EVALUATION OF BLOOD BIOCHEMISTRY IN TEXAS DIAMONDBACK TERRAPIN (*MALACLEMYS TERRAPIN LITTORALIS*) WITHIN THE MISSION-ARANSAS AND NUECES ESTUARIES

A Thesis

by

LINDSEY C. RAMIREZ

BS, Texas State University–San Marcos, 2014

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This thesis meets the standards for scope and quality of
Texas A&M University-Corpus Christi and is hereby approved.

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ABSTRACT

Freshwater inflow is essential for providing key nutrients for estuarine environments. However, reduction of freshwater inflow can alter the function and structure of estuarine conditions and resources with detrimental consequences. For example, construction of the Choke Canyon and Wesley Seale dams have altered the Nueces Bay by reducing freshwater inflow causing increased salinity in the upper portion of the estuary. Brackish waters require osmoregulating organisms, such as diamondback terrapin (*Malaclemys terrapin*), to expend a great deal of energy maintaining homeostasis in the face of the widely varying salinities. In South Texas, areas in the upper estuary that are typically brackish may become hypersaline during summers or 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.

This study evaluated the physiological effects of salinity on stress hormone production and assessed baseline blood chemistry and electrolyte values in the Texas diamondback terrapin (*Malaclemys terrapin littoralis*) within the Nueces and Mission-Aransas Estuaries. Terrapins (n = 110) were captured during April 2015 – November 2015 and May 2016 – August 2016 from Aransas Bay, Nueces Bay, and Oso Bay. Water parameters were recorded for each sampling event. A blood sample was drawn from the subcarapacial sinus vein; initial blood glucose concentrations and morphometric data were recorded for each individual captured. 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. Subsamples of plasma were analyzed using an

electrolyte panel (Na+, Cl-, K+, and CO_2) and colorimetric techniques with commercially available reagents to determine plasma concentrations of glucose, albumin, total protein, total bilirubin, creatinine, blood urea nitrogen, and uric acid. Salinity and blood biochemistry measurements for terrapins captured on the same day in the same trap were averaged to provide an accurate representation of each salinity.

Using canonical discrimination, each bay was successfully separated with canonical axis 1 accounting for 93% of the variance and canonical axis 2 accounting for 7% of the variance. Data for all stress hormone and blood biochemistry variables were tested using discriminant analysis resulting in a 100% classification accuracy for each of the bays. Significant correlations were found between salinity in Nueces Bay and plasma concentrations of sodium, chloride, and blood urea nitrogen, and salinity in Oso Bay had a significant correlation with aldosterone plasma concentrations. Significant differences in stress hormone and blood biochemistry concentrations were observed for each bay using ANOVA and Duncan's multiple range test. The results of this research provide the first physiological assessment of Texas diamondback terrapins under variable salinity conditions utilizing hormones and blood biochemistry concentrations.

DEDICATION

In loving memory of my grandpa,

Gary Lee LaCount

(December 1, 1946 – January 25, 2017),

whose love of animals and nature made an everlasting impression on me and inspired me to chase my dreams.

ACKNOWLEDGEMENTS

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I would also like to thank my committee chair, Dr. Paul Zimba, committee co-chair, Dr. Kim Withers, and committee member, Dr. Judy Metcalf, for their wisdom and guidance throughout the course my research and for their support in submitting my veterinary school applications. I owe much appreciation to Dr. Michael R. J. Forstner from Texas State University-San Marcos and Dr. Tim Tristan, DVM, DABVP, Director of the Texas Sea Life Center in Corpus Christi, Texas, for taking the time to provide me with the training required for proper handling, tagging, and blood collection techniques for chelonians. I would also like to acknowledge and express my sincere gratitude to Barbara Herro, Lab Manager at Christus Spohn Hospital-Beeville, for running electrolyte samples for my blood chemistry analysis.

With my utmost appreciation and respect, I would like to thank Aaron Baxter for inspiring my passion for diamondback terrapins, his invaluable knowledge, advice, and encouragement throughout the course of my research, and for investing a considerable amount of time in the field assisting with terrapin captures. Thank you to Beth Almaraz and Robert Duke from the Center for Coastal Studies for their assistance in the field, and to I-Shuo Huang for advising me in the laboratory.

I would like to give a special thank you to my mom and dad, my brother and sister, my grandparents, the rest of my family, and my best friends for reminding me that the sky is the limit and always encouraging me to chase my dreams. I would not be where I am today without their love and support. I also want to extend my gratitude to Gerald and Glenda Swierc for their endless support throughout graduate school and while applying for veterinary school, as well as taking care of my animals so I could stay late at the office while completing my thesis manuscript.

Finally, thank you to Shantel Swierc, for not only being my field partner, but for being my best friend and my support system. Thank you for accepting me at my best and my worst, but most importantly, thank you for believing in me and encouraging me throughout all my endeavors.

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BACKGROUND & RELEVANCE

Diamondback terrapin (*Malaclemys terrapin*) populations are declining throughout their range (Spivey, 1998; Koza, 2006; Baxter, 2013; Glenos, 2013). They are one of a few species that live exclusively in the brackish water zone (Wood, 1977; Koza, 2006; Baxter, 2013; Glenos, 2013), which ranges from fresh (< 0.5 ppt) to about 10 ppt (Britton and Morton, 1989). Within an estuarine system, species diversity tends to decrease in the transition from polyhaline (16–30 ppt) to mesohaline (3–16 ppt), with few species found in oligohaline environments (0.5–3 ppt) (Britton and Morton, 1989; Cotton, 2014). Brackish waters require osmoregulating organisms, such as terrapin, to expend a great deal of energy maintaining homeostasis in the face of the 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 kinds of physiological stress, such as salinity stress, may exacerbate mortality and morbidity associated with other environmental stressors.

Texas diamondback terrapins (*Malaclemys terrapin littoralis*) live in waters ranging from ~4 ppt to 22 ppt (Bishop, 1983; Halbrook, 2003) and can tolerate salinities ranging from fresh (< 0.5 ppt) to marine (35 ppt) (Coker, 1951; Dunson, 1970; Halbrook, 2003). 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 summers or 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 upper Nueces Estuary, terrapins have been documented in Nueces River where salinities are as low as 2 ppt to areas in Nueces Bay where the salinity was 50 ppt (A. Baxter, pers. comm.). The goal of this research was to measure relevant blood chemistry parameters (e.g., electrolytes, stress hormones) in 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." Selye 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 and will be under 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. For example, 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 ppt) 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 large

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 ppt) in the laboratory also results in reduced food intake which affects growth and development (Dunson, 1985; Davenport and Ward, 1993; Holliday et al., 2009). However, even salinities that are just outside terrapin's range of tolerance can be stressful. Holliday et al. (2009) found that when terrapins were exposed to stressor salinities of 0 ppt, 20 ppt, and 30 ppt, they exhibited an initial decrease in growth compared to terrapins held at 10 ppt. 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 ppt. Dunson and Mazzotti (1989) reported that terrapin growth stops when salinities exceed 21 ppt; increased salinity also negatively affects liver growth (Ford, 2005). Davenport and Ward (1993) reported that terrapins reduce seawater and prey intake 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 will also ingest water, which increases the effects of dehydration. When exposed to seawater (~35 ppt) 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 during nesting season 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.

