Physiological Stress and Genetic Differences in Diamondback Terrapin in the Coastal Bend

Publication CBBEP – 119
Project Number – 1620
January 2018

Prepared by

Aaron S. Baxter, M.S., Principal Investigator

Center for Coastal Studies
Texas A&M University-Corpus Christi
Natural Resources Center
6300 Ocean Drive, Suite 3200
Corpus Christi, Texas 78412

Submitted to:
Coastal Bend Bays & Estuaries Program
615 N. Upper Broadway, Suite 1200
Corpus Christi, TX 78401-0749

Physiological Stress and Genetic Differences in Diamondback Terrapin in the Coastal Bend

Prepared for:

Coastal Bend Bays and Estuaries Program
615 N. Upper Broadway, Suite 1200
Corpus Christi, Texas 78401

Contract No. 1620

Aaron S. Baxter, M.S., Principal Investigator

Center for Coastal Studies
Texas A&M University-Corpus Christi
Natural Resources Center
6300 Ocean Drive, Suite 3200
Corpus Christi, Texas 78412

January 2018

Table of Contents

List of Figures

List of Tables

Acknowledgements

We would like to thank the Coastal Bend Bays and Estuaries Program (CBBEP) for funding this work. In particular, we express our gratitude to Rosario Martinez, Project Manager (CBBEP), for her assistance and guidance throughout the study. Assistance in the field was provided by Beth Almaraz and for that we are thankful. Lindsey Ramirez and Shantel Swierc were instrumental in all aspects of this project and the work could not have been accomplished without them.

Physiological Stress and Genetic Differences in Diamondback Terrapin in the Coastal Bend

Aaron S. Baxter, M.S., Principal Investigator

Executive Summary

This project aimed to quantify physiological stress in diamondback terrapin as a result of fluctuating, or hyper, salinity. Microsatellite DNA analyses were also performed to determine genetic relatedness between the three sampled populations. The project occurred from April 2015 – August 2016 and focused on three local terrapin populations: Nueces Estuary, Mission-Aransas Estuary (Goose Island), and Oso Bay. Stress hormones (Corticosterone, Aldosterone, and Prolactin) were used as markers to determine the effects of salinity on this estuarine species. Although, hypersalinity was not observed during this study, the results provide a physiological baseline for comparison when these conditions do occur in the future. Significant differences in stress hormone concentrations, in relation to salinity, were observed between sampling locations. Genetic analyses suggested that the three sampled populations were significantly different and that there is little, to no, gene flow between them. The results of this study provide the first data describing the effects of salinity in relation to physiological stress for free-ranging terrapins in Texas. The results of the DNA portion of the project will add to the very limited data currently available for terrapins and their genetics for Texas diamondback terrapins.

Introduction

Diamondback terrapin (*Malaclemys terrapin*) populations are declining throughout their range (Spivey 1998). They are the only turtle species that lives exclusively in brackish water (Wood 1977). Within an estuarine system, species diversity tends to decrease in the transition from polyhaline (16–30 PSU) to mesohaline (3–16 PSU), with few species found in oligohaline environments (0.5–3 PSU) (Britton and Morton 1989; Cotton 2014). Brackish waters require organisms, such as terrapin, to expend a great deal of energy maintaining homeostasis due to widely varying salinities. Sources of terrapin mortality include drowning in blue crab traps, nest predation, and vehicular mortality, as well as exposure to polychlorinated biphenyls (PCBs) and other pollutants/contaminants (Bishop 1983; Draud et al. 2004; Ford 2005; Haskett and Guillen 2008; Grosse et al. 2009). Sublethal effects from other types of physiological stress, such as salinity stress, may exacerbate mortality and morbidity associated with other environmental stressors.

Physiological Stress

Texas diamondback terrapins (*Malaclemys terrapin littoralis*) live in waters ranging from ~4 PSU to 22 PSU (Bishop 1983) and can tolerate salinities ranging from fresh (< 0.5 PSU) to marine (35 PSU) (Coker 1931; Dunson 1970). They generally live in the upper portions of estuaries (e.g., tidal creeks, embayments, salt marshes) (Brennessel 2006) where salinities tend to be relatively low (Britton and Morton 1989). In South Texas, areas in the upper estuary that are typically brackish may become hypersaline during times of drought. Thus, terrapins living in South Texas estuaries may be subject to a great deal of physiological stress from high and/or varying salinities.

For example, in the Nueces Estuary, terrapins have been documented at salinities ranging from 2.8 PSU up to 48.3 PSU. (Baxter et al. 2013). The goal of this project was to measure stress hormone parameters in wild Texas diamondback terrapins, living in three Texas bay systems to provide a baseline so that the physiological effects of stressors, such as fluctuating or hypersalinity, could be documented.

Effects of Stress

Stress can be defined as any factor that alters energy allocation or acquisition for reproduction or maintenance of an organism, as a threat to maintaining homeostasis, or any factor that can reduce fitness via fecundity, survivorship, or both (Grime 1989; Sibly and Calow 1989; Beyers et al. 1999; Barton 2002; Ford 2005). For the purpose of this research, stress will be defined as any nonspecific response of an organism's body to any factor or demand placed upon the organism (Selye 1976). Hans Selye (1976) was the first to identify the general adaptation syndrome (G.A.S.), now termed "stress syndrome." He concluded that the response to stress occurs in three phases: the alarm reaction, the stage of resistance, and the stage of exhaustion. In the alarm reaction phase, the organism's nervous system detects a stressor (Selye 1976; Ford 2005). In the stage of resistance, the organism attempts to reduce the effects of a stressor through behavioral or internal responses. If the organism is unable to reduce the stressor via behavioral responses (migration, burrowing into mud, etc.), the body will attempt to maintain homeostasis via the production of adaptive hormones (glucocorticoids or anti-inflammatory hormones). If the organism is unsuccessful and experiences prolonged exposure to a stressor they will enter the stage of exhaustion resulting in chronic stress (Selye 1976; Ford 2005).

In vertebrates, the presence of a stressor, such as hypersalinity, prompts the adrenal glands to secrete glucocorticoids and catecholamines. These hormone secretions provide a defense mechanism for animals in stressful situations (Mostl and Palme 2002). However, not all stress has negative impacts on an organism. In short-term stress situations, the production of glucocorticoids can improve the fitness of an organism via energy mobilization. But during prolonged periods of stress, or increased cortisol production, the fitness of an organism can decline due to immunosuppression, reduced reproduction rates, and reduced growth and metabolic rates (Mostl and Palme 2002; Ford 2005). The secretion of glucocorticoids combined with physical stress regulates cytochrome P450 (CYT P450) within the liver where its role is to metabolize and eliminate toxins from the body (Iber et al. 1997).

Salinity Stress in Terrapins

Organisms become dehydrated when exposed to hypersaline or full seawater conditions over extended periods of time (Davenport and Ward 1993). Davenport and Macedo (1990) showed that terrapins cannot live exclusively in full seawater (~35 PSU) because their orbital salt glands are not as powerful as those of sea turtles. Their laboratory experiments revealed that terrapins exposed only to seawater spent more time on the land area of their enclosures compared to those which had access to freshwater. Davenport and Magill (1996) also reported that terrapins spent more time on land when their access to freshwater was reduced. Adult terrapins can decrease water loss and keep sodium uptake at minimal levels in the initial stage of dehydration via physiological adaptations such as increasing interstitial fluid to high concentrations, accumulation of urea in the plasma, and increasing ammonia and amino acids (Gilles-Baillien 1973; Robinson and Dunson 1976; Dunson 1985).

Prolonged exposure to full seawater conditions (~35 PSU) in the laboratory also resulted in reduced food intake which affects growth and development (Dunson 1985; Davenport and Ward 1993; Holliday et al. 2009). Yet, even salinities that are slightly outside of the terrapin's tolerance range can be stressful. Holliday et al. (2009) found that when terrapins were exposed to salinities of 0 PSU, 20 PSU, and 30 PSU, they exhibited an initial decrease in growth compared to terrapins held at 10 PSU. Although growth rates returned to normal by day 120 and were comparable across treatments, terrapins held at stressor salinities were smaller than terrapins held at 10 PSU. Dunson and Mazzotti (1989) reported that terrapin growth stopped when salinities exceeded 21 PSU. Increased salinity also negatively affects liver growth (Ford 2005). Davenport and Ward (1993) reported that terrapins reduce the intake of seawater and prey to compensate for high salinities. Prey organisms captured in hypersaline environments contain higher concentrations of salts compared to tissue concentrations in terrapins (Davenport and Ward 1993). In addition, while consuming prey, terrapins also ingest water, which increases the effects

of dehydration. When exposed to seawater (~35 PSU) without access to freshwater, terrapin appetites gradually decrease (Davenport and Ward 1993).

The effects of increased salinity may be very different in wild terrapins because hypothetically, they can relocate to areas where salinities are more favorable. For example, a female terrapin in New York was recaptured 8 km from her initial capture site and at the Kiawah River in South Carolina, nesting female terrapins have been recaptured 1.4 km from their original capture sites (Spivey 1998). Studies by Halbrook (2003) and Baxter et al. (2013) indicated that the average distance between capture and recapture locations within the Nueces Bay, Texas was 0.4 km. Koza (2006) reported an average capture and recapture distance of 0.6 km, with the maximum distance of 3.2 km. These distances may often be far enough for terrapins to find salinities that are less stressful.

