variability in egg size and egg number has been shown to affect hatchling fitness (Packard and Packard, 2000).

Maternal investment can also influence the rate of growth of offspring and, as such, other life history events are affected. For example, growth rates can affect life history events such as age at first reproduction, as well as cohort generation time (Roosenburg and Kelley, 1996). They found that terrapin eggs incubated at warmer temperatures produced hatchlings that grew faster than terrapin eggs incubated at cooler temperatures. This was attributed to an egg size effect that persisted to at least three years of age. Maternal investment may also impact growth rates of future generations thereby affecting hatchling fitness (Roosenburg and Kelley, 1996).

In the current study, these theories were examined in of the diamondback terrapin (*Malaclemys terrapin pileata*) inhabiting the salt marshes of Alabama. The number of eggs per clutch, size of eggs and hatchlings, and growth rates of hatchlings over a one-year period were examined in 11-19 clutches per nesting season over four nesting seasons. The results provide insight on the strategy that the diamondback terrapin utilizes in an attempt to maximize reproductive fitness of their offspring.

METHODS

The adult female terrapins (*Malaclemys terrapin pileata*) used in the study were captured on a nesting beach bordering the western margin of Cedar Point Marsh, which is located approximately 2 km north of Dauphin Island, AL. This represents the largest aggregation of terrapins and the most important nesting beach that have been identified in AL (Coleman, 2011). Adult female terrapin were captured in bucket traps while they were attempting to nest during the 2010 through 2013 nesting seasons (May through

July). The females were taken into the laboratory and induced to lay eggs by using oxytocin injections (Ewert and Legler, 1978). After laying eggs, the females were released near the location of their capture. From 11 to 19 clutches were obtained each nesting season. After chalking all eggs were weighed and measured (length and width), and each clutch was divided into two groups, with one group being incubated at a female-producing temperature of 31°C, and the other group incubated at a male-producing temperature of 26°C. Hatchlings were weighed and measured (straight carapace length, straight carapace width, plastron length) and were then subsequently weighed and measured at approximately two week intervals.

All nesting female morphometrics, including straight carapace width (CW), straight carapace length (CL), curved carapace width, curved carapace length, plastron length, depth, mass, tail lengths and head measurements, were found to be normally distributed. The flat nature of the plastron in comparison to the carapace resulted in more consistent and precise measurements, so plastron length (PL) was used as an indicator of the size of the nesting females in the statistical analysis. Egg measurements included egg length, egg width, and egg mass. Eggs were measured within 48 hours of laying. Egg mass measurements were more precise than egg length or width measurements, so egg mass measurements were used as an indicator of egg size. The weight of eggs was analyzed relative to clutch and by year using ANOVA. Egg weight was also evaluated relative to maternal female size by regression analysis. The weight and size of hatchings was analyzed relative to clutch, year, and sex by ANOVA. Growth rates of hatchlings and post hatchings were evaluated by examining changes in weight, carapace length, carapace width, and plastron length over time using, repeated measures

ANOVA. Average growth rates of hatchings and post hatchlings were also examined relative to maternal female size, using regression analysis. All of the statistical analyses were completed using IBM SPSS statistical software.

RESULTS

The number of clutches used in this study ranged from 11 to 19 per year (2010 = 11, 2012 = 11, 2012 = 19, 2013 = 17). The number of eggs obtained ranged from 84 to 138 per year (2010 = 84, 2011 = 102, 2012 = 142, 2013 = 138), with a total of 466 eggs. Over the four years of the study, hatching success was 94.0%, 70.59%, 94.37%, and 82.61%, for 2010, 2011, 2012, and 2013, respectively, with an overall average of 85.6%. Over the four years of this study the average clutch size was 7.7 eggs per clutch (range 4 to 11) and the average egg size was 11.9 grams (range = 8.52 to 14.62). Within years, average clutch size was 7.4 eggs per clutch for 2010, 7.4 eggs per clutch for 2011, 8 eggs per clutch for 2012, and 8.1 eggs per clutch for 2013. Average egg size was 11.5 grams for 2010, 12.07 grams for 2011, 11.76 for 2012, and 12.25 grams for 2013.

Egg weight varied significantly between clutches (ANOVA, $P<0.001$). Egg weight did not vary significantly by year (ANOVA, $P>0.05$). Regression analysis indicated that egg weight was significantly related to female size ($P<0.005$) (Figure 1). The number of eggs per clutch did not vary significantly between females (Chi Square goodness of fit test, $P=0.06$) (Figure 2). Hatchling size varied significantly by sex (ANOVA, $P<0.05$). The growth of hatchlings and post-hatchlings based on carapace length is shown by year in Figures 3 – 6. The growth of hatchlings and post-hatchlings varied significantly by clutch relative to CL, CW, PL, and mass, (repeated measures ANOVA, $P<0.001$), and by sex ($P<0.05$).

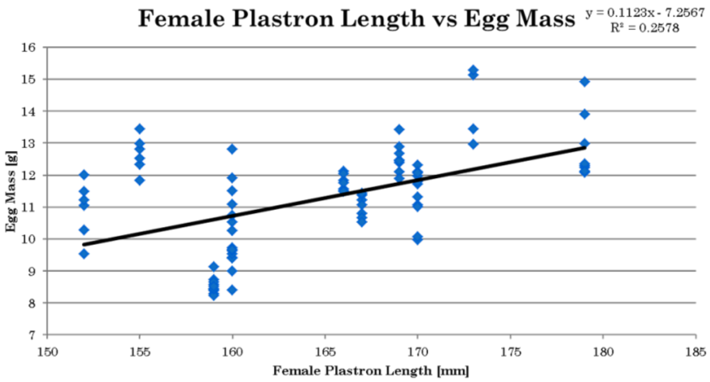

Figure 1: Female size versus egg weight; regression analysis showing significant difference in FPL (mm) relative to egg mass (grams)

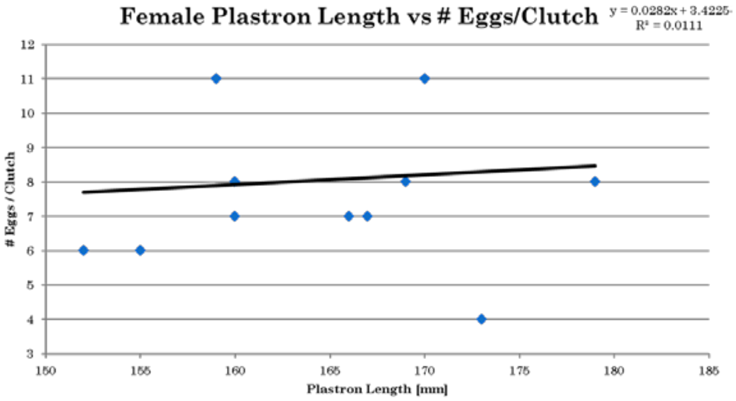

Figure 2: Female size versus number of eggs per clutch; regression analysis showing no significant different detected in eggs numbers per clutch in relation to FPL (mm).