RESEARCH OBJECTIVES

The objective of this study was to compare diamondback terrapin blood biochemistry and stress hormone production among three bays that represent a range of expected salinities: Aransas Bay, Nueces Bay, and Oso Bay. The specific research questions were:

1) Are there are differences in environmental conditions (i.e., salinity, water and air temperature) or blood biochemistry and stress hormone concentrations in diamondback terrapin among the bays.
2) Are there are differences in environmental conditions or blood biochemistry and stress hormone concentrations between male and female diamondback terrapins among the bays.

Hypotheses.—The null hypotheses tested were:

1) There is no difference in environmental conditions or blood biochemistry and stress hormone concentrations in terrapins among Aransas, Nueces, and Oso bays.
2) There is no overall difference in environmental conditions or blood biochemistry and stress hormone concentrations between male and female diamondback terrapins.
3) There is no difference in environmental conditions or blood biochemistry and stress hormone concentrations in either sex of diamondback terrapins among Aransas, Nueces, and Oso bays.

STUDY AREA

The study area consisted of three locations in the Texas Coastal Bend: Nueces Bay and Oso Bay, both within the Nueces Estuary, and Aransas Bay within the Mission-Aransas Estuary (Fig. 1). These locations were selected based on estimated population size, terrapin sightings, previous terrapin research, and likely salinity regime.

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). 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 (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; 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 ppt in Redfish Bay, 20-30 ppt in Aransas Bay and Mesquite Bay, and 10–20 ppt in St. Charles Bay, Copano Bay, Port Bay, and Mission 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 Canyon Reservoir along the Nueces River provide drinking water for the City of Corpus Christi, Texas (Hill et al., 2011). The Nueces River also receives effluent discharge from the Allison Wastewater Treatment Plant (City of Corpus Christi) (Coastal Bend Regional Water Planning Group, 2001). Effluent-dominated Oso Creek receives treated discharge from the Robstown Wastewater Treatment Plant (City of Robstown), Roloff Evangelistic Enterprises, Inc., Texas A&M Extension Service, Greenwood Wastewater Treatment Plant (City of Corpus Christi), and Central Power & Light-Barney Davis Plant (water cooling discharge) (Nicolau, 2001). In addition to the discharge into Oso Creek, Oso Bay receives effluent from the Oso Wastewater Treatment Plant located west of Ward Island near the mouth of the bay (Nicolau, 2001). Often referred to as a reverse estuarine system, salinity in the Nueces Estuary is typically greater in the Nueces Delta to the west and often decreases through the estuary moving towards the Gulf of Mexico to the east. Salinities range from 5–50 ppt in Nueces Bay, 1–51 ppt in Oso Bay, and 15–30 ppt 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.

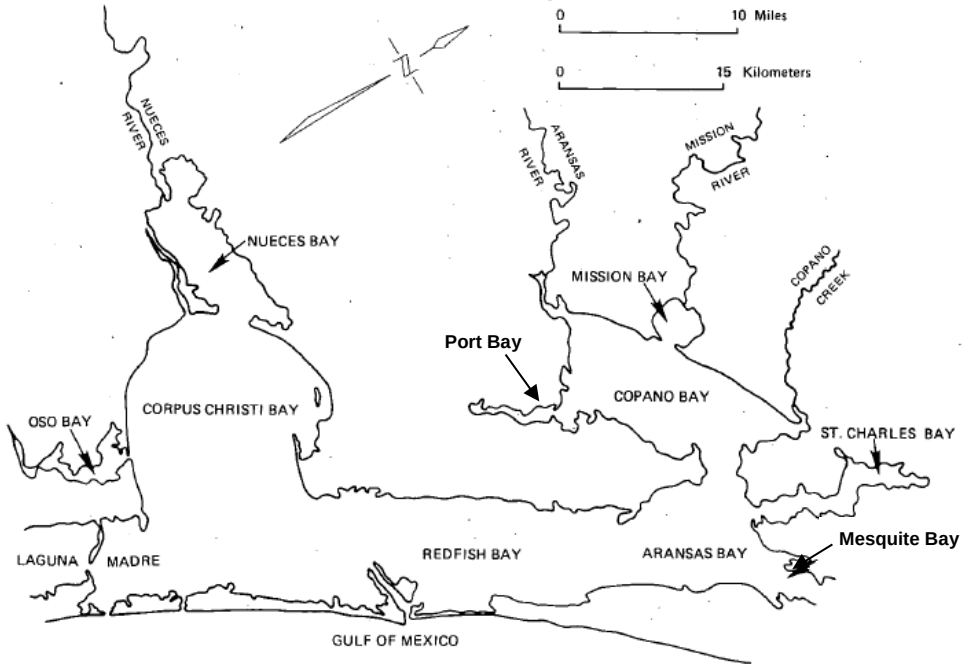

FIG. 1. Map of the Mission-Aransas Estuary and Nueces Estuary study areas (Texas Department of Water Resources, 1981b).

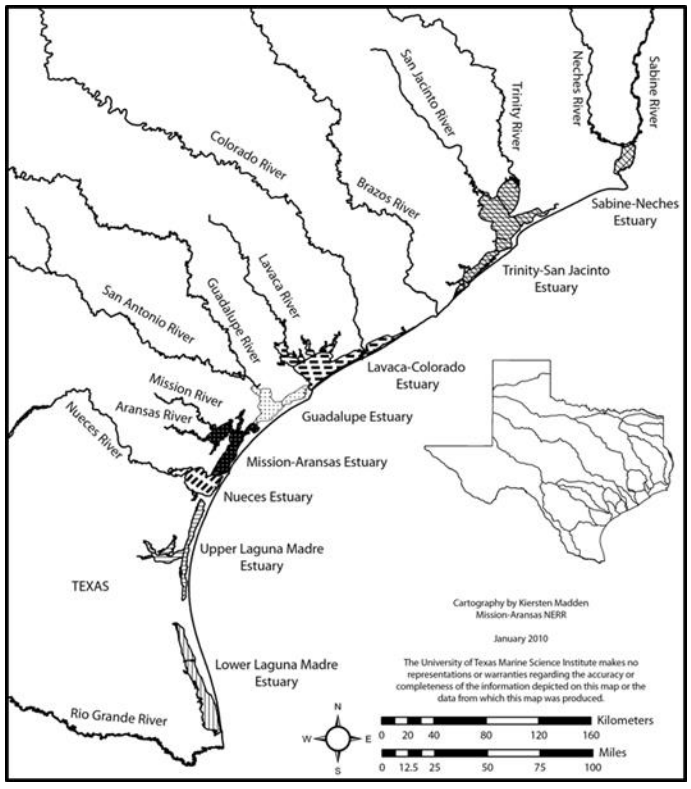

FIG. 2. Map of the major rivers that provide freshwater inflow to the major estuaries on the Texas coast (Evans et al., 2012).