Genetics

During the 1800's and early 1900's, diamondback terrapins were commercially harvested as a food source, resulting in the near-extinction of the species. However, in the 1920's and 1930's several economic and political occurrences, including Prohibition, decreased the demand on terrapin harvest (Carr 1952; Brennessel 2006; Hart and Lee 2006; Schaffer et al. 2008; Texas Parks and Wildlife Department 2014). Fortunately, the decreased demand allowed terrapin populations to recover in many areas (Brennessel 2006; Ernst and Lovich 2009). Nevertheless, these increases and decreases in market demand during this period influenced several anthropogenic induced genetic changes amongst terrapin populations along the U.S. Gulf and eastern coasts. These genetic changes created challenges for researchers today who are trying to understand the relationships between the historical and current population genetics, range-wide. (Hauswaldt and Glenn 2005; Glenos 2013; Hart et al. 2014).

Furthermore, understanding this species population genetics is now deemed critical because of a rise in pressure from multiple threats that began affecting terrapins across their range during the 1980's. The investigation into all extant populations along with the historical record of the species is

highly significant for management and conservation efforts to preserve diversity. Terrapins are currently divided into seven subspecies, based mostly on morphological characteristics, although a genetic approach is been recently undertaken (Hart 2005). Many areas, including Texas, lack sufficient genetics data.

Today, declining terrapin populations result from extensive exportation to Asian markets and for the pet trade (Butler 2000; Cheung and Dudgeon 2006). Habitat fragmentation/alteration, nest predation, drowning in crab traps, vehicular mortality, and environmental stressors such as pollution and agriculture runoff are also contributing to these declines (Bishop 1983; Hogan 2003; Draud et al. 2004; Ford 2005; Hart and Lee 2006; Haskett and Guillen 2008; Grosse et al. 2009; Brown et al. 2011). Additionally, environmental and anthropogenic stressors can have significant impacts on vulnerable turtle species, of which the terrapin is one, having a state listing of S2 (Critically Imperiled) (Texas Parks and Wildlife Department 2017). The combination of these factors, and the resultant changes to populations, makes conducting research on local terrapin populations crucial. By filling these information gaps, resource managers will be able to more responsibly manage this species at the state, and federal, level.

Methods

Study Area

The study area consisted of three locations within the Texas Coastal Bend: Nueces Bay and Oso Bay, in the Nueces Estuary, and Aransas Bay in the Mission-Aransas Estuary (Fig. 1). These locations were selected based on previous terrapin research and recent hydrological regimes.

Mission-Aransas Estuary

The Mission-Aransas Estuary is located on the South Texas coast between San Antonio Bay and Corpus Christi Bay. The Mission-Aransas Estuary consists of three primary bays: Aransas Bay, Mesquite Bay, and Redfish Bay; three secondary bays: St. Charles Bay, Port Bay, and Copano Bay; and a single

tertiary bay: Mission Bay (Armstrong 1987; Britton and Morton 1989; Chen 2010; Evans et al. 2012; Moretzsohn et al. 2016).

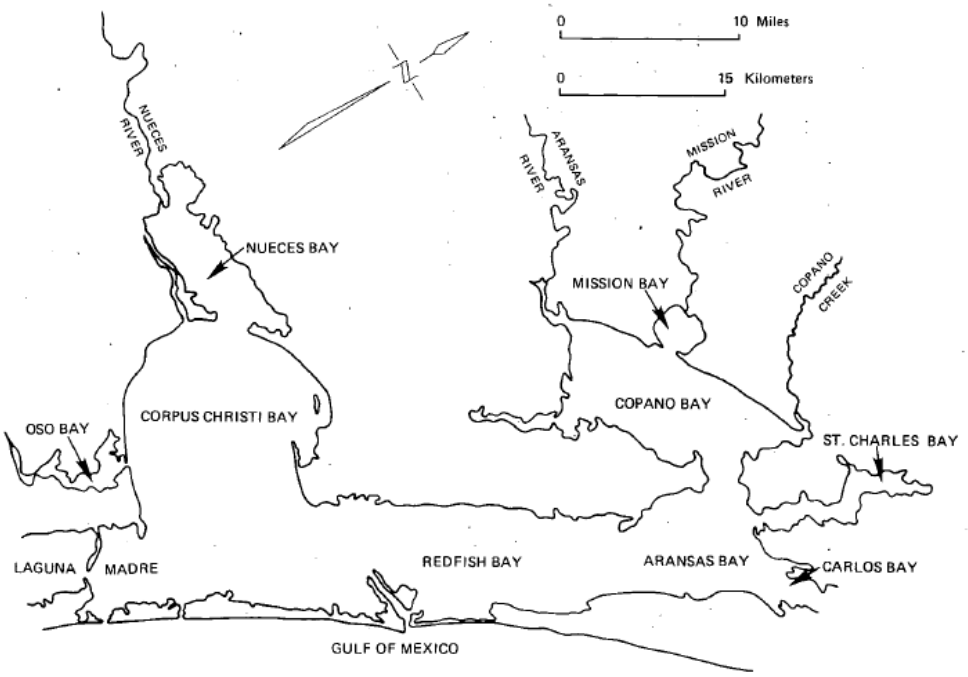

Figure 1. Map of the Mission-Aransas Estuary and Nueces Estuary study areas (Texas Department of Water Resources, 1981b).

Aransas Pass and Cedar Bayou directly connect the primary bays to the Gulf of Mexico on the north and south ends of San Jose Island, respectively (Chen 2010; Bittler 2011). The secondary bays, which drain into the primary bays, and the tertiary bay, connected to the secondary bay at head of the estuary, do not directly exchange water with the Gulf of Mexico (Texas Department of Water Resources 1981a). The Mission-Aransas Estuary receives freshwater inflow to Copano Bay from the Mission River, Aransas River, and Copano Creek (Fig. 2). No sources of freshwater inflow to the system are utilized to supply drinking water to municipalities and neither of the rivers are restricted by manmade diversions such as dams or other structures (Evans et al. 2012). Salinity typically ranges from 25–35 PSU in Redfish Bay, 20-30 PSU in Aransas Bay and Mesquite Bay, and 10–20 PSU in St. Charles Bay, Copano Bay, Port Bay, and Mission

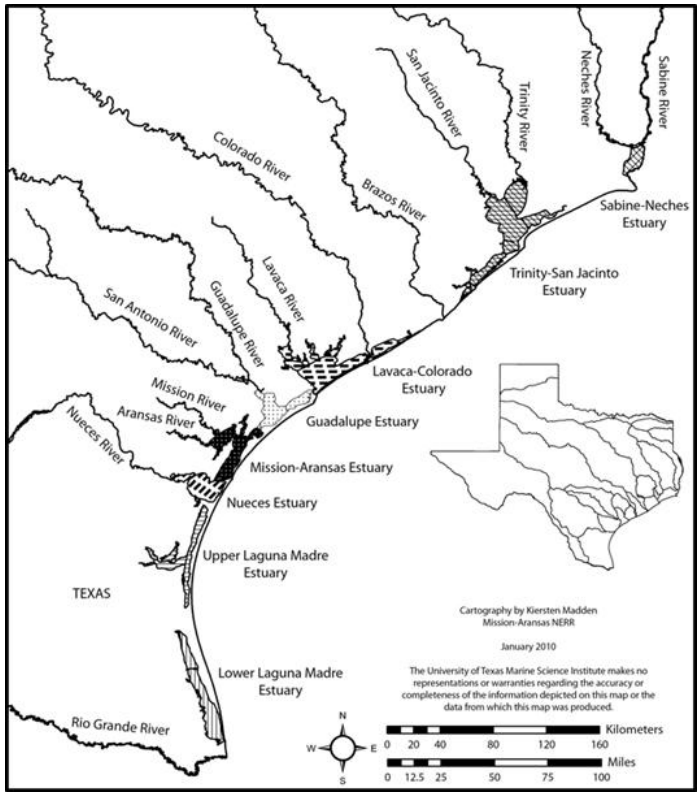

Figure 1. Map of the major rivers that provide freshwater inflow to the major estuaries on the Texas coast (Evans et al., 2012).

Bay (Chen 2010). For this study, sampling in the Mission-Aransas Estuary occurred in, and around, Goose Island State Park (GISP) located at the confluence of St. Charles Bay and Aransas Bay near Rockport, Texas (Fig. 3).

Nueces Estuary

The Nueces Estuary is hydrologically connected to the Mission-Aransas Estuary to the north by way of Redfish Bay. The Nueces Estuary consists of Corpus Christi Bay, a primary bay, and two secondary/tertiary bays, Oso Bay and Nueces Bay (Fig. 4). Freshwater inflow enters this system through the Nueces River and Oso Creek (Fig. 2). Impoundments, including the Calallen Diversion Dam, Wesley Seale Dam, and Choke

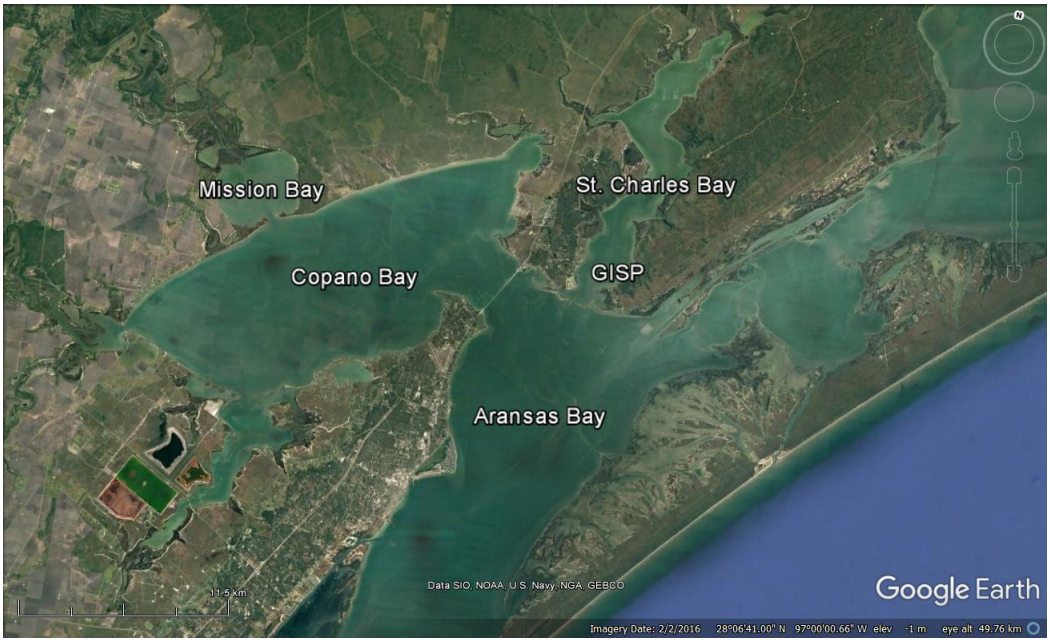

Figure 2. Map of the location of Goose Island State Park (GISP) study area within the Mission-Aransas Estuary, Texas (Google Earth 2017).