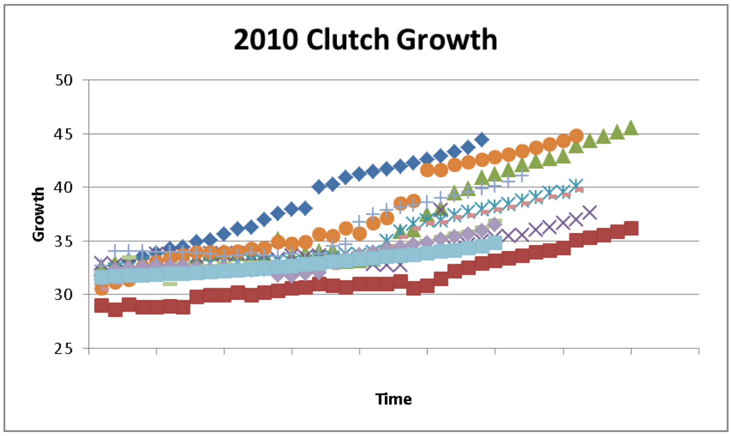

Figure 3: 2010 growth of hatchling and post-hatchling terrapin relative to carapace length.

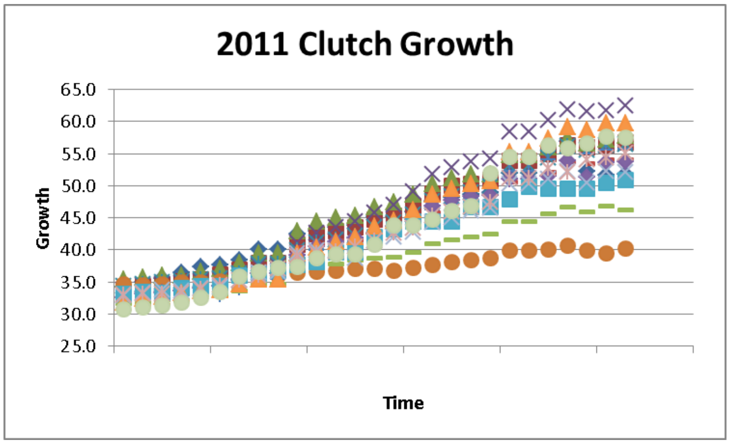

Figure 4: 2011 growth of hatchling and post-hatchling terrapin relative to carapace length.

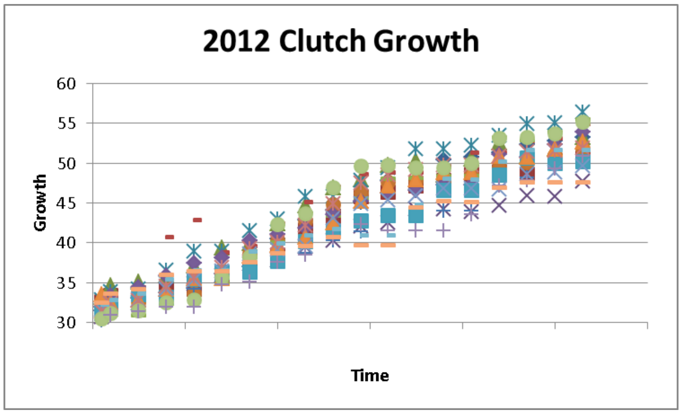

Figure 5: 2012 growth of hatchling and post-hatchling terrapin relative to carapace length.

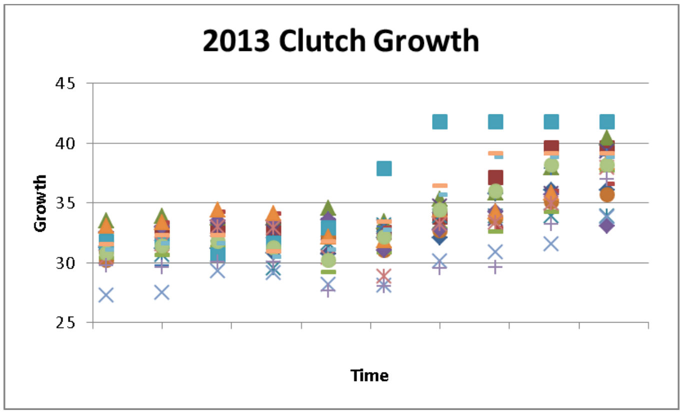

Figure 6: 2013 growth of hatchling and post-hatchling terrapin relative to carapace length.

DISCUSSION

The drift fences/bucket trap system proved to be an effective means of capturing approximately 15 to 20 terrapins per season. A high hatching success was obtained in the study, averaging 85.6% over the four years, indicating that the incubation regimen using laboratory incubators was an effective means for producing hatchlings. The results indicate that the diamondback terrapins from Cedar Point Marsh produced an average clutch size of 7.7 eggs with an average egg weight of 11.90 g. Significant variation was detected between egg weights from different clutches, but not within clutches. Previous studies have obtained similar results, indicating that variability in egg size is much greater between clutches than within clutches (Montevecchi and Burger, 1975). The clutch sizes recorded in the current study (ranging from 4 to 11 eggs) were within the ranges of clutch sizes reported for diamondback terrapins in other locations (i.e. 4 to 18 eggs) (Ernst et al., 1994). However, it should be noted that oviposition in the current study was induced with oxytocin (Ewert and Legler, 1978), so it is possible that some eggs may have been retained by females.

Egg weights recorded in the current study (ranging from 8.2 to 14.9 g) were similar to the range of egg weights (5.0 to 13.2 g) reported for terrapin eggs along the Atlantic coast of Florida (Seigel, 1980). Thus, the ranges of egg size and number of eggs per clutch appear comparable between the terrapins at Cedar Point Marsh and those that have been studied in other locations (reviewed by Ernst et al. 2004). It has been hypothesized that egg size may vary geographically among terrapin populations, with females in southern populations producing fewer but larger eggs (Siegel, 1980; Montevecchi and Burger, 1975; Allman, 2006). Seigel (1980) reported an average clutch size of 6.7 with an average egg weight of 12.48 g in Florida, whereas Montevecchi and Burger (1975) reported an average clutch size of 9.7 eggs with an average egg weight of 7.7 g in New Jersey. The data from the current study is consistent with this hypothesis, with the average clutch size (7.7 eggs) and average egg weight (11.90 g) for terrapins from Cedar Point Marsh being similar to those reported for a southern population from Florida (Seigel, 1980). This latitudinal trend is consistent with results previously reported for terrapins from Cedar Point Marsh by Coleman (2011).