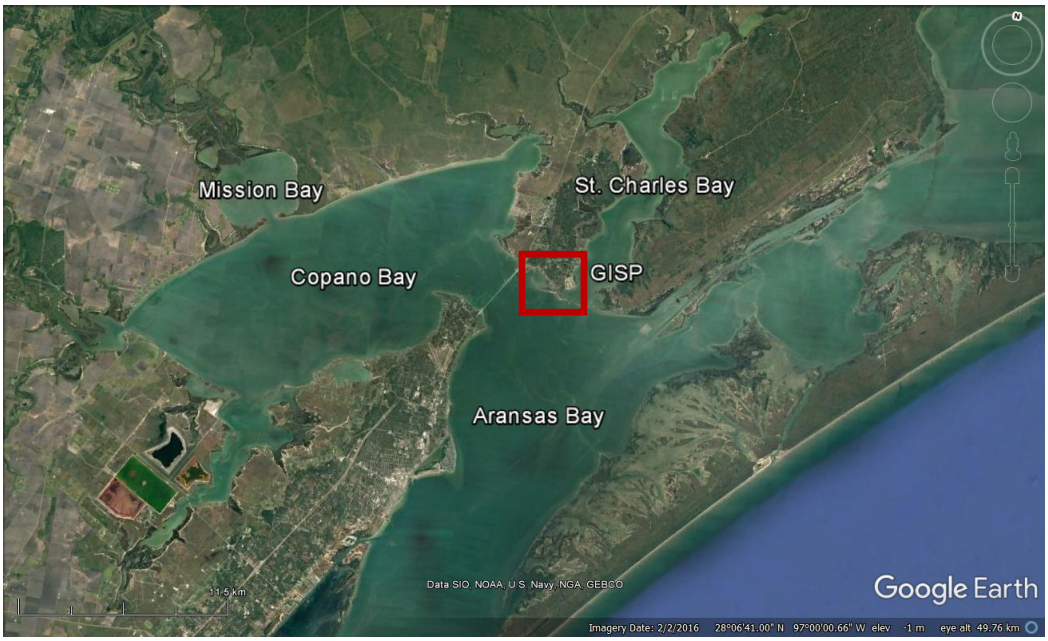

FIG. 3. Map of the location of Goose Island State Park (GISP) study area within the Mission-Aransas Estuary, Texas (Google Earth, 2016).

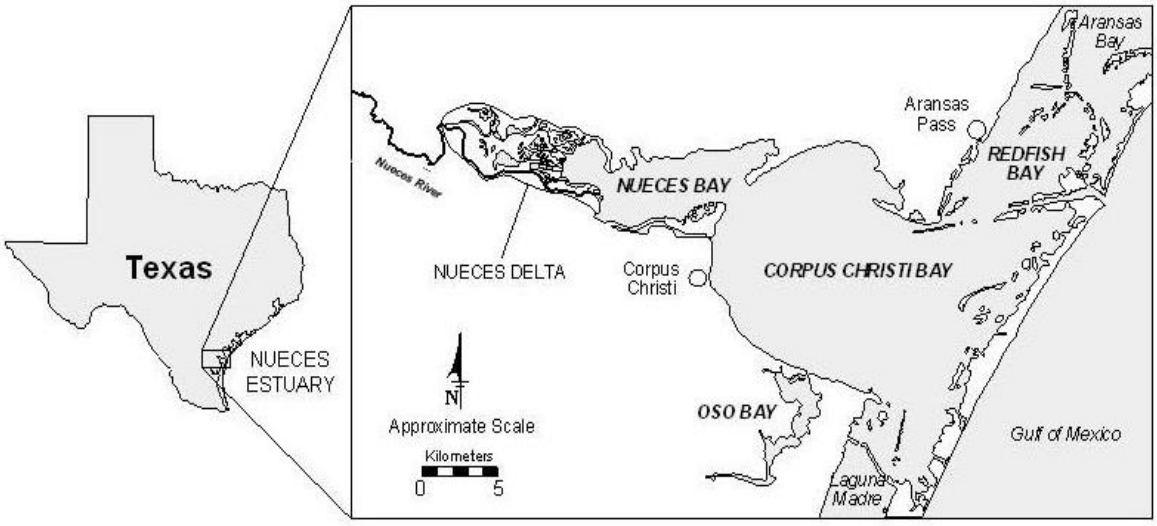

FIG. 4. Map of the location and major components of the Nueces Estuary system (Bureau of Reclamation, 2000).

METHODS

Terrapin Captures.—Trapping was conducted under scientific research permit (Permit No. SPR–0910–148; Appendix 1) obtained from Texas Parks and Wildlife. 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 pots, and commercial crab traps modified with chimneys to allow terrapins to surface for air (Fig. 6). These methods have been successful in capturing terrapins in previous studies (Forstner et al., 2000; Butler, 2002; Baxter, 2013; Baxter et al., 2013; Glenos, 2013; Baxter, 2014; Baxter, 2015). Traps were set at various sites of terrapin activity 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 to 20. Traps were retrieved after 24 h. General locality, GPS location, habitat type, trap type, and trap depth (m) was recorded for each trap deployed. Hydrological data was collected at each location at deployment and retrieval using a YSI® 6920 V2 Multiparameter Sonde. Hydrological parameters included salinity (ppt) and water temperature (°C).

FIG. 5. Photograph of the custom turtle trap used to capture diamondback terrapin in the Mission-Aransas and Nueces Estuaries (photo courtesy of S. Swierc).

FIG. 6. Photograph of a modified commercial crab trap used to capture diamondback terrapin in the Mission-Aransas and Nueces Estuaries (photo courtesy of S. Swierc).