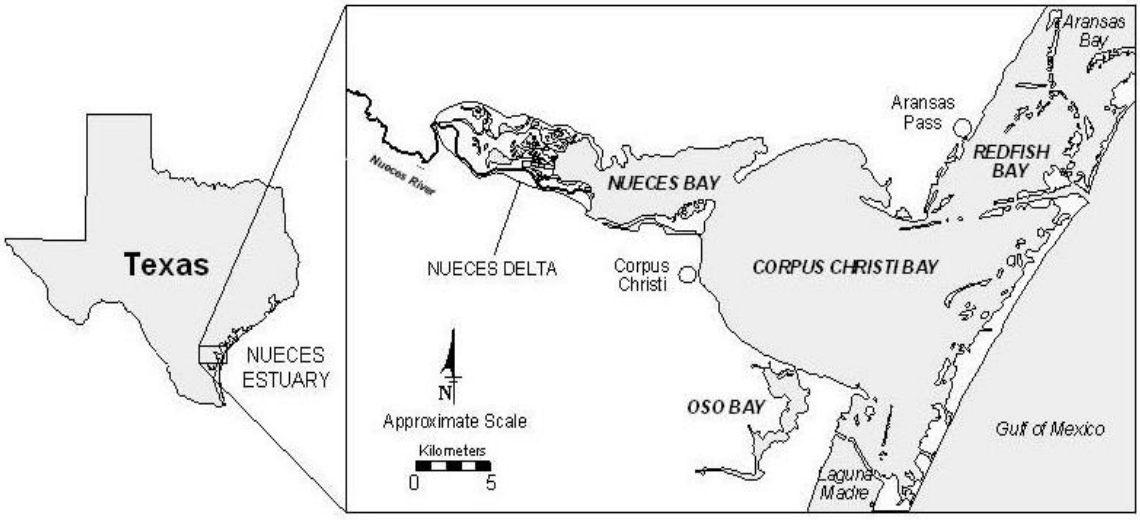

Figure 3. Map of the location and major components of the Nueces Estuary system (Bureau of Reclamation 2000).

Canyon Reservoir along the Nueces River provide drinking water for the City of Corpus Christi, Texas (Hill et al. 2011).

The Nueces River receives effluent discharge from the City of Corpus Christi Allison Wastewater Treatment Plant (Coastal Bend Regional Water Planning Group, 2001). Effluent-dominated Oso Creek receives treated discharge from the City of Robstown Wastewater Treatment Plant, Roloff Evangelistic Enterprises, Inc., Texas A&M Extension Service, City of Corpus Christi Greenwood Wastewater Treatment Plant, and Central Power & Light-Barney Davis Power Plant (Nicolau 2001). In addition to the discharge into Oso Creek, Oso Bay receives effluent from the City of Corpus Christi Oso Wastewater Treatment Plant located west of Ward Island near the mouth of the bay (Nicolau 2001). Often referred to as a reverse estuary, salinity in the Nueces Estuary is typically greater in the Nueces Delta to the west and often decreases throughout the estuary moving towards the Gulf of Mexico to the east. Salinities range from 5–50 PSU in Nueces Bay, 1–51 PSU in Oso Bay, and 15–30 PSU in Corpus Christi Bay (Orlando et al. 1993; Nicolau 2001; Baxter 2013). This study took place within the Nueces River, the western end of Nueces Bay, and the northernmost end of Oso Bay, also known as the Blind Oso.

Field Procedures

Trapping was conducted under scientific research permit (Permit No. SPR–0910–148) obtained from the Texas Parks and Wildlife Department. Diamondback terrapins were captured between April – November 2015 and May – August 2016 using a custom turtle trap (Fig. 5), hoop nets, cast nets, 2-ring crab traps, and commercial crab traps modified with chimneys to allow terrapins to surface for air (Fig. 6).

These methods have proved successful in capturing terrapins in previous studies (Forstner et al. 2000; Butler 2002; Baxter et al. 2013; Glenos 2013). Traps were set at various sites within each location to capture a minimum of 30 terrapins (total) per site. Traps were baited with dead finfish, dog food, or a

mixture of both and were deployed at depths ranging between 0.3 m and 0.9 m. During each sampling event, traps were deployed between 0800 h–1600 h and the number of traps deployed ranged from 10

Figure 4. Photograph of the custom turtle trap used to capture diamondback terrapin in the Mission-Aransas and Nueces Estuaries (photo courtesy of S. Swierc).

Figure 5. Photograph of a modified commercial crab trap used to capture diamondback terrapin in the Mission-Aransas and Nueces Estuaries (photo courtesy of S. Swierc).

to 20. Traps were retrieved within 24 hours of being set. General locality, latitude/longitude, habitat type, trap type, and trap depth (m) were recorded for each trap deployed. Hydrological data was collected at each location at deployment and retrieval with a YSI® 6920 V2 Multiparameter Sonde. Hydrological parameters included salinity (PSU) and water temperature (°C).

Upon arrival at each trapping location, the trap was quickly pulled into the boat and the initial time was recorded. Following the protocol developed by Cash et al. (1997), each terrapin was weighed and a blood sample was collected within the first 10 min of capture to control for the effects of capture and handling stress and to ensure basal hormone levels.

Blood samples were drawn from the subcarapacial sinus vein following the protocol developed by Hernandez-Divers et al. (2002), and used previously for terrapins by Glenos (2013) and Sheridan et al. (2010). All procedures were approved by the Texas A&M University-Corpus Christi (TAMUCC) Institutional Animal Care and Use Committee (IACUC Protocol No. 01–15). Training in chelonian blood collection techniques and PIT tag implantation was provided by Dr. Tim Tristan, veterinarian and Director of the Texas SeaLife Center in Corpus Christi, Texas and by Dr. Michael R. J. Forstner, Regent's Professor at Texas State University-San Marcos, Texas.

According to Beaupre et al. (2004), total blood volume is 4–8% of the overall body weight in chelonians, and the amount of blood that can be collected will depend upon the weight and health of the species. In healthy reptiles, up to 10% of total blood volume can be collected without negative consequences (Beaupre et al., 2004). In accordance with Beaupre et al. (2004), 2 mL of blood can safely be collected from diamondback terrapins that weigh at least 200 g. Following the methods approved by the IACUC, each individual was immediately weighed upon capture to ensure each terrapin weighed more than 200 g prior to blood being collected.

Upon cleaning the venipuncture site with iodine (Fig. 7), a 2 mL blood sample was drawn using a sterile, disposable 22-gauge 1½-inch long needle for females and a 22-gauge or 23-gauge ½-inch long needle for males, attached to a 3 mL syringe (Fig. 8). Following collection, antibiotic ointment was applied to the venipuncture site and each individual was monitored to ensure the venipuncture site was not bleeding prior to release. If bleeding did occur, liquid bandage was applied to the venipuncture site before the individual was released.

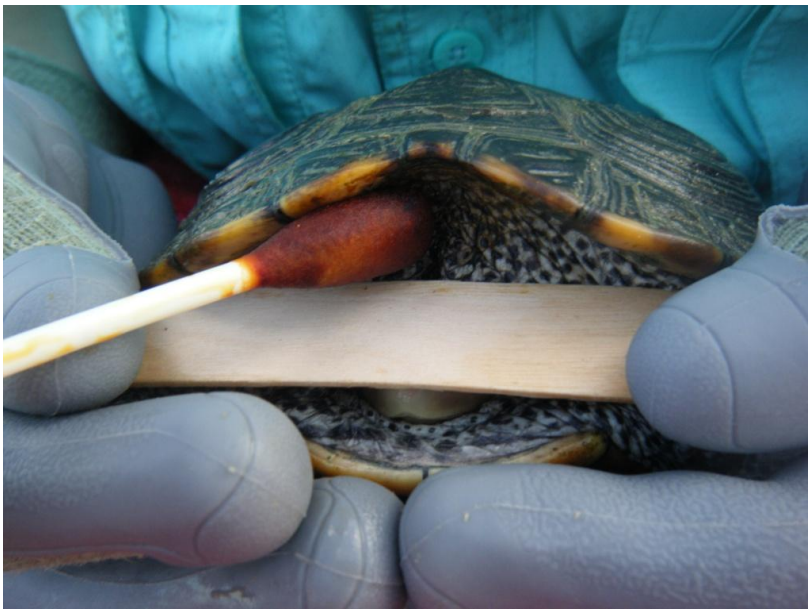

Figure 6. Photograph of a diamondback terrapin illustrating proper restraint techniques to clean the venipuncture site with iodine prior to blood collection (photo courtesy of A. Baxter).