The OES theory suggests that larger females should produce more eggs of an optimal size than smaller females (Smith and Fretwell, 1974). The effects of female allocation to eggs have been studied in a variety of previous studies of diamondback terrapin populations (Montevecchi and Burger, 1975; Seigel, 1980; Roosenburg, 1996; Roosenburg and Kelley, 1996; Roosenburg and Dunham, 1997; Roosenburg and Dennis, 2005, Allman 2006; Coleman 2011). Consistent with this theory, several studies have reported no significant variation in egg size relative to the size of adult female terrapins (Montevecchi and Burger 1975; Seigel, 1980; Roosenburg and Dunham, 1997). In contrast, the results of the current study are not consistent with the OES theory, since egg size varied with female size, with the larger females producing larger eggs. A previous study by Coleman (2011) obtained similar results to the current study, with larger females producing larger eggs.

The OES hypothesis also suggests that larger females should produce more eggs. Consistent with this theory, it was reported in a terrapin population in New Jersey, larger females tended to lay more eggs (Montevecchi and Burger, 1975). However, this was not detected in a terrapin population in Florida (Seigel, 1980) nor in the current study in which no significant variation was detected between egg number per clutch and the size of nesting females (Figure 2). Further, a previous study of terrapins in Cedar Point Marsh obtained similar results, with no significant variation being detected (Coleman, 2011).

The results of the current study suggest that larger females can produce larger eggs, and this finding does not support the OES theory. It is potentially consistent with the "developmental plasticity" hypothesis in which females could vary their allocation to offspring in relation to environmental conditions and available resources (Roosenburg and Dunham, 1997). It is plausible that producing larger offspring could provide a survival advantage, depending on the specific environmental conditions. A previous study of diamondback terrapins suggest that egg and clutch size varied considerably within individual females, thus supporting the development plasticity hypothesis, but that study also reported greater coefficients of variation for clutch size than for egg mass, thus supporting the OES theory (Roosenburg and Dunham, 1997). Thus, it is plausible that neither OES nor developmental plasticity theory alone may provide a precise explanation for clutch size and egg size produced by diamondback terrapin. A wide variety of factors may need to be examined relative to reproductive output, including environmental conditions, resource availability, and the physiological state of the terrapins in order to better understand the reproductive strategy of the diamondback terrapin in regards to clutch and egg size. For example, one aspect of reproductive physiology that could affect egg number and egg size is the number of clutches produced each year by a female. Terrapin have been reported to produce 1 -3 clutches per season in nature and up to 5 clutches per season in captivity (Montevicchi and Burger, 1975; Lazell, 1979; Seigel, 1980). It is plausible that there may be a physiological trade-off between the numbers of clutches per season versus the number of eggs per clutch.

The results indicate that eggs incubated at a male-producing temperature (26°C) produced significantly larger hatchlings than eggs incubated at female-producing temperature (31°C). Previous studies have shown turtle embryos incubated at cooler incubation temperatures, such as 26°C, consumed more yolk and grew to a larger size than embryos incubated at warmer incubation temperatures such as 31°C (Packard, 1987). The size difference at hatching has ecological and evolutionary implications. It has been suggested that a larger hatchling may have certain survival advantages over smaller hatchlings, such as predator avoidance (Barrows and Schwarz, 1895), while warmer incubation temperatures could also provide an advantages, such as greater yolk reserve upon hatching (Gibbons and Nelson, 1978).

The results of the current study also provide insight on the growth rates of hatchlings and post-hatchlings during a year of captive rearing. There was significant variation between the growth rates of hatchings from different clutches (Figure 3) and hatchlings of different sexes (Figure 4). The sex-specific growth rates indicate that the effect of incubation temperatures can potentially affect the subsequent physiology of hatchling and post-hatchlings. The between-clutch variation in growth rates suggest that clutch-specific (e.g. maternal, genetic, or egg-specific) factors can have long-term effects on growth.

Collectively, the results of the current study provide insight on the maternal investment, egg size, hatchling size, and growth rates in diamondback terrapin. Coleman, *et al*, (2011) noted that numerous studies had been conducted in order to understand female allocation strategies in turtles and they all came up with varying results. The result from these studies together with those of the current study suggest that the diamondback terrapin could be utilizing developmental plasticity, or possibly multiple strategies that may dependent upon the environment resources. To optimally address this issue, it might be possible to record the seasonal reproductive output of individual females from Cedar Point Marsh relative to the environmental conditions/resources for each year. That could lead to a better understanding of how a specific female allocates her resources to offspring over multiple years with varying environmental conditions.

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CHAPTER 2

EVALUATION OF CARAPACE MORPHOLOGY AS A POTENTIAL SEXING TECHNIQUE FOR DIAMONDBACK TERRAPIN (*MALACLEMYS TERRAPIN PILEATA*)

by

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ABSTRACT

Several previous studies have suggested that shell morphology of some turtle species may vary relative to factors such as incubation temperature and/or sex. The current study utilized previously reported methodology to evaluate if shell shape could be used as an indicator of the sex of hatchling and post-hatchlings diamondback terrapin (*Malaclemys terrapin pileata*). A total of 19 clutches of eggs were obtained during the 2012 nesting season from females nesting at Cedar Point Marsh, AL. The eggs from each clutch were divided into two groups, one incubated at male-producing temperature (26°C) and the other at female-producing temperatures (31°C) (69 females and 65 males). The carapace of each terrapin was photographed a total of 11 times at approximate 6 week intervals ranging from August 2012 to January 2014. To evaluate carapace shape, software was used to plot the coordinates of the 34 digital landmarks at specific junctions of scutes on each carapace. The coordinates of the digitized landmarks were compared between males versus females using MANOVA. No significant differences between the carapace shape of males versus females were detected at any of the 11 time periods analyzed (P>0.05). The results suggest that alternative methods for identifying the sex of hatchling and post-hatchling terrapin should be evaluated.

Keywords: diamondback terrapin–sex determination–carapace morphology–sexing technique

INRODUCTION

A variety of turtles possess temperature-dependent sex determination (TSD) in which incubation temperature determines sex (Charnier, 1966; Yntema and Mrosovsky, 1980; Pieau, 1996; Wibbels, 2003). TSD can produce a variety of sex ratios and therefore has implications for ecology, evolution, and conservation of turtles (Wibbels, 2003). For example, naturally occurring sex ratios resulting from TSD do not always conform to a 1:1 male to female sex ratio that is often predicted by evolutionary theory (Mrosovsky, 1994; Wibbels, 2003). Additionally, sex ratios affect the reproductive output of a population and can therefore affect the recovery of an endangered species (Coyne, 2000; Wibbels, 2003; 2007).