Upon arrival at each trap location, the trap was quickly pulled into the boat and the initial time the trap was first disturbed was recorded. A study by Cash et al. (1997) measured the effects of capture and handling on the corticosterone response in free-living red-eared sliders (*Trachemys scripta elegans*). Their results indicate that plasma corticosterone concentrations significantly increase with prolonged handling time. The greatest increase occurs within the initial 30 min of capture and handling. The concentration then declines between 30 min and 60 min following capture and handling. Cash et al. (1997) suggest that an initial blood sample collected within the first 10 min of capture and handling represents basal corticosterone levels. 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 Collection.—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). The site of the sinus vein collection is located where the dorsal base of the terrapin's neck connects below the carapace between the two center anterior marginal scutes (Glenos, 2013). All procedures were approved by the Texas A&M University-Corpus Christi (TAMUCC) Institutional Animal Care and Use Committee (IACUC Protocol No. 01–15; Appendix 2). Training in chelonian blood collection techniques and PIT tag implantation was provided by Dr. Tim Tristan, Veterinarian and Director of the Texas Sea Life 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 from the subcarapacial sinus vein 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 at the site of capture.

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. Upon returning to the Center for Coastal Studies Laboratory at TAMUCC, samples were centrifuged at 2180 x g for 10 min. The plasma was removed from the whole blood pellet and transferred to a new microcentrifuge tube, and both the plasma and the whole blood pellet were stored at -80 °C for subsequent analyses.

FIG. 7. Photograph of a diamondback terrapin illustrating proper restraint techniques to clean the venipuncture site with iodine prior to blood collection.

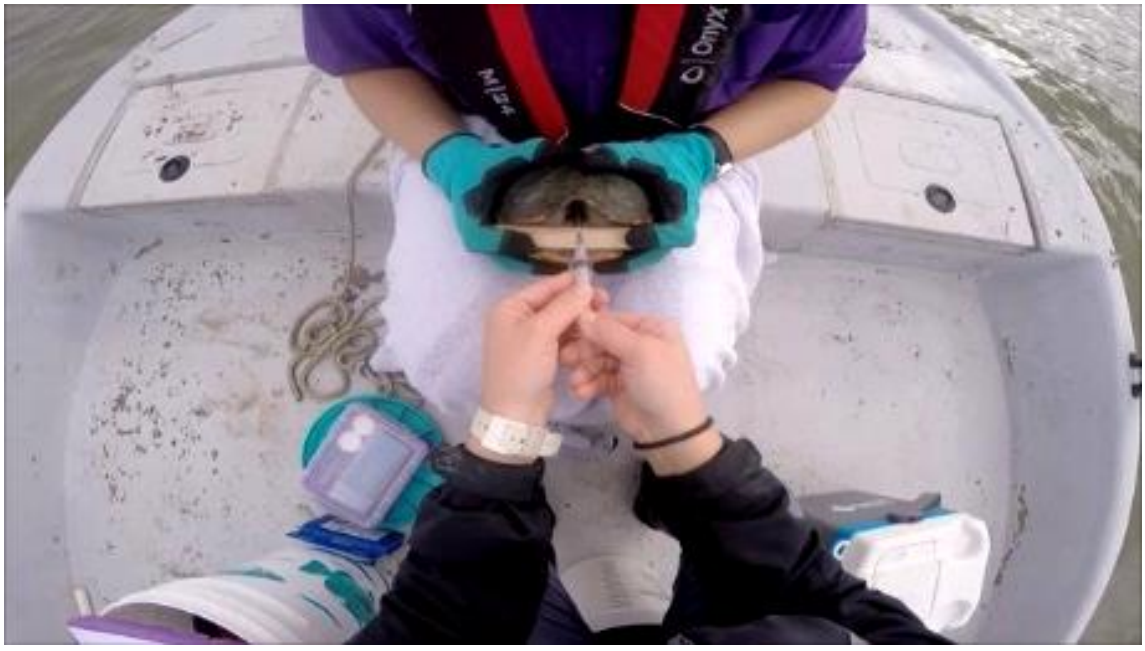

FIG. 8. Photograph of the venipuncture site used to collect blood samples from diamondback terrapin in the Mission-Aransas and Nueces Estuaries (photo courtesy of S. Swierc).

Morphological Measurements, Age, and Sex Determinations.—Each terrapin was evaluated upon capture to record weight (g), activity levels, and body condition scores (BCS; Table 1; Appendix 3). Sex was determined for each individual by the position of the cloacal opening from the posterior end of the carapace (Lovich and Gibbons, 1990; Baxter et al., 2013). Females were palpated to determine gravidity. Standard measurements of each individual were 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 the growth rings on the costal or vertebral scutes on the carapace. All individuals were photographed and physical characteristics, abnormalities, injuries, lesions, or wounds were recorded.

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 the plasma from each individual was used to determine the concentration of three different stress hormones. Due to the limited volume of plasma for each individual, samples could not be assayed in duplicate. However, out of every 40 samples assayed, 4–5 samples were randomly selected to run in duplicate. Standards and buffer solutions were run in duplicate and with each assay of plasma samples.

TABLE 1. Description of codes 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.

Corticosterone.—The initial concentration of the corticosterone ELISA standard bulk solution was 50 ng/mL. This bulk solution was serially diluted to create the eight standards (S1–S8) used to determine the relationship between the plasma concentration of corticosterone and the absorbance. Prior to analysis, each plasma sample was purified by adding four times the sample volume of methylene chloride. The samples were vortexed and allowed to separate into layers. The lower layer of methylene chloride was transferred into a new test tube. This process was repeated three times. The combined methylene chloride extracts were then evaporated under a stream of nitrogen and the

extract was dissolved in 300 µL of ELISA buffer solution. Following the addition of reagents and plasma samples to the plate wells, the plate was incubated at 4 °C for 16–20 h. The wells were then rinsed five times with wash buffer to remove all unbound reagents and 200 µL of Ellman's Reagent was added to each well. The assay was developed in the dark for 90–120 min using a Thermolyne Aros 160™ Adjustable Reciprocating Orbital Shaker (Barnstead/Themolyne Corp., Dubuque, IA, USA) and the absorbance of the reaction was read between 405 and 420 nm using a Synergy™ HT Multi-Detection Microplate Reader with Gen5™ version 2.03.1 software package (Bio-Tek Instruments, Winooski, VT, USA). Fig. 9 provides an example of a developed assay plate.