Figure 7. Photograph of the venipuncture site used to collect blood samples from diamondback terrapin in the Mission-Aransas and Nueces Estuaries (photo courtesy of S. Swierc).

Initial blood glucose was measured by placing a drop of blood on a glucose test strip attached to a FreeStyle Precision Neo® blood glucose monitoring system. The remaining sample was immediately transferred to a 3 mL BD® lithium heparin vial, gently inverted several times to mix the blood and anticoagulant, and was stored on ice in the field.

Each terrapin was evaluated upon capture to record weight (g), activity levels, and body condition scores (BCS) (Table 1). Sex was determined for each individual by the distance of the cloacal opening from the posterior edge of the carapace (Lovich and Gibbons 1990). Females were palpated to determine gravidity. Standard measurements recorded to the nearest millimeter using tree calipers. Measurements included carapace width (CW, mm), carapace length (CL, mm), carapace height (CH, mm), plastron length (PL, mm), plastron width (PW, mm), and head width (HW, mm). An approximate age was determined by counting growth rings on the carapacial scutes. All individuals were photographed and physical characteristics, abnormalities, injuries, lesions, or wounds were recorded.

Upon return to the Center for Coastal Studies Laboratory at TAMUCC, blood samples were centrifuged at 2180 x g for 10 min. The plasma was removed from the whole blood pellet and

transferred to a separate microcentrifuge tube, and both the plasma and whole blood pellet were stored at -80 °C for subsequent analyses.

Stress Hormone Analyses

Plasma samples were analyzed to determine the stress hormone concentrations of corticosterone, aldosterone, and prolactin using spectrophotometry techniques and commercially available enzyme-linked immunosorbent assay (ELISA) kits (Cayman Chemical Company, Ann Arbor, MI, USA; Corticosterone Item No. 501320; Aldosterone Item No. 501090; Rat Prolactin Item No. A05101). A limited volume of plasma (approximately 0.5–1.5 mL) was recovered from the blood sample for each individual and was used to determine the concentration of three different stress hormones. Five samples out of every 40 were randomly selected to run in duplicate.

Table 1. Criteria used to score activity level and body condition score (BCS) of diamondback terrapins within the Mission-Aransas and Nueces Estuaries.

Parameter	Parameter Code	Description
Activity Level Upon Arrival		
No Activity	1	Not moving or DOA (dead on arrival).
Mild Activity	2	Little movement or weak/lethargic.
Normal Activity	3	Active, alert, vigorous body movements.
Excessive Activity	4	Excitable, extremely active body movements, or agitated.
Body Condition Score (BCS)		
Emaciated	1	Lethargic, sunken eyes, loss of shoulder and neck musculature and fat, muscle tone loose, skeletal elements prominent on skull and plastron, loss of soft tissue between bones of carapace and plastron is evident.
Thin	2	Loss of fat stores in shoulder, neck, and groin, plastron slightly sunken in.
Adequate	3	Normal muscle tone, fat stores present, plastron flat/appears normal.

Robust	4	Fat store present and notable in neck, shoulder, and groin, plastron may appear bowed.

Statistical Analyses: Stress Hormones

To determine differences in diamondback terrapin characteristics between Nueces, Oso, and Aransas Bays, canonical discrimination and discriminant analysis with re-substitution was performed using the CANDISC and DISCRIM Procedures in SAS version 9.4 software. These multivariate analyses were then repeated to determine if there were differences between males and females, and to determine if there were differences in each sex between Nueces, Oso, and Aransas Bays. An analysis of variance (ANOVA) was performed using the GLM Procedure in SAS version 9.4 software to compare mean concentrations of stress hormones, electrolytes, and blood chemistry by location. The Duncan's Multiple Range Test was applied if significant locational effects were shown.

Stress hormone data were exported to Microsoft Excel® 2016 (Microsoft Corporation) and linearized using logit transformation with the following equation: logit $(B/B_0) = \ln[B/B_0/(1-B/B_0)]$, where B/B_0 is equal to the sample or standard bound divided by the maximum bound. Standard concentrations were plotted against the logit (B/B_0) values to create the standard curve and data were fit to a logarithmic regression line. Stress hormone concentrations were then calculated using the constants from the log-regression line, logit value, and dilution factor for each sample.

The generalized Shapiro-Wilk test for multivariate normality (Villasenor-Alva and Gonzalez-Estrada, 2009) and q-q plots were used to evaluate the distribution of the data. A log10 + 1 transformation was applied to stress hormone concentrations to meet the assumption of normal distribution. Salinity (PSU) measurements for terrapins captured on the same day in the same trap were averaged to provide an accurate representation of that salinity. Pearson product-moment correlation coefficients were calculated to determine whether salinity (PSU) was significantly correlated with plasma stress hormone concentrations. Only mean data which had complete observations for all plasma

stress hormones (n = 21) were used to assess Pearson's correlation coefficient. Statistical analyses were performed using R version 3.2.5 software (R Core Team 2016). Data were considered statistically significant at $P < 0.05$.

Genetic Analyses

Genomic DNA from all captured individuals was extracted using the QIAGEN DNeasy® Blood and Tissue Kit (QIAGEN, Louisville, KY, USA; Item No. 69506) following the appropriate protocol for the given sample type. The QIAGEN DNeasy® Blood and Tissue Kit has been used in previous research for this species and provides high-quality purification for Polymerase Chain Reaction (PCR) (Glenos 2013; Drabeck et al. 2014; Dominy 2015; Petre et al. 2015).

The quantity of DNA within each sample vial was unknown and predicted to range widely due to field collection conditions and preservation method. Thus, an initial test to measure DNA content within a range of volumes was conducted using two samples from recaptured individuals. A run through of the protocol was performed on these samples using 5 µL, 10 µL, and 20 µL volumes of starting material each. The results showed that the starting material should be greater than 20 µL to produce an appropriate amount of DNA for PCR. Therefore, adjustments were made for the starting material to be 30 µL or greater. Additionally, the DNeasy® membranes from each samples spin column were kept for long-term preservation in case it is needed for future research opportunities involving the species.

To determine whether the extraction was successful and to visualize the quality of the genomic DNA present, samples were assessed by gel electrophoresis. Electrophoresis was run on a 1% agarose gel stained with 500X GelStar™ Nucleic Acid Gel Stain (Lonza, Rockland, ME, USA; Cat. No. 50535. Gel electrophoresis was conducted with a Mini-Sub® Cell GT Horizontal Electrophoresis System (Bio-Rad, Hercules, CA, USA; Cat. No. 170-4466) powered by a PowerPac™ Basic Power Supply (Bio-Rad, Hercules, CA, USA; Cat. No. 164-5050). This study utilized the same 12 polymorphic simple sequence repeat (SSR)

markers or microsatellite loci selected by Hart (2005) and Glenos (2013) in previous genetic work on terrapins.

Once successful PCR products were observed, amplified PCR products were prepared for capillary electrophoresis. Preparation of the samples was made to utilize the DNA analyzers ability to read multiple dye colors at the same time within a single capillary, also known a multiplex strategy. These PCR products were run on an ABI 3730xl DNA Analyzer (Applied Biosystems™, Foster City, CA, USA. Once the samples were prepared, they were then taken to the Genomics Core Lab at TAMUCC for fragment length analysis using the ABI 3730xl DNA Analyzer with the Liz 600 Size Standard.

Statistical Analyses: Genetics

Microsatellite DNA fragments were analyzed using the same markers as previous Gulf Coast terrapin studies allowing the results to be compared to previous studies and to determine similarities and differences among regions. canonical discriminant analysis (CANDISC [SAS]) was used to determine: 1) if there were significant differences in the genetic structure among bays, and 2) which genes contributed most to any significant differences between bay systems. Canonical discriminant analysis is analogous to multivariate analysis of variance and finds canonical variables which are linear combinations of quantitative variables, in this case genes, which summarize the differences between classification variables (i.e. bay system).

Results

Environmental Conditions

Salinities ranged from 0.4 PSU to 28.8 PSU, with terrapins captured throughout this range. Hypersalinity was not observed during this study. Mean salinities (± SE) for Aransas Bay, Nueces Bay, and Oso Bay were 16.0 ± 1.3 PSU, 10.4 ± 4.4 PSU, and 7.0 ± 2.7 PSU, respectively. Salinity was not

significantly different between study areas (F = 1.46; P = 0.26). Overall, mean air temperature was 27.8 ± 0.5 °C (range = 22.6–32.5 °C) and no significant differences were observed between study sites (F = 0.29; P = 0.75). Mean water temperature was 27.6 ± 0.6 °C (range = 22.4–32.7 °C) and water temperature was not significantly different between study areas (F = 1.21; P = 0.32).

Terrapin Captures

One hundred fifteen terrapins were captured between April 2015 – November 2015 and May 2016 – August 2016. Of the 115 captured terrapins, 110 were live captures The remaining five were killed by predators before researchers could retrieve them. Of the 110 live captures, 101 were unique individuals (44 M and 57 F), and nine were recaptures (Table 2). No males were recaptured during this study. Within Aransas Bay, one individual was recaptured three times, another individual was recaptured twice, and a third individual was recaptured once. Three individuals were each recaptured once in Nueces Bay and no individuals were recaptured in Oso Bay.

On average, female diamondback terrapins were larger than and weighed 3.5 times more than male terrapins (Table 3). The overall M:F sex ratio was 0.8:1 and the recapture sex ratio was 0:9. The sex ratios for Nueces Bay and Aransas Bay were female skewed (0.3:1, n = 42; and 0.4:1, n = 32), and male skewed in Oso Bay (8:1, n = 27).