Although turtle sex ratios resulting from TSD are of interest, there are often logistical difficulties associated with assessing population sex ratios (Wibbels, 2003). The sex of adult turtles is often indicated by secondary sexual characteristics. For example, in the case of the diamondback terrapin *(Malaclemys terrapin*), males develop a longer and muscular tail, whereas females are significantly larger than males (Ernst et al., 1994). However, sampling bias can be a problem in assessing adult sex ratios in turtles due to sex-specific migration patterns (Wibbels, 2003). Studying the sex ratios of the hatchling and immature portions of a turtle population can also be logistically difficult. In the case of hatchlings, sampling bias can again be a problem because sex ratios can vary greatly depending upon the time of the nesting season, nest location, and local weather patterns (Wibbels, 2003). The immature portion of a population represents a condensation of many years of hatchling production, and is not confounded by sex-specific migratory patterns. However, in the case of both hatchings and immature turtles, a common problem encountered in sex ratio studies is the inability to determine the sex of individuals based on external morphology. The secondary sexual characteristics of adults typically develop at puberty, so they are not useful in determining the sex of hatchlings and immature turtles (Wibbels et al., 2000). Traditionally, the definitive method of sexing most hatching and immature turtles is through direct or histological evaluation of the gonads, which requires euthanizing and dissecting of turtles. A variety of other non-lethal methodologies have been evaluated in turtles, and laparoscopy and blood testosterone levels appear to be reliable methods for sexing immature turtles (Wibbels et al., 2000). However, they are both invasive, logistically difficult, and require special equipment and training.

As a potential alternative method, several studies have suggested that shell morphology of some turtle species may vary relative to factors such as incubation temperature and/or sex (Ferreira-Júnior, Treichel, Scaramussa, & Scalfoni, 2011; Michel-Morfin, Muñoz, & Rodríguez, 2001; Myers, Janzen, Adams, & Tucker, 2007; Valenzuela, Adams, Bowden, & Gauger, 2004). The current study applies this methodology to hatching and post-hatchling diamondback terrapins to evaluate if shell (carapace) shape can be used as an indicator of the sex of individual turtles.

METHODS

Adult female terrapins were captured in bucket traps while they were attempting to nest on the nesting beach at Cedar Point Marsh, AL, during the spring and summer of 2012. The females were taken into captivity and induced to lay eggs with oxytocin injections (Ewert and Legler, 1978). All eggs were weighed and measured (length and width), and each clutch was divided equally into two groups, with one group being incubated at a female-producing temperature (31°C), and the other group incubated at a male-producing temperature (26°C). Upon hatching, each terrapin was weighed and measured (straight carapace length, straight carapace width, plastron length). Each hatchling was placed on a grid background with $1cm^2$ reference squares and reference rulers on both X and Y axes (Figure 1). Photographs were taken of their carapace utilizing a 12.1 megapixel Sony cybershot camera. The same methodology was used to photograph each terrapin's carapace at six week intervals for approximately 1.5 years after hatching.

Figure1. Photographs of each terrapin's carapace were taken with 12.1 megapixel camera.

All carapace images captured were uploaded into tpsUtil software (F. James Rohlf, Stony Brook University, 1997) to create an analysis file capable of being digitized. TpsDig2 software was used to plot the coordinates of the 34 digital landmarks at specific junctions of scutes on each carapace (Figure 2). The coordinates of the digitized landmarks were uploaded to a statistical format using tpsRelw, and then converted to numerical nomenclature using MatLab. Statistical analysis was completed using MANOVA (R statistical software) for comparing the shape of the carapace relative to sex at each of eleven time periods over their first year of growth.

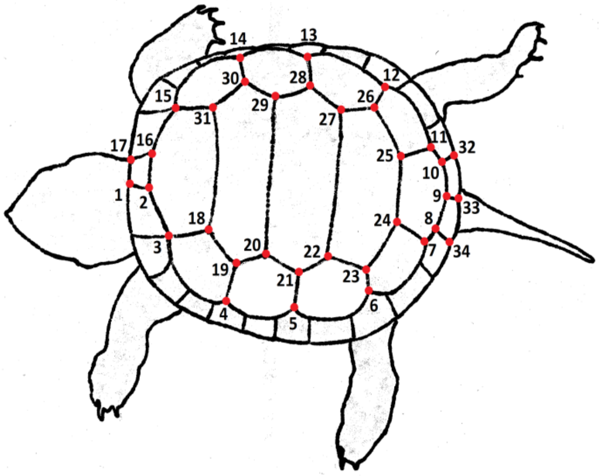

Figure 2. A total of 34 landmarks were digitally plotted at specific suture junctions on each carapace photo.

RESULTS

A total of 19 clutches of eggs were obtained during the 2012 nesting season. These clutched produced a total of 134 hatchings (69 females and 65 females). The carapace of each terrapin was recorded a total of 11 times at approximate 6 week intervals ranging from August 2012 to January 2014. The MANOVA analysis indicated that there were no significant differences between the shell shape of males versus females at any of the 11 time periods analyzed (P>0.05).

DISCUSSON

Several studies have suggested that shell morphology of some turtle species may vary relative to factors such as incubation temperature and/or sex. In an initial study of olive ridley sea turtles, *Lepidochelys olivacea*, 30 morphometric variables were recorded and analyzed in a search for sex-specific characteristics (Michel-Morfin *et al*., 2001). That study used hatchlings produced in laboratory incubators with constant temperature settings, at either a male-producing or a female-producing temperature. Based on nine of the characteristics they were able to classify the turtles by sex with 95% confidence. That study did not evaluate if the technique was applicable to natural nests or to incubation temperatures producing a mixture of males and females. A second study evaluated two species of turtles (*Chrysemys picta* and *Podonemis expansa*) using a landmarked based system based on the location of junctions between carapace scutes, and reported an 85% confidence in assigning sex (Valenzuela et al., 2004). That study utilized animals from previous studies, some from natural nests and others hatched in laboratory incubators. A study of shell shape in loggerhead sea turtles (*Caretta caretta*) found that the duration of incubation correlated with the carapace shape of the hatchings (Ferreira-Júnior et al. 2011). The results indicated that shorter incubation periods (55 days) resulted in hatchlings that were rounder than hatchlings with longer incubation durations (67 days). The sex of the hatchlings was not verified in that study, but previous studies suggest that mostly females would have been produced in the shorter duration incubation and mostly males would have been produced by the longer incubation duration (Ferreira-Júnior et al. 2011).

While these studies indicate that the shell morphology can vary in some species due to differences in incubation temperatures and/or sex, there have been a variety of other studies of turtle species that have used similar techniques and have not been able to detect significant variation between the shell shapes of males versus female hatchings. An earlier study of *Podnemis expansa* examined a variety of morphological characteristics, including carapace and plastron dimensions, but was not able to detect any significant morphological variation between the sexes (Hildebrand et al., 1997). Additionally, evaluations of the desert tortoise (*Gopherus agassizii*), and the gopher tortoise (*Gopherus polyphemus*) were also unsuccessful in determining morphological differences that could be used to accurately sex hatchlings (Burke *et al*., 1994; Boone *et al*., 1998).