Aldosterone.—The initial concentration of the aldosterone ELISA standard bulk solution was 20 ng/mL. This bulk solution was serially diluted to create the eight standards (S1–S8) used to determine the relationship between the plasma concentration of aldosterone and the absorbance. Prior to analysis, each plasma sample was purified by adding four times the sample volume of chloroform. The samples were vortexed and allowed to separate into layers. To ensure desirable separation, samples were centrifuged at 30 x g for 5 min and 90% of the lower layer of chloroform was transferred into a new test tube. This process was repeated three times. The combined chloroform extracts were then evaporated at 30 °C under a stream of nitrogen and the extract was dissolved in 600 µL of ELISA buffer solution. Following the addition of reagents and plasma samples to the plate wells, the plate was incubated at 4 °C for 18 h. The wells were then rinsed five times with wash buffer to remove all unbound reagents and 200 µL of Ellman's Reagent was added to each well.

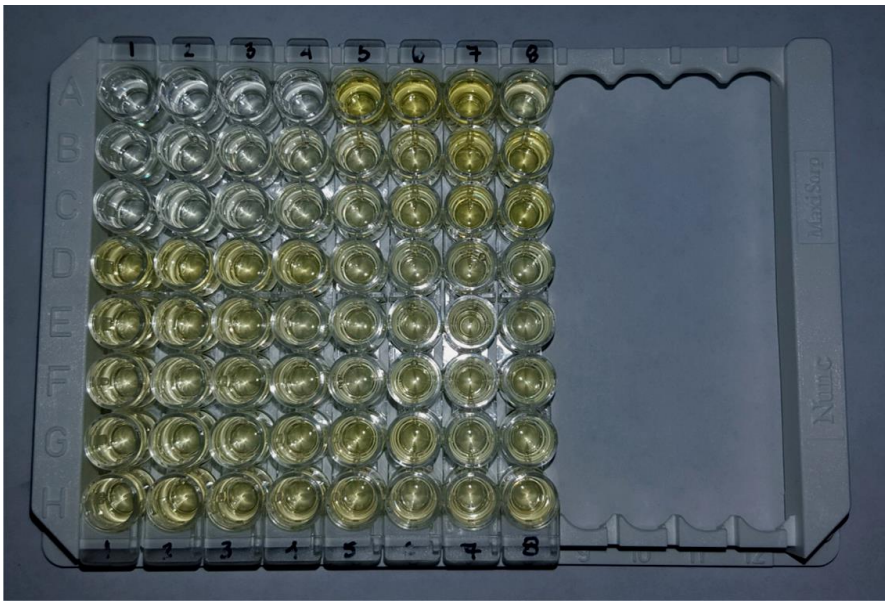

FIG. 9. Photograph of a developed corticosterone assay plate used to determine the concentration of corticosterone in diamondback terrapin plasma samples from the Mission-Aransas and Nueces Estuaries.

The assay was developed in the dark for 90–120 min using a Thermolyne Aros 160™ Adjustable Reciprocating Orbital Shaker (Barnstead/Themolyne Corp., Dubuque, IA, USA) and the absorbance of the reaction was read between 405 and 420 nm using a Synergy™ HT Multi-Detection Microplate Reader with Gen5™ version 2.03.1 software package (Bio-Tek Instruments, Winooski, VT, USA).

Prolactin.—The initial concentration of the prolactin ELISA standard bulk solution was 50 ng/mL. This bulk solution was serially diluted to create the eight standards (S1–S8) used to determine the relationship between the plasma concentration of prolactin and the absorbance. Prior to analysis, each plasma sample was vortexed and centrifuged at 1600 x g for 20 min. Use of a purification protocol was not necessary to measure the concentration of prolactin in the plasma samples. Once the assay plate wells were rinsed five times with wash buffer, the reagents and samples were added to the

appropriate wells and the plate was incubated at room temperature for 16-20 h. Following incubation, the wells were rinsed five times with wash buffer to remove all unbound reagents and 200 µL of Ellman's Reagent was added to each well. The assay was developed in the dark for 90–120 min using a Thermolyne Aros 160™ Adjustable Reciprocating Orbital Shaker (Barnstead/Themolyne Corp., Dubuque, IA, USA) and the absorbance of the reaction was read between 405 and 414 nm using a Synergy™ HT Multi-Detection Microplate Reader with Gen5™ version 2.03.1 software package (Bio-Tek Instruments, Winooski, VT, USA).

Electrolyte and Blood Chemistry Analyses.—A 0.5 mL subsample of plasma from each individual was sent to the CHRISTUS® Spohn Hospital Beeville Laboratory (CHRISTUS® Spohn Health System, Beeville, TX, USA) for electrolyte analysis (Na^+, Cl^-, K^+, and CO_2). These plasma subsamples were further analyzed using colorimetric techniques and commercially available reagents to determine plasma concentrations of glucose, albumin, total protein, total bilirubin, creatinine, blood urea nitrogen, and uric acid using a Sirrus® Clinical Chemistry Analyzer (Stanbio® Laboratory, Boerne, TX, USA; Glucose Liqui-UV® Ref. No. S1060-600; Albumin LiquiColor® Ref. No. S0285-750; Total Protein LiquiColor® Ref. No. S0250-750; Total Bilirubin LiquiColor® Ref. No. S0230-600; Creatinine LiquiColor® Ref. No. S0430-600; Urea Nitrogen Liqui-UV® Ref. No. S2020-600; Uric Acid LiquiColor® Ref. No. S1045-750).

Statistical Analyses.—To determine if there were differences in diamondback terrapin characteristics between Nueces, Oso, and Aransas Bays, canonical discrimination and discriminant analysis with resubstitution 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 location effects were determined.

Stress Hormones.—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 (ppt) 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 (ppt) 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 $P < 0.05$.

Electrolytes and Blood Chemistry.—Pearson product-moment correlation coefficients were calculated to determine whether salinity (ppt) was significantly correlated with electrolyte concentrations and blood chemistry concentrations. Salinity (ppt) measurements for terrapins captured on the same day in the same trap were averaged to provide an accurate representation of that salinity. Statistical analyses were performed using R version 3.2.5 software (R Core Team, 2016). Results were considered statistically significant at $P < 0.05$.

RESULTS

Environmental Conditions.—Salinities during course of this study ranged from 0.4 ppt to 28.8 ppt, 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 ppt, 10.4 ± 4.4 ppt, and 7.0 ± 2.7 ppt, 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 and five were individuals that had been killed by predators. From 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 (Recap)	# of Individuals Captured (Individ. Recap)	# of Males Captured (Recap)	# of Females Captured (Recap)
Aransas Bay	38 (6)	32 (3)	10 (0)	28 (6)
Nueces Bay	45 (3)	42 (3)	10 (0)	35 (3)
Oso Bay	27 (0)	27 (0)	24 (0)	3 (0)
Total	110 (9)	101 (6)	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 Characteristics.—Canonical discrimination and discriminant analysis with resubstitution was performed to determine if there were differences in diamondback terrapin characteristics (1) between location, (2) between males and

females, and (3) between females among location and between males among location. The linear discriminant functions are provided in Appendix 4.