Table 2. A comparison of live capture and recapture data for Nueces Bay, Oso Bay, and Aransas Bay between April 2015 – November 2015 and May 2016 – August 2016.

Location	Total Captured	Individuals Captured	Males Captured (Recaptured)	Females Captured (Recaptured)
Aransas Bay	38	32	10 (0)	28 (6)
Nueces Bay	45	42	10 (0)	35 (3)
Oso Bay	27	27	24 (0)	3 (0)
Total	110	101	44 (0)	66 (9)

Table 3. Mean (± SE) morphometrics for male and female Texas diamondback terrapins within the Mission-Aransas and Nueces Estuaries. WT = weight; CL = carapace length; CW = carapace width; CH = carapace height; PL = plastron length; PW = plastron width.

	WT (g)	CL (mm)	CW (mm)	CH (mm)	PL (mm)	PW (mm)
Aransas Bay						
M (*n* = 10)	427.5 ± 12.0	139.8 ± 1.7	101.0 ± 1.0	55.4 ± 0.8	116.7 ± 1.3	89.0 ± 0.8
F (*n* = 28)	1504.6 ± 49.0	200.0 ± 3.4	145.4 ± 1.4	88.4 ± 1.0	173.7 ± 3.9	129.1 ± 1.1
Nueces Bay						
M (*n* = 10)	352.0 ± 15.0	131.8 ± 3.0	99.3 ± 1.9	53.7 ± 1.1	113.0 ± 2.6	86.6 ± 2.1
F (*n* = 35)	1229.2 ± 50.1	193.3 ± 3.1	143.6 ± 2.3	82.7 ± 1.5	171.4 ± 2.8	128.6 ± 2.0
Oso Bay						
M (*n* = 24)	377.5 ± 8.0	136.1 ± 1.0	100.2 ± 0.8	52.5 ± 0.5	113.2 ± 1.0	89.4 ± 1.0
F (*n* = 3)	1410.0 ± 132.0	200.3 ± 5.2	148.0 ± 3.1	87.0 ± 4.2	177.0 ± 5.1	133.3 ± 4.3
Overall						
M (*n* = 44)	383.1 ± 7.2	136.0 ± 1.0	100.2 ± 0.7	53.5 ± 0.4	113.9 ± 0.9	88.7 ± 0.8
F (*n* = 66)	1354.3 ± 37.7	196.5 ± 2.2	144.6 ± 1.4	85.3 ± 1.0	172.6 ± 2.2	129.1 ± 1.2

Physiological

Canonical discrimination indicated there were significant differences by location (F = 8.03; P = 0.0023) and each location could be successfully separated with canonical axis 1 accounting for 93% of the variance and canonical axis 2 accounting for 7% of the variance (Fig. 9).

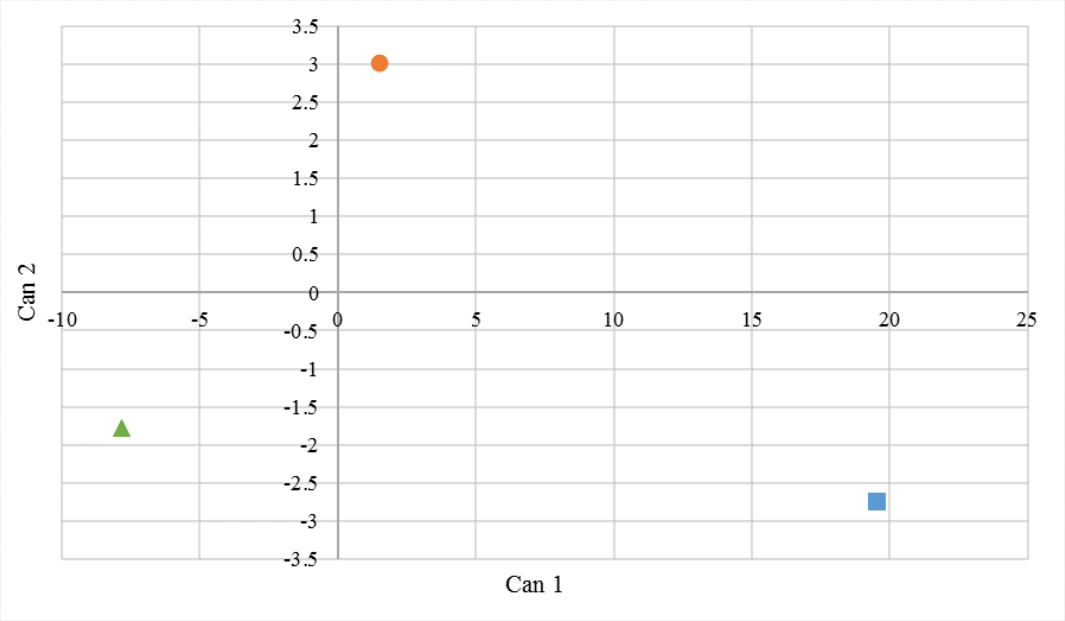

Figure 9. Mean canonical coefficients for terrapins captured in Oso Bay (■), Nueces Bay (▲), and Aransas Bay (●).

The mean canonical coefficients for each location were clearly separated, with CW (mm), CH (mm), PL (mm), and PW (mm) accounting for most of the separation. Discriminant analyses of location resulted in a classification accuracy of 100% with re-substitution for Aransas, Nueces, and Oso bays. Canonical discrimination indicated there were no significant differences between males and females (F = 1.08; P = 0.4513) or between females among locations (F = 1.08; P = 0.4809). The small sample size of male terrapins (n = 6) prevented the use of canonical discrimination and discriminant analyses between locations.

An analysis of variance was performed to compare mean plasma concentrations of stress hormones by location, and results indicated there were significant differences between locations (F = 8.97; P = < 0.0001). Further analyses using Duncan's Multiple Range Test indicated that Oso Bay corticosterone concentrations were significantly greater than Nueces Bay and Aransas Bay. Oso Bay aldosterone concentrations were significantly greater than Nueces Bay, and prolactin concentrations

were significantly less than Aransas Bay (Table 4). Means within a row with the same letter are not significantly different (Duncan, $P \leq 0.05$).

A Pearson's correlation was performed to determine the relationship between salinity and concentrations of stress hormones (Table 5). Salinity within Oso Bay was significantly positively correlated with plasma aldosterone concentrations ($r = 0.98$; $P = 0.02$).

Table 4. Mean concentrations of stress hormones for Texas diamondback terrapins within the Mission-Aransas and Nueces Estuaries. Means within a row with the same letter are not significantly different (Duncan, $P \leq 0.05$). CORT = corticosterone; ALD = aldosterone; PRL = prolactin

	Aransas Bay	Nueces Bay	Oso Bay
CORT (pg/mL)*	4.11**a**	4.44**a**	5.00**b**
ALDOST (pg/mL)*	2.91**ab**	2.72**a**	3.40**b**
PRL (ng/mL)*	1.46**a**	1.36**ab**	1.28**b**

*Means of transformed values.

Table 5. Pearson's correlation coefficients for relationships between salinity and measurements of stress hormones and blood biochemistry. CORT = corticosterone; ALD = aldosterone; PRL = prolactin

	CORT	ALD	PRL
Aransas Bay	-0.54	-0.7	0.32
Nueces Bay	0.35	0.01	0.09
Oso Bay	0.73	0.98*	0.85
Overall	-0.1	-0.19	0.29

* Significance (2-tailed; $P < 0.05$).

Genetics

There were significant differences in the genetic structure among the three bay systems (Wilk's Lambda, DF = 40, 152; F=3.13; P <0.0001; Figure 10). The two canonical axes depicted in the graph accounted for ~78% of the observed variation (Table 6).

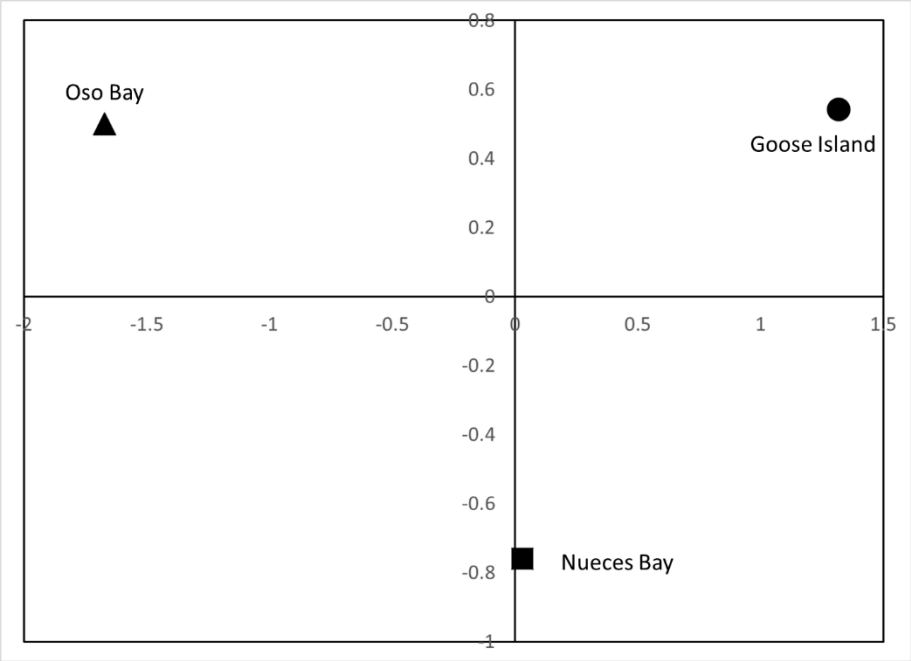

Figure 10. Canonical axes 1 and 2 showing the significant differences among the genetic structure of terrapins in Oso, Nueces, and Aransas (Goose Island) bays.