The results of the current study indicate that the analysis of carapace morphology does not appear to be a good predictor of the sex of hatchling and post-hatchling diamondback terrapin (up to 1 year of age). It is noteworthy that constant incubation temperatures were used in the current study, and that only two incubation temperatures (26°C and 31°C) were utilized. It is plausible that the carapace of hatchlings from other incubation temperatures or from natural fluctuating temperature regimes could produce shell shape variations, but such differences were not evident in the current study. Interestingly, the size of males and females hatchling terrapins varies, with males typically being larger than females (Chapter 1 of this thesis). However, the results suggest, that although a size difference occurs, the relative shapes of the shells are similar.

The ability to sex hatchling or immature diamondback terrapin would provide a very useful tool for ecological and evolutionary studies of this species. The results of the current study suggest that alternative methods should be evaluated for sexing hatchling and immature diamondback terrapins. Laparoscopy has been used successfully on a variety of turtle species (Limpus and Reed, 1985; Wibbels et al., 1987; 1990; Rostal et al., 1990, 1994; Wyneken et al. 2007). Although this method requires specific equipment and training, it can be used to directly verify the sex based on the morphology of the gonads and reproductive ducts. However, it is labor intensive and is more practical for laboratory studies in contrast to field studies. Another alternative method would be the evaluation of blood testosterone levels in immature turtles. This method has been used to accurately predict the sex of immature turtles (Owens et al, 1978; Wibbels et al., 1987; 2000; Lance et al., 1992; Valenzuela, 2001). The blood testosterone method uses a radioimmunoassay or ELISA to determine blood testosterone levels of females and male immature turtles. The method would need to be validated for diamondback terrapin using laparoscopy and or necropsy (Wibbels et al., 2000). Once validated, this method is applicable to field studies since it only requires blood sampling of terrapin in the field, and then subsequent analysis of the blood in the laboratory. Although this method has been shown to be accurate for immature turtles in a variety of studies, its usefulness for sexing hatchling turtles has only been addressed in a single study (Gross et al., 1995), and therefore would require rigorous evaluation for hatchling terrapin. Thus, laparoscopy and blood testosterone levels appear to be the most promising non-lethal approaches that are currently available for accurately determining the sex of diamondback terrapin.

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CHAPTER 3

EVALUATION OF HEAD-STARTED DIAMONDBACK TERRAPIN, *MALACLEMYS TERRAPIN PILEATA*, FOR FITNESS AND ABILITY TO ADAPT TO A NATURAL PREY ITEM

by

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In preparation for Journal of Herpetology
Format adapted for thesis

ABSTRACT

Head-starting of turtles represents an experimental conservation strategy that is dependent upon the ability to successfully rear healthy turtles in captivity, and then subsequently have those turtles adapt to the wild after head-starting. The current study includes two experiments that address fitness of head-start terrapins (*Malaclemys terrapin pileata*) (1) the evaluation of locomotor ability through a "righting response" assay, and (2) their ability to adapt to a live food source following a relatively prolonged period of captive rearing (1 – 2 years) in which they were fed a commercially-processed turtle food. In the first experiment, the fitness of terrapin was examined using a "righting response" test in which terrapins were inverted and then the time required to "right" themselves was recorded. Trials were conducted at hatching and then at six more time periods ranging up to 90 days of development. The righting response times did not differ between clutches or by sex at the first sampling period (i.e., hatchling stage), but did vary significantly by sex at the other time periods tested, with males righting themselves faster than females. A significant difference was also detected between the time periods at which terrapins were tested, with terrapins showing significantly longer times during the first trial (i.e. hatchlings) apparently due to great latency periods prior to movements. In contrast, older terrapin had relatively consistent and short righting times. In the second experiment, 1 – 2 year old terrapins which had been continually fed on commercially-available turtle food were monitored for five days after being provided with crickets as a potential live food source. Of the 68 terrapins tested, 81% adapted to eating crickets on the first feeding (day 1) and 97% were feeding on crickets by the second feeding (day 2) over a five-day experimental period. Two of the terrapins in this experiment did not eat

crickets. The results of these two experiments indicate relatively good locomotor abilities and food adaptability in head-start diamondback terrapin.

INTRODUCTION

The **“**head-starting” of turtles represents a conservation practice that has been the subject of a variety of previous studies (Klima and McVey, 1982; Huff, 1989; Haskell et al., 1996; Spinks et al., 2003; Bell et al., 2005; Mitrus, 2005; Shaver and Wibbels, 2007). However, head-start programs have often been controversial and are considered experimental, since data on the survival of head-start turtles are often unknown or have often not been well evaluated (Frazer, 1992; Bowen et al., 1994). However, there are data indicating that some head-start turtles survive and reproduce (Spinks et al., 2003; Shaver and Wibbels, 2007). Ultimately, the evaluation of head-start programs should include the comparison of stage-specific survival and mortality of both head-start turtles versus non-head-start turtles in the natural environment (Heppell *et al.*, 1996). However, this is often not practical or logistically possible, depending on the species of interest.

In the current study, diamondback terrapins (*Malaclemys terrapin pileata*) were head-started as part of a terrapin conservation program in Alabama in an effort to avoid high predation rates of eggs and early stage terrapins at Cedar Point Marsh, AL. A long-term goal of this program is to monitor survival to adulthood and evaluate the percentage of head-start terrapin that recruit into the adult reproductive population. However, the long-term evaluation is beyond the scope of the current study. In the short term, the current study includes two experiments which evaluate two measures of fitness of head-start terrapins.

It has been previously proposed that fitness can be assessed through the study of specific phenotypic traits (Arnold, 1983; Garland and Losos, 1994). In particular, it was noted that locomotor function was of major importance to fitness (Arnold, 1983). For example, in the case of reptiles, running or swimming speed has often been used as a measure of fitness in lizards and snakes (Burger,1989; Garland *et al*., 1990; Huey *et al*., 1990; Thibodeaux and Hancock, 2004). In turtles, righting response behavior has been shown to be a practical method of assessing fitness (Delmas *et al*., 2007). In this method, the turtle is inverted and then its ability to right itself is timed. This has been used in a variety of previous turtle studies (Du and Ji, 2003; Janzen, 1993; Rhen and Lang, 1999; Freedberg *et al*., 2001; 2004; Steyermark and Spotila, 2001; Paitz *et al*., 2010; Micheli-Campbell *et al*., 2011). In an initial experiment in the current study, righting response is evaluated as measure of fitness at various time periods ranging from hatchlings to 90 days of development.