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. 10). The mean canonical coefficients for each location were clearly separated, with CW (mm), CH (mm), PL (mm), PW (mm), and BUN (mg/dL) accounting for most of the separation. Discriminant analyses of location resulted in a classification accuracy of 100% with resubstitution 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 and blood biochemistry by location, and the 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).

Nueces Bay sodium concentrations were significantly less than both Aransas and Oso bays, and chloride concentrations were significantly less than those in Aransas Bay (Table 4). Aransas Bay total protein concentrations were significantly greater than

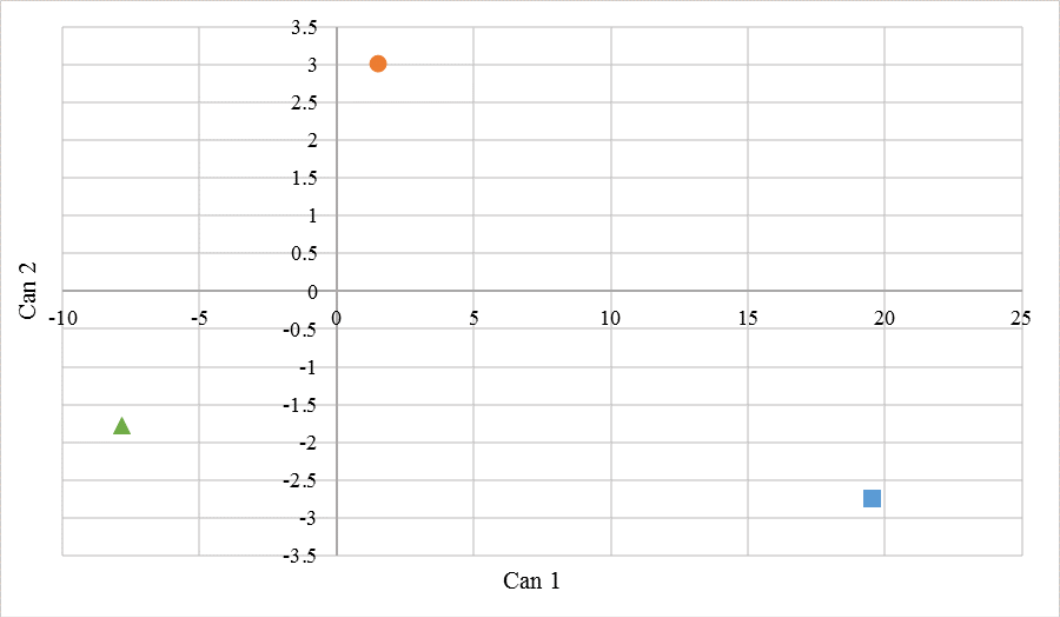

FIG. 10. Mean canonical coefficients for terrapins captured in Oso Bay (■), Nueces Bay (▲), and Aransas Bay (●).

Nueces Bay. Blood urea nitrogen concentrations were significantly different among all three bays, and glucose, uric acid, and total bilirubin concentrations for terrapins within Oso Bay were significantly greater than those within Nueces Bay and Aransas Bay (Table 4).

A Pearson's correlation was performed to determine the relationship between salinity and concentrations of stress hormones, electrolytes, and blood chemistry (Table 5). Overall, salinity was significantly positively correlated with plasma concentrations of sodium (r = 0.47; P = 0.03) and blood urea nitrogen (r = 0.53; P = 0.014). Salinity within Oso Bay was significantly positively correlated with plasma aldosterone concentrations (r = 0.98; P = 0.02). Nueces Bay salinity was significantly positively correlated with plasma concentrations of sodium (r = 0.72; P = 0.03), chloride (r = 0.78; P = 0.014) and blood urea nitrogen (r = 0.67; P = 0.049).

TABLE 4. Mean concentrations of stress hormones and blood biochemistry variables 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; GLU = glucose; Na+ = sodium; K+ = potassium; Cl- = chloride; CO_2 = carbon dioxide; BUN = blood urea nitrogen; UA = uric acid; CREA = creatinine; ALB = albumin; TPRO = total protein; and TBILI = total bilirubin.

	Location		
Variable	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**
Na+ (mmol/L)	145.90**a**	140.45**b**	148.60**a**
K+ (mmol/L)	3.84**a**	3.73**a**	3.90**a**
Cl- (mmol/L)	116.45**a**	105.43**b**	111.60**ab**
CO_2 (mmol/L)	24.89**a**	24.79**a**	20.20**a**
Initial GLU (mg/dL)	78.74**a**	76.35**a**	167.00**b**
GLU (mg/dL)	72.44**a**	67.53**a**	75.60**a**
BUN (mg/dL)	88.82**a**	36.89**b**	61.20**c**
UA (mg/dL)	0.57**a**	1.31**a**	2.20**b**
CREA (mg/dL)	0.001**a**	0.000**a**	0.002**a**
ALB (g/dL)	2.34**a**	2.01**a**	2.26**a**
TPRO (g/dL)	3.03**a**	2.25**b**	2.64**ab**
TBILI (mg/dL)	0.00**a**	0.00**a**	0.04**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; GLU = glucose; Na+ = sodium; K+ = potassium; Cl- = chloride; CO_2 = carbon dioxide; BUN = blood urea nitrogen; UA = uric acid; CREA = creatinine; ALB = albumin; TPRO = total protein; and TBILI = total bilirubin.

	Stress Hormones			Electrolytes				Blood Chemistry							
	CORT	ALD	PRL	Na+	K+	Cl-	CO_2	INIT GLU	GLU	BUN	UA	CREA	ALB	TPRO	TBILI
Aransas Bay Salinity	-0.54	-0.70	0.32	-0.45	-0.28	0.25	0.58	-0.33	-0.64	0.32	-0.5	-0.03	0.07	-0.13	-
Nueces Bay Salinity	0.35	0.01	0.09	0.72*	0.48	0.78*	0.10	0.44	0.28	0.67*	-0.23	-	-0.14	-0.10	-
Oso Bay Salinity	0.73	0.98*	0.85	0.54	0.25	0.73	-0.90	-0.19	-0.09	0.50	0.49	0.45	0.16	0.49	0.45
Overall Salinity	-0.10	-0.19	0.29	0.47*	0.26	0.42	0.18	0.25	-0.15	0.53*	-0.33	0.06	0.01	0.11	-0.03

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

DISCUSSION

This study is the first to quantify the effects of salinity on stress hormone production and blood biochemistry in free-ranging Texas diamondback terrapins found in typical estuarine salinities (~0.5–35 ppt) at the southernmost end of their range and is also the first known report of aldosterone and prolactin concentrations in free-ranging diamondback terrapins. Although hypersaline conditions were expected based on historical trends, hypersalinity was not observed during the 2015 and 2016 sampling periods. 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.