Table 6. Standardized canonical coefficients for variables contributing most to the differences in genetic structure among the bays. Canonical axis 1 accounted for ~76% of the variation.

Canonical Axis 1		Canonical Axis 2	
Variable	Coefficient	Variable	Coefficient
FAMA18r1	0.96	VICD62	-1.93
PETD55	-0.75	FAMD87	-1.0
nedd21r2	-0.64	VICD114	1.52

Discussion

Physiological

Although hypersaline conditions were expected based on historical trends, hypersalinity was not observed during this study. Studies investigating the physiological effects of elevated salinity in free-ranging diamondback terrapins are overdue and this study provides a baseline for comparison when hypersaline conditions arise again.

Stress Hormones

Comparisons in stress hormone concentrations from this study were compared to other chelonian species from previously published research. Mean (± SE) plasma concentrations of corticosterone (4.66 ± 1.01 ng/mL) were comparable to the range of initial corticosterone concentrations (~0–10 ng/mL) for blood collected within 10 min of initial disturbance reported for active free-ranging red-eared sliders (*Trachemys scripta elegans*) (Cash et al. 1997). However, mean plasma corticosterone concentrations were greater than those reported in a laboratory study for diamondback terrapins (0.08–1.35 ng/mL) exposed to varying salinity levels and PCB 126, a toxic dioxin-like polychlorinated organic chemical (Ford 2005). Similarly, plasma corticosterone concentrations were also greater than those reported for overwintering diamondback terrapins within an open-air salt marsh enclosure (0.76 ng/mL ± 0.58 SD) and overwintering free-ranging individuals (0.55 ng/mL ± 0.45 SD; Harden et al. 2015). Interestingly, this study's salinity (range = 0.23–28.8 PSU) was similar to the salinity treatments (0 PSU, 10 PSU, 20 PSU, and 30 PSU) by Ford (2005) and salinity fluctuations (range = 25–35 PSU; mean = 33.3 PSU ± 1.8 SD) reported by Harden et al. (2015).

Generally, during a stress response, an acute stressor will cause an initial increase in corticosterone concentrations followed by a subsequent increase until it plateaus. Once the stressor is removed, the concentrations will decline to baseline levels (Cash et al. 1997; Ford 2005). Differences in plasma corticosterone levels may be attributed to the environmental differences between the controlled laboratory study by Ford (2005), the overwintering study by Harden et al. (2015), and this study, where free-ranging terrapins were exposed to natural variations within the estuarine environment during their active season. In the controlled laboratory study by Ford (2005), terrapins were housed individually at a constant temperature with regulated salinities and light cycle. Terrapins were cycled through 30 d salinity treatments for a total of six months, with the initial 10 d of each cycle spent at an acclimation salinity. Corticosterone concentrations were measured at the end of the sixth salinity cycle (sixth month). The combination of stable laboratory conditions (temperature, light cycle,

food, etc.) and acclimation time likely resulted in habituation. Therefore, measurement of corticosterone concentrations at the end of the six-month period, following habituation, would allow corticosterone concentrations to return to baseline levels. Furthermore, the overwintering study by Harden et al. (2015) indicates that dormant terrapins experience reduced water exchange such as saltwater ingestion during feeding and fresh rainwater uptake, and evidence suggests they are hypophagic over winter. Taken together, these results suggest that corticosterone concentrations in active free-ranging terrapins would be greater than corticosterone concentrations in overwintering terrapins since active free-ranging individuals would experience multiple stressors including increased water exchange and natural reproductive stress (Valverde et al. 1999; Ford 2005).

Mean (± SE) plasma concentrations of aldosterone (0.13 ± 0.02 ng/mL) were greater than aldosterone concentrations in the female tortoise *Testudo hermanni* (0.08 ± 0.01 ng/mL; Uva et al., 1982). However, plasma concentrations of aldosterone reported in this study for diamondback terrapin are similar to concentrations in mammals, which typically range between 0.1 ng/mL and 1.0 ng/mL depending on the animal's sodium (Blair-West et al. 1968; Bradshaw and Grenot 1976). According to Bradshaw and Grenot (1976), considerable variation exists between species of reptiles in the extent to which aldosterone is involved in sodium balance regulation. In the terrestrial Mediterranean tortoise (*Testudo hermanni*), Uva et al. (1982) found that increased sodium intake resulted in depressed aldosterone concentrations and that the loss of sodium via diuresis is an influential stimuli for aldosterone secretion. Red-eared sliders (*Trachemys scripta elegans*), held in freshwater then exposed to various salinity treatments, exhibited a decrease in aldosterone with increasing salinity (Hong et al. 2014). In this study, although not statistically significant ($P > 0.05$), aldosterone was negatively correlated with Aransas Bay salinity and the overall study salinity, and is similar to the results found by Hong et al. (2014) and Uva et al. (1982). However, aldosterone concentrations in diamondback terrapins within Nueces Bay and Oso Bay increased with increasing salinity (Table 5).

Prolactin has a wide spectrum of functions in vertebrates, many related to osmoregulation (Mancera and McCormick 2007). However, very little is known about the osmoregulatory effects of prolactin in reptiles and even less is known about the effects of prolactin in free-ranging terrapins. Chan et al. (1970) reported a synergistic relationship between prolactin and corticosterone in restoring tissue and plasma compositions in hypophysectomized lizards. Chan et al. (1970) also suggested the possibility that the function of prolactin is regulated or enhanced by corticosterone and aldosterone. Brewer and Ensor (1980) studied the effects of prolactin, corticosterone, and aldosterone in freshwater chelonians and results indicated that osmoregulation in freshwater chelonians may be controlled by a combination of corticosteroids and prolactin, where prolactin induced diuresis with subsequent electrolyte loss is corrected by the corticosteroids. Although sodium retention was observed in dehydrated painted turtles (*Chrysemys picta*), Brewer and Ensor (1980) concluded that prolactin had a diuretic effect in *C. picta*, a freshwater chelonian, in contrast to its role in water retention in the Greek tortoise (*Testudo graeca*), a terrestrial chelonian. In the present study, prolactin concentrations, though not statistically significant ($P > 0.05$), were positively correlated with salinities in Aransas, Nueces, and Oso bays. However, the direct relationship between prolactin and corticosterone seen in hypophysectomized lizards was not apparent in diamondback terrapins in this study. Prolactin concentrations were expected to mirror the elevated corticosterone concentrations measured in active, free-ranging terrapins but this trend was not observed. Further investigation is required to determine whether prolactin affects sodium or water retention in diamondback terrapin.

This study was the first to quantify the effects of salinity on stress hormone production in free-ranging Texas diamondback terrapins, and is first to report on aldosterone and prolactin plasma concentrations in free-ranging diamondback terrapins in Texas. Here, I have shown that typical estuarine salinities (~0.5–35 PSU) do not induce a physiological response in stress hormone concentrations characteristic of severe or chronic stress. This indicates that these terrapins may be in the second phase

of Selye's (1976) "stress syndrome", known as the stage of resistance. Free-ranging terrapins in South Texas may be relying on behavioral adaptations more than physiological mechanisms to mitigate salinity stress. However, it is important to note that it is difficult to document and control for the unknown effects of stress in free-ranging terrapins, such as the actual amount of time each individual spent in the traps, the type of traps used, the length of time each individual was exposed to specific salinities, season, weather patterns, reproductive state and/or success, energy expenditure, availability of food sources, and the effects of predators.

To better understand the effects of salinity on free-ranging terrapins, future studies should evaluate the effects of capture and handling on stress and include a time series of blood sampling to build a stress profile to determine the diamondback terrapin's stress sensitivity. Additionally, satellite and/or acoustic telemetry should be utilized to determine the relationship between movement patterns and salinity. A telemetry study of sufficient duration would also allow for the evaluation of total body water changes between spring emergence and winter dormancy and would allow for additional blood biochemistry measurements, including urea concentrations, to determine osmotic balance in Texas diamondback terrapins. This study provides physiological data for free-ranging terrapins under typical estuarine salinities which can serve as a baseline for comparison when hypersaline conditions arise again.

Genetics

Genetic diversity is essential for the survival of a species. In general, genetic diversity is low within populations that have a low number of individuals or are separated from other populations resulting in low recruitment and interbreeding. Therefore, it is important to determine which populations of diamondback terrapin are isolated, low in number, and are losing genetic diversity so that proper management plans can be created for the species (Frankham et al. 2010). Additionally, populations that have been geographically separated overtime can develop unique alleles, which can

eventually lead to speciation. Populations may also lose alleles within their population due to lack of genetic diversity, called absent alleles. If any populations possess unique or absent alleles, it would be significant to the future management of the species (Glenos 2013; Drabeck et al. 2014).

Hauswaldt and Glenn (2003) showed that East coast terrapin populations were more genetically similar to Nueces Bay terrapins than to populations found in Florida. These similarities have been attributed to the farming and translocation of terrapins from Texas to the Carolinas during the mid-20th century. The artificial propagation of terrapins was short-lived and numerous terrapins were released into the wild, as the farms were closed down. This could have resulted in the genetic mixing of wild terrapins, resulting in the observed genetic similarity.