In a second experiment, adaption to a live food source was evaluated. Due to logistical considerations, head-start programs often use species-appropriate commercially available foods to rear turtles in captivity. In the head-start program for terrapin in Alabama, the commercially-available floating turtle food Reptomin (Tetra, Blacksburg, VA) has been used as a standard feed for the diamondback terrapins. The purpose of the experiment was to evaluate if head-start terrapins could readily adapt to a live food source after a prolonged captive rearing (approximately 1 to 2 years).

METHODS

Adult female terrapins were captured in bucket traps while they were attempting to nest on the nesting beach at Cedar Point Marsh, AL, during the spring and summer of

2011 and 2012. The females were taken into captivity and induced to lay eggs with oxytocin injections (Ewert and Legler, 1978). Each clutch was divided into two groups, with one group being incubated at a female-producing temperature (31°C), and the other group incubated at a male-producing temperature (26°C). All turtles were captively-reared at UAB using methodology previously described by Coleman, 2011. During their captive-rearing, terrapins were fed daily with a commercially-available turtle food (Reptomin, Tetra, Blacksburg, Virginia).

Fitness Experiment

During 2012, 134 terrapins were analyzed for relative fitness by sex using standard righting response assay. Trials were performed between 0800 to 0930 A.M. Terrapins were tested as hatchling terrapin and then subsequently at 30, 40, 50, 60, 75, and 90 days of development. Trials were conducted in a 33 by 18 cm plastic box that was semi-transparent in a room with an ambient temperature of approximately 29 C. Terrapins were inverted in the bottom of the box, and the observer then moved to a distant part of the room approximately 3 to 4 m away from the box. “Righting” was defined as a 180 degree turn and the plastron became flush with the flat surface. The time required for each terrapin to right itself was recorded. If the terrapin did not move for 120 seconds, the trial was terminated, and the data from those terrapins were not used in the analysis.

Feeding Experiment

Approximately one week prior to release, the terrapins were monitored after their exposure to a live food source (crickets from a commercial cricket farm). Every morning approximately 3-4 large crickets were placed in each terrapin’s container, which was then closed to prevent the escape of the crickets. Terrapin were initially monitored for approximately 15 min to record initial feeding behavior. If any crickets had not been consumed in 15 min period, the terrapin containers were checked approximately 4 hours later to record the number of crickets that were still present, and they were removed. Since these terrapins were to be released after the experiment, they were also fed Reptomin in the afternoon to maximize their feeding before release.

RESULTS

Righting Response Experiment

The number of terrapins recorded at each sampling period ranged from 84 to 127 (due to terrapins available at time of each trial), with similar numbers of males and females (Figure 1). There was no significant difference detected in righting times between males versus females as hatchlings (t-test, $p>0.05$). However significant differences were detected between males and females at all other sampling periods (t-tests, $P<0.05$), with average righting response times being less in males than females (Figure 1). Additionally, there was no significant differences between hatchling righting response between clutches ($P>0.05$). The righting response time varied significantly over the multiple sampling periods, with hatchling righting responses being significantly longer than those in the subsequent sampling periods (ANOVA, $P<0.05$, Tukey Post hoc test ($P<0.05$ Figure 2).

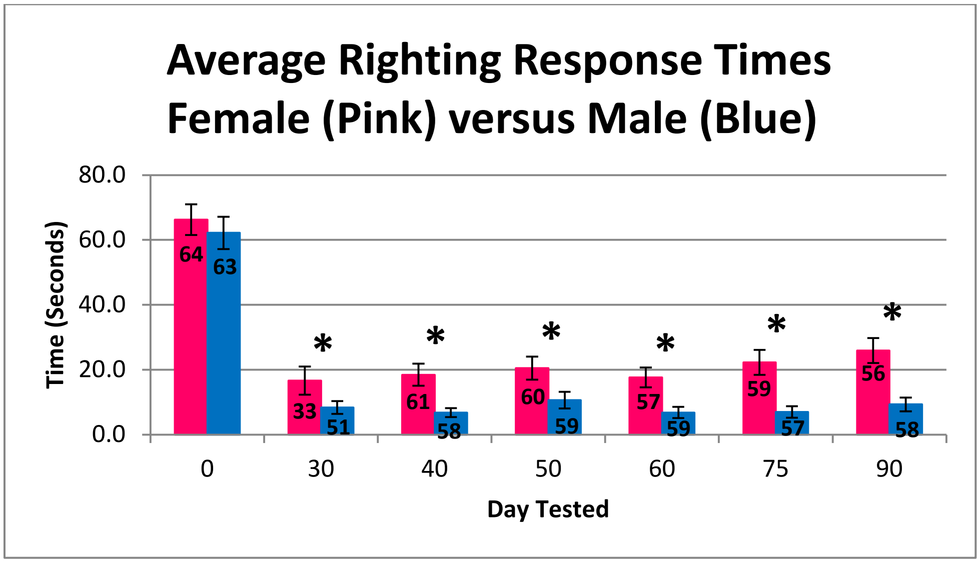

Figure 1. The righting response times of males versus females at the various sampling periods. Pink bars are females, blue bars are males. The number above each bar represents the number of terrapins tested. Asterisks denote significant variation between males versus females.

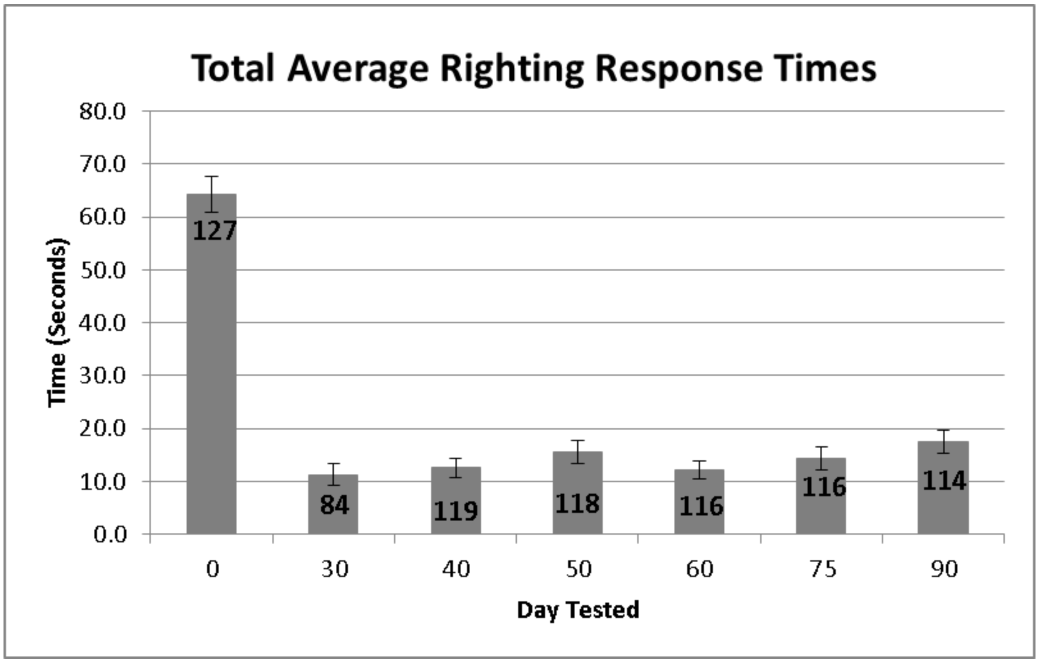

Figure 2. Total average response times for the various sampling periods. The righting response for time 0 (hatchlings) was significantly greater than the other time periods.