Diamondback terrapins living in South Texas estuaries are subjected to physiological stress from high and/or varying salinities. Evaluation of stress hormone and blood biochemistry concentrations across terrapin populations can identify if differences exist between locations. For example, Nueces Bay terrapins exhibited the strongest salinity response with positive significant correlations with sodium, chloride, and blood urea nitrogen, whereas in Aransas Bay and Oso Bay no trends were evident. The majority of terrapins captured in Aransas Bay were captured in Goose Island State Park in close proximity to the boat ramp and fish cleaning station, which is frequented on a regular basis by charter fishermen and the public. Freshwater inflow and a diet of saltwater fish is made readily available at the fish cleaning station due to the cleaning/gutting of fishes and the disposal of scraps and carcasses into the water. According to McCormick (2001), the plasma osmotic concentration in teleost fishes is maintained at approximately one-third of that of seawater (~35 ppt), regardless of environmental salinity, and the fish's

gills are actively secreting salts in seawater and taking up salts in freshwater. The lack of correlation between Aransas Bay salinity and the blood biochemistry variables may be attributed to the freshwater inflow from the fish cleaning station and consumption of hypo-osmotic marine fishes.

Similarly, terrapins in Oso Bay were captured in the area referred to as the "Blind Oso" in close proximity to the Oso Wastewater Treatment Plant (OWWTP) located west of Ward Island. Approximately 16.2 million gallons of wastewater effluent is discharged per day into Oso Bay from the OWWTP (Nicolau, 2001). This influx of freshwater combined with the probable consumption of hypo-osmotic marine fishes lends itself to the lack of correlation between Oso Bay salinity and the blood biochemistry variables, with the exception of aldosterone.

Stress Hormones.—Non-transformed stress hormone data used to compare this study's mean (± SE) plasma concentrations to previous studies are provided in Appendix 5. 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 ppt) was similar to the salinity treatments (0 ppt, 10 ppt, 20 ppt, and 30 ppt) by Ford (2005) and salinity fluctuations (range = 25–35 ppt; mean = 33.3 ppt ± 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, and 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), among others.

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 the hypophysectomized lizard. 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 their 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 the hypophysectomized lizard 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.

Electrolytes.—Nueces Bay exhibited the strongest salinity response with significant positive correlations between salinity and sodium and chloride concentrations. Dunson (1985) evaluated the effects of salinity on water and ion content in terrapin

hatchlings and found that sodium concentrations significantly increased as salinity increased between ~0–18 ppt and concentrations remained constant at salinities ≥ 18 ppt. The direct relationship between increasing salinity and sodium is supported by the results of this study.

Plasma concentrations of sodium, potassium, and chloride measured in active Texas diamondback terrapin were comparable to concentrations measured in overwintering terrapins in southeastern North Carolina (Harden et al., 2015) and concentrations measured in a controlled laboratory study on terrapin hibernation and osmoregulation (Gilles-Baillien, 1973). However, the minimum plasma concentrations of sodium, potassium, and chloride were less than the minimum concentrations measured in the aforementioned studies with the exception of the minimum potassium concentration in the controlled laboratory study (Table 6). Terrapins in the controlled laboratory study had the greatest plasma concentration of potassium between the months of April and September. Between April and September, plasma sodium increased while plasma chloride decreased (Gilles-Baillien, 1973). According to Gilles-Baillien (1973), greater concentrations of sodium and chloride during the summer months (i.e., active season) suggests that active transport of sodium in the large intestine, small intestine, and colon is more effective during the summer months.

According to Bertolero et al. (2009), dehydration in estuarine or marine reptiles is due to hypernatremia, which is the acclimation of sodium. Harden et al. (2015) concluded that overwintering terrapins did not exhibit noticeable signs of dehydration (i.e., no change in mean total body water between pre- and post-dormancy). Furthermore, Dunson (1985) concluded that terrapins held at ~26 ppt had high rates of sodium efflux when fed

a salty diet of clams or fathead minnows, which was likely due the amount of food ingested and thus the incidental ingestion of seawater during feeding. However, Dunson (1985) determined that only 13.5% of the sodium efflux for terrapins fed whole clams, the saltiest diet offered, was the direct result of sodium intake via diet. Taken together, the results of this study suggest that (1) terrapins may use similar mechanisms to regulate blood biochemistry during winter dormancy and the active season (Gilles-Baillien, 1973; Harden et al., 2015), and (2) the differences in sodium concentrations between the overwintering terrapins and this study's active free-ranging terrapins may be attributed to the ingestion of seawater during feeding and not dehydration. These data can serve as a baseline for terrapin physiological condition under normal or average salinity conditions. Future studies will be needed to evaluate hypersalinity effects on osmotic balance and stress responses. Additional research is required to evaluate total body water changes between spring emergence and winter dormancy in addition to measuring urea concentrations to determine osmotic balance in Texas diamondback terrapins.

Blood Chemistry.—Blood glucose levels are often used to monitor metabolism and physiology (Foster, 2017). In this study, plasma concentrations of glucose were substantially greater than those measured in overwintering terrapins (Harden et al., 2015; Table 6). However, blood glucose levels were within the range reported for other chelonians, though variations may exist depending on the environmental and physiological conditions of the animals studied (Pagés et al., 1992; Rosenthal and Heinze, 2002; Kirchgessner and Mitchell, 2009; Flower et al., 2015; Appendix 5). Differences in plasma glucose concentrations are likely due to seasonality differences between the overwintering study and this study, in which data were collected during the

TABLE 6. Mean (± SE) plasma concentrations (mmol/L) for active and dormant diamondback terrapins reported in literature. Values in parentheses represent the range.