In 2004, King and Julian outlined 27 loci to be used in genetic analysis of the bog turtle. Twelve of the 27 loci developed by King and Julian (2004) were selected for microsatellite analysis in this study. Although these primers were developed for use in bog turtles, the results of cross-species amplification tests reflected high levels of polymorphism in terrapin. Other studies (Forstner et al. 2000; Hauswaldt & Glenn 2003) have developed species-specific markers for terrapin, but neither study resulted in polymorphic levels as high as those found by King and Julian (2004). For this reason, previous Gulf Coast studies (Hart 2005; Coleman 2011; Glenos 2013; Drabeck et al. 2014; Petre et al. 2015) used the primers developed by King and Julian (2004). For this study, the same 12 SSR markers were used so that results could be compared with other Gulf Coast studies.

Previous genetic analyses for diamondback terrapins has included samples from Nueces Bay, although the sample size in these studies were small (Hart 2005, n = 15; Glenos 2013, n = 8). Despite the small sample sizes for Nueces Bay, both Hart (2005) and Glenos (2013) reported similar results. However, both studies lacked robust sample sizes for Nueces Bay, therefore, the reliability of both estimates for the genetic diversity of Nueces terrapins are questionable and a future reassessment with a more robust dataset was suggested by Glenos (2013). The current study presents results from a more

robust sample size for this population, along with two others. Glenos (2013) reported that Nueces Bay terrapins, Galveston Bay terrapins, and LA/AL terrapin as three distinct genetic metapopulations. Nueces Bay, located at the far western end of the Texas terrapin (*M. t. littoralis*) subspecies range, is also near the southernmost boundary for the entire species' range. Therefore, this population would be expected to exhibit somewhat lower levels of genetic diversity relative to other Gulf Coast populations, as it is assumed to be subject to unilateral gene flow, limiting its genetic exchange to the nearest neighboring populations located to the north (Glenos 2013). This is the first genetics study for terrapins from the Goose Island and Oso Bay terrapin populations.

The results of this study showed genetics differentiation between all three local populations. This would be expected if gene flow were limited. There are behavioral and geographical barriers that could restrict gene flow between these three populations, resulting in significant genetic differences between populations. Diamondback terrapins have small home ranges and exhibit high site fidelity (Gibbons et al. 2001). Although individual movements in the Nueces Estuary have been documented at longer distances (>5 km) than other populations from outside of Texas (Baxter unpublished), these movements are still less than would be required to encounter other individuals from the Oso or Mission-Aransas populations. These three local populations are separated by large over-land distances (>25 km), and even greater distances by water. Terrapins rarely come ashore and haven't been shown to travel overland, except for forays to nesting beaches. Even if overland travel was possible from a behavioral standpoint, physical barriers, such as roadways, buildings, and farmland, would prevent mixing in these three distinct populations.

Genetic mixing could occur as a result of events such as tropical storms, hurricanes, and flooding. The transport of large volumes of water could result in the displacement of terrapins, allowing for gene flow to occur between these three local populations. Based on the genetic differences shown during this study, this gene flow between local populations has been minimal, at best.

How Gulf Coast terrapin populations are genetically related is pertinent information that must be obtained and assessed when considering management decisions for the species. For instance, if managers were interested in establishing terrapin conservation banks from which individuals could be translocated to enhance or attempt to restore a neighboring population in critical decline, the genetic relationship between those populations would need to first be determined. With populations declining range-wide, the issue of genetic similarity/difference will play a large role in preventing the extirpation of small, localized populations and the ultimate decline of the species as a whole.

Literature Cited

Armstrong, N.E. 1987. The ecology of open-bay bottoms of Texas: a community profile. U.S. Fish and Wildlife Service Biological Report 85(7.12). 104 pp.

Barton, B.A. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology 42:517-525.

Baxter, A.S., E.M. Hill, and K. Withers. 2013. Population assessment of Texas diamondback terrapin (*Malaclemys terrapin littoralis*) in the Nueces Estuary, Texas. Center for Coastal Studies, Texas A&M University-Corpus Christi, TX.

Beaupre, S.B., E.R. Jacobson, H.B. Lillywhite, and K. Zamudio. 2004. Guidelines for use of live amphibians and reptiles in field and laboratory research. A publication of the American Society of Ichthyologists and Herpetologists, approved by the Board of Governors.

Beyers, D.W., Rice, J.A., and W.H. Clements. 1999. Evaluating biological significance of chemical exposure to fish using bioenergetics-based stressor response model. Canadian Journal of Fisheries and Aquatic Science 56:823-829.

Bishop, J.M. 1983. Incidental capture of diamondback terrapin by crab pots. Estuaries 6:426-430.

Bittler, K. 2011. Salinity gradients in the Mission-Aransas National Estuarine Research Reserve. GIS and Water Resources. 19 pp.

Blair-West, J.R., J.P. Coghlan, D.A. Denton , J.F. Nelson, E. Orchard, B.A. Scoggins, R.D. Wright, K. Myers, and C.L. Junqueira. 1968. Physiological, morphological and behavioural adaptation to a sodium-deficient environment by wild native Australian and introduced species of animals. Nature (Lond.) 217:922-928.

Bradshaw, S.D., and C.J. Grenot. 1976. Plasma aldosterone in two reptilian species, *Uromastix acanthinurus* and *Tiliqua rugosa*, and the effect of several experimental treatments. Journal of Comparative Physiology B 111:71-76.

Brennessel, B. 2006. Diamonds in the Marsh. University Press of New England, Lebanon, NH. 219 pp.

Brewer, K.J., and D.M Ensor. 1980. Hormonal control of osmoregulation in the chelonian: The effects of prolactin and interrenal steroids in freshwater chelonians. General and Comparative Endocrinology 42:304-309.

Britton, J.C., and B. Morton. 1989. Shore Ecology of the Gulf of Mexico. University of Texas Press, Austin, TX. 387 pp.

Brown, D.J., V.R. Farallo, J.R. Dixon, J.T. Baccus, T.R. Simpson, and M.R.J. Forstner. 2011. Freshwater turtle conservation in Texas: harvest effects and efficacy of the current management regime. Journal of Wildlife Management 75(3):486-494

Butler, J.A. 2000. Status and distribution of the Carolina diamondback terrapin, *Malaclemys terrapin centrata*, in Duval County. Final Report Florida Fish and Wildlife Conservation Commission Project NG94-103. 52 pp.

Butler, J.A. 2002. Population ecology, home range, and seasonal movements of the Carolina diamondback terrapin, *Malaclemys terrapin centrata*, in Northeastern Florida. Prepared for Florida Fish and Wildlife Conservation Commission. Project NG96-007. Tallahassee, FL, USA.

Carr, A. 1952. Handbook of turtles: the turtles of the United States, Canada, and Baja California. Cornell University Press, Ithaca, NY. 169 pp.

Cash, W.B., R.L. Holberton, and S.S. Knight. 1997. Corticosterone secretion in response to capture and handling in free-living red-eared slider turtles. General and Comparative Endocrinology 108:427-433.

Chan, D.K.O., I.P. Callard, and I.C. Jones. 1970. Observations on the water and electrolyte composition of the iguanid lizard *Dipsosaurus dorsalis dorsalis* (Baird and Girard) with special reference to the control by the pituitary gland and the adrenal cortex. General Comparative Endocrinology 15:374-387.

Chen, G.F. 2010. Freshwater inflow recommendations for the Mission-Aransas Estuarine System with appendix by Texas Water Development Board. Texas Parks and Wildlife Department, Coastal Fisheries Division, Austin, TX. 120 pp.

Cheung, S.M., and D. Dudgeon. 2006. Quantifying the Asian turtle crisis: market surveys in southern China, 2000-2003. Aquatic Conservation 16:751-770.

Coastal Bend Regional Water Planning Group (CBRWPG). 2001. 5A.4 Reclaimed wastewater supplies. Pp. 5A.4-1–5A.4-35 in Coastal Bend Regional Water Plan Volume II: Water management strategies. Prepared for Texas Water Development Board. Texas, USA. http://www.twdb.texas.gov/waterplanning/rwp/plans/2001/N/Region_N_2001_RWP_Vol2.pdf.

Coker, R.E. 1931. The diamondback terrapin in North Carolina. Pp. 219-230 in H.F. Taylor (Ed.), Survey of Marine Fisheries of North Carolina. University of North Carolina Press, Chapel Hill, NC.

Coleman, A.T. 2011. Biology and conservation of the diamondback terrapin, *Malaclemys terrapin pileata*, in Alabama. Ph.D. Dissertation, University of Alabama, AL.

Cotton, D. 2014. Estuaries. Natural History of Sligo and Leitrim. http://staffweb.itsligo.ie/staff/dcotton/Estuaries_introduction_to_ecology.html. Accessed: 03 May 2015. (Archived by WebCite® at http://www.webcitation.org/6r0kDmAvd).

Davenport, J., and E.A. Macedo. 1990. Behavioral osmotic control in the euryhaline diamondback terrapin *Malaclemys terrapin*: responses to low salinity and rainfall. Journal of Zoology 220:487-496.

Davenport, J., and S.H. Magill. 1996. Osmotic control? Some preliminary observations on the function of emersion in the diamondback terrapin, *Malaclemys terrapin* (Latreille). Herpetological Journal 6:26-29.

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

Drabeck, D.H., M.W.H. Chatfield, and C.L. Richards-Zawacki. 2014. The status of Louisiana's diamondback terrapin (*Malaclemys terrapin*) populations in the wake of the Deepwater Horizon oil spill: insight from population genetic and contaminant analyses. Journal of Herpetology 48:125-136.

Draud, M., M. Bossert, and S. Zimnavoda. 2004. Predation on hatchling and juvenile diamondback terrapins (*Malaclemys terrapin*) by the Norway rat (*Rattus norvegicus*). Journal of Herpetology 38:467-470.