Feeding Experiment

Of the 68 terrapins tested, 56 (82%) ate cricket upon the first feeding (day 1 of study), and 66 (97%) were eating crickets by the second feeding (day 2 of study). Two terrapins (3%) did not eat crickets over the 5 days of the study. In the majority of cases, it was noted that terrapins would quickly pursue crickets after they were placed into their containers and consume them within the initial 15 minute time period.

DISCUSSION

The results of this study provide insight on several aspects of terrapin biology and the head-starting of terrapins. In regards to biology, the initial data on hatchling righting responses provides insight on potential maternal, egg-specific, and/or temperature effects on fitness. Several previous studies have suggested that the efficiency of righting responses may be heritable (Steyermark and Spotila, 2001; Ashmore and Janzen, 2003; Delmas *et al*., 2007), There was no detectable difference at time of hatching between sexes, and no detectable difference among clutches. However, righting times for hatchlings were significantly longer than those for the same terrapins at later points in their development. This was potentially due to “latent periods” during which the terrapins would simply not move after being placed on their carapace. In contrast, the older terrapins often reacted much more rapidly in their response (Figure 2). The latent period could have greatly added to the variability that was recorded in righting response trials at the time of hatching and could have contributed to the inability to detect any potential differences in righting response at that time period. Latent periods have been noted in other fitness studies of turtles (Ashmore and Janzen, 2003; Delmas *et al*, 2007), In order to obtain more resolute data for the time of hatching, it may be advantageous to change the experimental protocol to include a video camera for documenting the righting response of the hatchlings. In that way, the precise timing of the latency period could be scrutinized and accurately determined. The use of an observer in the current study prevented close evaluation of the latency period due to possible effects on terrapin behavior.

In contrast to the hatchlings, the older terrapins exhibited relatively quick righting responses. Further, those responses suggested consistent and significant differences between terrapins that were incubated at 26°C (male-producing) versus 31°C (female-producing) temperatures. These results are consistent with the hypothesis that incubation temperature and/or sex could significantly affect the fitness of turtles with temperature-dependent sex determination (Shine, 1999). In this case, the results suggest that males consistently righted themselves faster than females. A previous study of one month old red-eared sliders (*Trachemys scripta*) did not detect any significant difference in the “righting times” of males versus females, but that study subtracted latency time periods in their analysis. The significant results of the current study suggest that further studies are warranted. If this is not a spurious result, it could provide another example of how incubation temperature may affect phenotypic plasticity in reptiles (Shine, 1999; Ashmore and Janzen, 2003; Delmas *et al*, 2007).

As indicated above and in Figure 2, the righting times of the older terrapins were relatively fast. This suggests that these head-start turtles are relatively active and agile. Further, their righting times did not significantly vary over the six sampling periods after the hatchling stage. The relatively quick righting times and no notable decrease over the 6 trials suggest that the captive-rearing was not adversely impacting this ability over the study period.

The results of the feeding experiment indicate that most of the terrapins examined adapted to a natural food source (crickets) very rapidly after being fed commercial processed turtle food for 1 – 2 years. Although crickets are not noted as a primary prey item for diamondback terrapins (Ernst *et al*., 1994), they show the head-start terrapins’ ability to maintain plasticity in their prey identification. Of the 68 terrapins tested, 97% were feeding on the crickets by the second feeding period, and their ability to capture and eat a highly mobile prey item. The results of the feeding experiment suggest that the head-start terrapin represent an opportunistic and relatively agile feeder after 1 – 2 years in captivity.

The results of these two experiments in the current study are consistent with the hypothesis that head-start terrapin may be capable of adapting to the salt marsh after their release. However, long term tag-recapture studies will be the true verification of this hypothesis. Previous studies have shown the head-start turtles can survive and reproduce in the wild (Bell *et al.*, 2000; Spinks et al., 2003; Shaver and Wibbels, 2007), including diamondback terrapins (Smeenk , 2010). However, optimal evaluation of head-starting will require knowledge of survival rates in specific populations relative to survival rates of natural terrapins. However, these studies need to include all life history stages (Heppell *et al.*, 1996). For example, head-start turtles could survive at a different rate than natural turtle. Therefore, avoiding the high mortality of early life stages could compensate for such differences in regards to the impact on the population.

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FINAL DISCUSSION

The diamondback terrapin are an obligate, estuarine turtle that ranges along the Atlantic and Gulf of Mexico coastal marshes from New Jersey to Texas. It is considered a keystone species in its habitat and typically feeds on grazing snails and mudcrabs. Throughout its range populations have declined, especially in Alabama. As such a head-start program was devised as a way to mitigate predation on hatchlings and provide a path for population recovery. The head-start program at UAB therefore provides a unique opportunity to study the ecology, evolution, and conservation of a keystone species.

Maternal investment strategies were evaluated in eggs and hatchlings of diamondback terrapin and the results suggest that diamondback terrapin are capable of using a variety of resource partitioning strategies that may be dependent upon the environment of the terrapin. The results also suggest that larger terrapin produce the same number but larger eggs, which is not consistent with the optimal egg size theory. Growth of hatchlings and post-hatchlings also showed significant clutch-specific differences and male terrapin were significantly larger at hatching than female terrapin. Sex-specific growth differences occurred, however, the patterns were not consistent from year to year.