Dunson, W.A. 1970. Some aspects of electrolyte and water balance in three estuarine reptiles, the diamondback terrapin, American and "salt water" crocodiles. Comparative Biochemistry and Physiology 32:161-174.

Dunson, W.A. 1985. Effect of water salinity and food salt content on growth and sodium efflux of hatchling diamondback terrapins (*Malaclemys*). Physiological Zoology 58:736–747.

Dunson, W.A., and F.J. Mazzotti. 1989. Salinity as a limiting factor in the distribution of reptiles in Florida Bay: a theory for the estuarine origin of marine snakes and turtles. Bulletin of Marine Science 44:229-244.

Ernst, C.H., and J.E. Lovich. 2009. Turtles of the United States and Canada, 2nd ed. Johns Hopkins University Press, Baltimore, MD. 827 pp.

Evans, A., K. Madden, and S. Morehead. 2012. The Ecology and Sociology of the Mission-Aransas Estuary: An Estuarine and Watershed Profile. University of Texas Marine Science Institute, Port Aransas, TX. 183 pp.

Ford, D.K. 2005. Sublethal effects of stressors on physiological and morphological parameters in the diamondback terrapin, *Malaclemys terrapin*. Ph.D. Dissertation, College of Arts and Sciences of Ohio University, OH.

Forstner, M.R.J., G. Parks, L. Miller, B. Herbert, K. Halbrook, and B.K. Mealey. 2000. Genetic variability and geographic structure of *Malaclemys terrapin* populations in Texas and south Florida. A report to the Species at Risk Program of the United States Geological Survey.

Frankham, R., J.D. Ballou, and D.A. Briscoe. 2010. Introduction to conservation genetics, 2nd ed. Cambridge University Press, NY. 618 pp.

Gilles-Baillien, M. 1973. Hibernation and osmoregulation in the diamondback terrapin *Malaclemys centrata centrata* (Latreille). Journal of Experimental Biology 59:45-51.

Glenos, S. 2013. A comparative assessment of genetic variation of diamondback terrapin (*Malaclemys terrapin*) in Galveston Bay, Texas in relation to other northern Gulf Coast populations. Thesis, The University of Houston, TX.

Grime, J.P. 1989. The stress debate: symptom of impending synthesis? Biological Journal of the Linnaean Society 37:3-17.

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

Halbrook, K.A. 2003. Population estimate of Texas Diamondback Terrapin (*Malaclemys terrapin littoralis*) in Nueces Estuary and assessment of nesting habitat suitability. M.S. Thesis, Texas A&M University-Corpus Christi, TX.

Harden, L.A., S.R. Midway, and A.S. Williard. 2015. The blood biochemistry of overwintering diamondback terrapins (*Malaclemys terrapin*). Journal of Experimental Marine Biology and Ecology 466:34-41.

Hart, K.M. 2005. Population biology of diamondback terrapins (*Malaclemys terrapin*): defining and reducing threats across their range. Ph.D. Dissertation, Duke University, NC.

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.

Hart, K.M., M.E. Hunter, and T.L. King. 2014. Regional differentiation among populations of diamondback terrapin (*Malaclemys terrapin*). Conservation Genetics DOI 10.1007/s10592-014-0563-6.

Haskett, K., and G. Guillen. 2008. Population Characteristics of diamondback terrapin at the deer island complex: Galveston, Texas during 2008. Technical Report for the Houston Zoo No. 10-2. University of Houston-Clear Lake, TX.

Hauswaldt, J.S., and T.C. Glen. 2005. Population genetics of the diamondback terrapin (*Malaclemys terrapin*). Molecular Ecology 14:723-732.

Hernandez-Divers, S.M., S.J. Hernandez-Divers, and J. Wyneken. 2002. Angiographic, anatomic, and clinical technique descriptions of a subcarapacial venipuncture site for chelonians. Journal of Herpetological Medicine and Surgery 12:32-37.

Hill, E.M., B.A. Nicolau, and P.V. Zimba. 2011. History of water availability and habitat improvement in the Nueces Estuary, Texas, USA. Texas Water Journal 2:97-111.

Hogan, J.L. 2003. Occurrence of the diamondback terrapin (*Malaclemys terrapin littoralis*) at South Deer Island in Galveston Bay, Texas, April 2001–May 2002. USGS Open file report 03-022. http://water.usgs.gov/pubs/of/2003/ofr03-022. Accessed 8 December 2015.

Holliday, D.K., A.A. Elskus, and W.M. Roosenburg. 2009. Impacts of multiple stressors on growth and metabolic rate of *Malaclemys terrapin*. Environmental Toxicology and Chemistry 28(2):338-345.

Hong, M., K. Zhang, C. Shu, D. Xie, and H. Shi. 2014. Effect of salinity on the survival, ions and urea modulation in red-eared slider (*Trachemys scripta elegans*). Asian Herpetological Research 5(2):128-136.

Iber, H., Q. Chen, M. Sewer, and E.T. Morgan. 1997. Regulation of hepatic cytochrome P450 2C11 by glucocorticoids. Archives of Biochemistry and Biophysics 345:305-310.

King, T.L., and S.E. Julian. 2004. Conservation of microsatellite DNA flanking sequence across 13 Emydid genera assayed with novel bog turtle (*Glyptemys muhlenbergii*) loci. Conservation Genetics 5(5):719–725.

Koza, B.R. 2006. Distribution, habitat selection, and resource partitioning of Texas Diamondback terrapins (*Malaclemys terrapin littoralis*) in the Aransas National Wildlife Refuge Area, Texas. M.S. Thesis, Texas A&M University-Corpus Christi, TX.

Lovich, J.E., and J.W. Gibbons. 1990. Age at maturity influences adult sex ratio in the turtle *Malaclemys terrapin*. Oikos 59:126-134.

Mancera, J.M., and S.D. McCormick. 2007. Role of prolactin, growth hormone, insulin-like growth factor and cortisol in teleost osmoregulation. Pp. 497–515 in B. Baldisserotto, J.M. Mancera, and B.G. Kapoor (Eds.), Fish Osmoregulation. Science Publishers.

Moretzsohn, F., J.A. Sánchez Chávez, and J.W. Tunnell, Jr., Editors. 2016. GulfBase: Resource Database for Gulf of Mexico Research. http://www.gulfbase.org.

Mostl, E., and R. Palme. 2002. Hormones as indicators of stress. Domestic Animal Endocrinology 23:67-74.

Nicolau, B.A. 2001. Water quality and biological characterization of Oso Creek & Oso Bay, Corpus Christi, Texas. A report of the Coastal Coordination Council pursuant to National Oceanic and Atmospheric Administration Award No. NA97OZ017. Prepared for Texas General Land Office and Texas Natural Resource Conservation Commission. Contract No. 0102. Austin, TX. 138 pp.

Orlando, S.P. Jr., L.P. Rozas, G.H. Ward, and C.J. Klein. 1993. Salinity Characteristics of Gulf of Mexico Estuaries. National Oceanic and Atmospheric Administration, Office of Ocean Resources Conservation and Assessment, Silver Spring, MD. 209 pp.

Petre, C., W. Selman, B. Kreiser, S.H. Pearson, and J.J. Wiebe. 2015. Population genetics of the diamondback terrapin, *Malaclemys terrapin*, in Louisiana. Conservation Genetics 16:1243-1252.

Robinson, G.D., and W.A. Dunson. 1976. Water and sodium balance in the estuarine diamondback terrapin (Malaclemys). Journal of Comparative Physiology 105:129-152.

Schaffer, C., R.C. Wood, T.M. Norton, and R. Schaffer. 2008. Terrapins in the stew. Iguana 15:78-85.

Selye, H. 1976. The Stress of Life. pp. 3-56 in McGraw-Hill: New York, USA.

Sheridan, C.M., J.R. Spotila, W.F. Bien, and H.W. Avery. 2010. Sex-biased dispersal and natal philopatry in the diamondback terrapin, *Malaclemys terrapin*. Molecular Ecology 19:5497-5510.

Sibly, R.M., and P. Calow. 1989. A life-cycle theory of responses to stress. Biological Journal of the Linnaean Society 37:101-116.

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

Texas Department of Water Resources (TDWR). 1981. *Nueces and Mission-Aransas estuaries: A study of the influence of freshwater inflows.* LP-108. Texas Department of Water Resources, Austin, TX. 381 pp.

Texas Parks and Wildlife Department (TPWD). 2014. Texas diamondback terrapin (*Malaclemys terrapin littoralis*). http://www.tpwd.state.tx.us/huntwild/wild/ species/terrapin/. Accessed: 23 February 2014.

Texas Parks and Wildlife Department. 2017. Texas Natural Diversity Database. https://tpwd.texas.gov/huntwild/wild/wildlife_diversity/txndd/documents/20171004_TXNDD_tracked_animals.pdf (accessed 1 November 2017).

Uva, B., M. Vallarino, A. Mandich, and G. Isola. 1982. Plasma aldosterone levels in the female tortoise *Testudo hermanni* Gmelin in different experimental conditions. General and Comparative Endocrinology 46:116-123.

Valverde, R.A., D.W. Owens, D.S. Mackenzie, and M.S. Amoss. 1999. Basal and stress-induced corticosterone levels in olive Ridley sea turtles (*Lepidochelys olivacea*) in relation to their mass nesting behavior. Journal of Experimental Zoology 284:652-662.

Wood, R.C. 1977. Evolution of the emydine turtles *Graptemys* and *Malaclemys* (Reptilia, Testudines, Emydidae). Journal of Herpetology 11:415-421.