Several studies have suggested the shell shape morphology in some species of turtles may be an accurate method for sexing immature turtles. Diamondback terrapin hatchling and post-hatchling terrapin were used to assess this methodology. Carapace shape was used for morphometric analysis (tpsUtil, tpsDig, tpsRelw; F James Rohlfe, Stony Brook Morphometrics; 1997). The results indicated that carapace shape was not an accurate nor reliable method for sexing immature diamondback terrapin. Therefore, this methodology needs to be further assessed. Of note, this study operated at only 2 incubation temperatures. Other, multiple temperature regimes need to be conducted in order for a complete evaluation of shell shape as a sexing method of immature turtles

Fitness of the diamondback terrapins in the head-start program was evaluated using two measures. A first study evaluated hatchling and post-hatchling righting response times relative to temperature/sex and clutch. The results showed that there was no difference in hatchling (Day 0) righting time in either sex or clutch, however, at subsequence testing days post-hatchling terrapins significantly righted faster. Males also righted faster than females throughout all time periods. A second study evaluated live food adaptation in terrapin prior to their release. Diamondback terrapin aged 1-2 years were evaluated to observe their adaptation to a natural food source. Prior to releasing, terrapin were fed the normal floating stick food diet for reptiles, Reptomin. 1-2 weeks before releasing, terrapin were fed a live food item (crickets) during the morning, and their behavior was subsequently recorded up to 15 minutes after feeding. Within 2 day, 97% of terrapin adapted to the live food item. Out of 68 terrapin assessed, only 2 terrapin did not eat the live food item. All terrapin were fed the standard amount of Reptomin during the afternoons if they did not consume the live food item. These fitness evaluations provide feedback for the head-start program. These initial assessments show that the head-start program is capable of captive-rearing diamondback terrapin.

In conclusion, the diamondback terrapin head-start program is successfully hatching offspring and reincorporating them to the wild. Further studies need to be conducted to evaluate their success after releasing into the wild. However, during their captive-rearing, they continue to provide a unique opportunity to evaluate the ecology, evolution, and conservation of the diamondback terrapin in Alabama.

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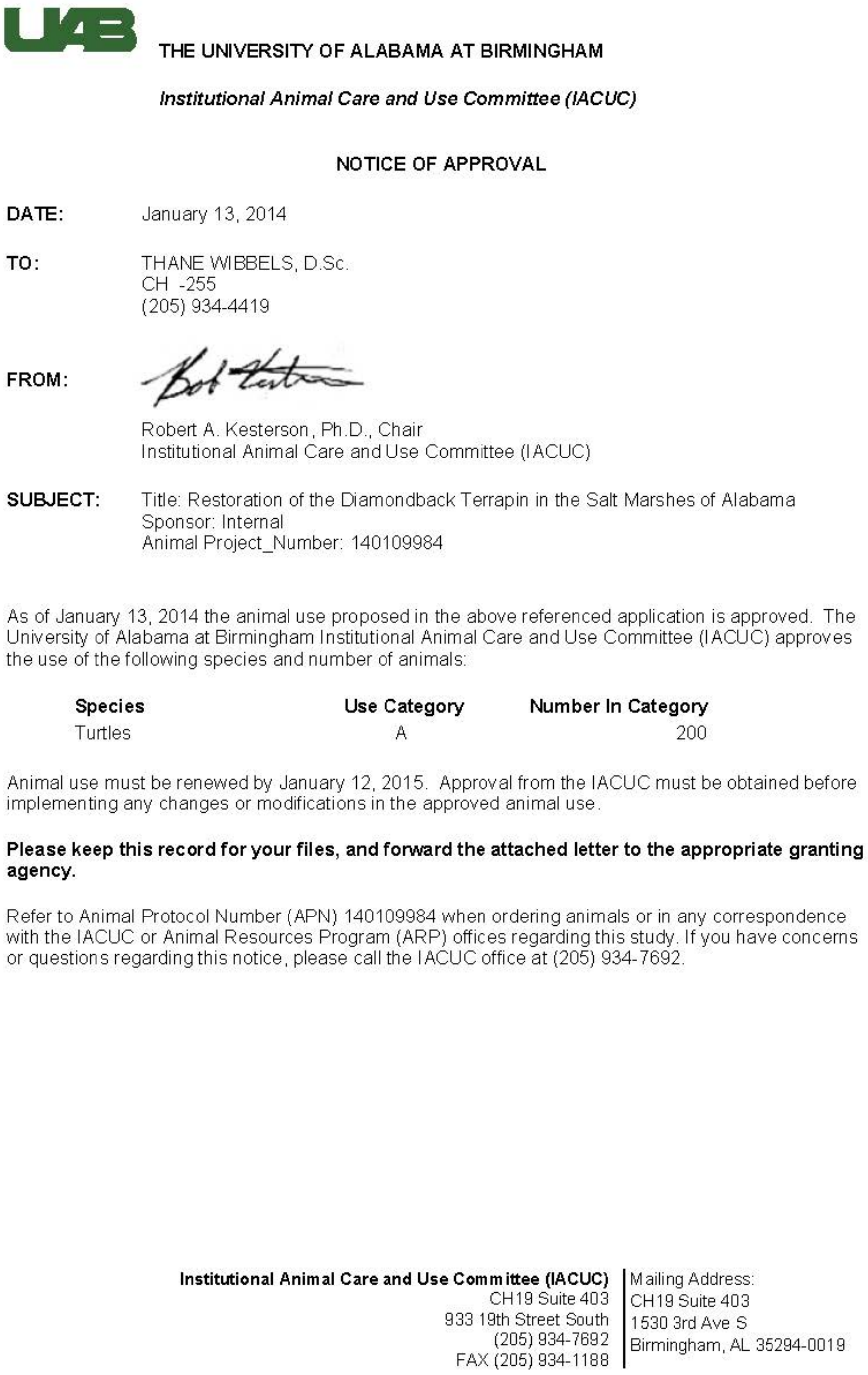
APPENDIX

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL



THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Animal Care and Use Committee (IACUC)

NOTICE OF APPROVAL

DATE: January 13, 2014

TO: THANE WIBBELS, D.Sc.
CH -255
(205) 934-4419

FROM:

Robert A. Kesterson, Ph.D., Chair
Institutional Animal Care and Use Committee (IACUC)

SUBJECT: Title: Restoration of the Diamondback Terrapin in the Salt Marshes of Alabama
Sponsor: Internal
Animal Project_Number: 140109984

As of January 13, 2014 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 number of animals:

Species	Use Category	Number In Category
Turtles	A	200

Animal use must be renewed by January 12, 2015. 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) 140109984 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.

Institutional Animal Care and Use Committee (IACUC)
CH19 Suite 403
933 19th Street South
(205) 934-7692
FAX (205) 934-1188

Mailing Address:
CH19 Suite 403
1530 3rd Ave S
Birmingham, AL 35294-0019

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Animal Care and Use Committee (IACUC)

MEMORANDUM

DATE: January 13, 2014

TO: THANE WIBBELS, D.Sc.
CH -255
(205) 934-4419

FROM:

Robert A. Kesterson, Ph.D., Chair
Institutional Animal Care and Use Committee (IACUC)

SUBJECT: **NOTICE OF APPROVAL - Please forward this notice to the appropriate granting agency.**

The following application was approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) on January 13, 2014.

Title:	Restoration of the Diamondback Terrapin in the Salt Marshes of Alabama
Sponsor:	Internal

This institution has an Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare (OLAW), is registered as a Research Facility with the USDA, and is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

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