	Na+	K+	Cl-	GLU	References
Active Season: Free-ranging	144.0 ± 1.2 (129.0 – 167.0)	3.8 ± 0.1 (2.7 – 5.5)	109.7 ± 1.8 (96.0 – 136.0)	70.0 ± 3.9 (21.0 – 161.0)	Present Study
Overwintering Females: Free-ranging	151.7 ± 2.2 (144.3 – 155.7)	2.9 ± 0.1 (2.8 – 3.1)	107.4 ± 2.3 (104.3 – 110.1)	2.7 ± 0.4 (2.1 – 3.3)	Harden et al. (2015)
Overwintering Females: Open-air enclosure	146.4 ± 1.8 (142.9 – 152.8)	2.8 ± 0.1 (2.6 – 3.5)	106.9 ± 2.3 (103.7 – 109.1)	2.5 ± 0.2 (2.1 – 3.3)	Harden et al. (2015)
Hibernation & Osmoregulation	173.5 ± 16.2 (152.4 – 199.6)	3.1 ± 0.7 (2.4 – 4.2)	143.0 ± 15.3 (134.0 – 150.5)	-	Gilles-Baillien (1973)

active season when rates of metabolic and physiological functions are elevated. Blood urea nitrogen is used as an indicator of renal function and/or dehydration (Foster, 2017). In the present study, significant positive correlations between salinity and plasma concentrations of blood urea nitrogen were observed for Nueces Bay and the overall salinity for all study areas. Blood urea nitrogen levels were substantially greater than those reported for free-ranging green sea turtles (*Chelonia mydas*) (Aguirre and Balazs, 2000; Fong et al., 2010), but were within the range reported for wild caught gopher tortoises (*Gopherus polyphemus*) that were maintained in a large outdoor enclosure (Taylor and Jacobson, 1982). Although not statistically significant ($P > 0.05$), a negative correlation was observed between the overall salinity for all three study areas and uric acid concentrations (see Table 5). With the exception of uric acid concentrations measured in Oso Bay, a male-biased population, concentrations were less than the range reported for overwintering terrapins (Harden et al., 2015) and the reference range

reported for other chelonians (Rosenthal and Heinze, 2002). Bolten and Bjorndal (1992) found that mean uric acid values between male and female green sea turtles were significantly different. Although the male-biased population in Oso Bay may have contributed to the differences in uric acid concentrations, the exact cause is currently unknown.

Creatinine is another indicator used to monitor renal function. With the exception of one female from Aransas Bay and two males from Oso Bay, each with creatinine concentrations of 0.1 mg/dL, creatinine concentrations were below detectable limits in active diamondback terrapins. However, the values for creatinine were within the range reported for the gopher tortoise (Taylor and Jacobson, 1982; Appendix 5) and captive New Guinea snapping turtles (*Elseya novaeguineae*) (Anderson et al., 1997), and values were similar to the means reported for wild green sea turtles (Aguirre and Balazs, 2000).

Albumin is an abundant plasma protein, which serves as a carrier and transport protein in addition to its role in blood volume regulation through the maintenance of oncotic pressure, a form of osmotic pressure, within body fluids (Osborne et al., 2010). Albumin concentrations were also within the range reported for other chelonians (Taylor and Jacobson, 1982; Aguirre and Balazs, 2000; Kirchgessner and Mitchell, 2009; Fong et al., 2010; Appendix 5). Total protein concentration is a combination of various protein fractions that includes albumin, α-globulin, β-globulin, and γ-globulin (Osborne et al., 2010). Total protein concentrations were slightly less than the typical range for reptiles (Dessauer, 1970; Pagés et al., 1992) and the reference range for chelonians (Rosenthal and Heinze, 2002). However, total protein concentrations were within the range reported for the desert tortoise (*Gopherus agassizii*), gopher tortoise, Aldabra tortoise (*Geochelone*

giganteus), and the pancake tortoise (*Kinixys erosa*) (Kirchgessner and Mitchell, 2009; Appendix 5). Bilirubin is byproduct of hemoglobin breakdown, the molecule in red blood cells that carries oxygen to the tissues (Foster, 2017). Total bilirubin concentrations were similar to the values reported in other chelonians (Taylor and Jacobson, 1982; Bolten and Bjorndal, 1992; Anderson et al., 1997; Aguirre and Balazs, 2000; Kirchgessner and Mitchell, 2009; Appendix 5).

Conclusion.—This study is the first to quantify the effects of salinity on stress hormone production and blood biochemistry in free-ranging Texas diamondback terrapins, and is the first known report of aldosterone and prolactin plasma concentrations in free-ranging diamondback terrapins in Corpus Christi, Texas. Here, I have shown that typical estuarine salinities (~0.5–35 ppt) do not induce a physiological response in stress hormone and blood biochemistry plasma 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.

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Appendix 1. Scientific Permit Number SPR-0910-148.

TEXAS PARKS & WILDLIFE

March 16, 2015

Erin Marie Hill
Texas A&M University – Corpus Christi
Center for Coastal Studies
6300 Ocean Drive, NRC 3200
Corpus Christi, TX 78412

Dear Mrs. Hill:

Enclosed is your amended Scientific Research Permit No. **SPR-0910-148**. Please review your permit for accuracy and make note of any restrictions. If additional authority is needed, you should request a permit amendment.

Although the permit does not have to be renewed each year, annual reports are required for the permit to remain valid. Please note that all mortalities, retained and discarded, must be documented and reported on annual reports. **Your next annual report will be due on September 24, 2015**. The report form is also available online: http://tpwd.texas.gov/business/permits/land/wildlife/research/.

If you have information on the location of a rare plant or animal and would like to help us build the Texas Natural Diversity Database, please contact the Wildlife Diversity Program at 512-389-8111.

Furthermore, it is necessary for you to carry this permit when conducting authorized activities. Sub-permittees may carry a copy in lieu of the original permit. It is also advisable to carry some additional corroborative identification such as a driver's license.

Please be sure to notify the Texas Parks and Wildlife Department Law Enforcement Office(s) in the region(s) of your field activities by telephone not less than 24 hours nor more than 72 hours prior to collection if collection techniques or devices being used are ordinarily classified as illegal (e.g. using gill nets or electro-shocking devices to collect fish, hunting/collecting along public roads and rights-of-way). **A confirmed response from the local game warden is required prior to collection if the sampling activities being conducted involve methods of capture ordinarily classified as illegal.** To determine appropriate regional office location and/or telephone number, please see http://www.tpwd.state.tx.us/warden/connect/offices. If the regional office(s) or telephone number(s) is unknown, the number(s) may be obtained at any time by calling a Parks and Wildlife Communication Center: Austin - (512) 389-4848; Houston - (281) 842-8100.

In addition, please be advised that collecting in a wildlife management area is not authorized without prior written permission from the area manager.

Please note that you will be required to obtain/hold a U.S. Fish & Wildlife Service federal permit for collection and/or handling of federally protected wildlife, including banding, possession, and/or salvage of migratory birds. Should you have any questions or require further assistance, please contact me via email, as it is the best way of communicating with me.

Sincerely,

Christopher Maldonado
Wildlife Permits Specialist

Appendix 1. Continued.

SCIENTIFIC PERMIT NUMBER SPR-0910-148
IS HEREBY ISSUED TO:

Erin Marie Hill
Texas A&M University – Corpus Christi

UNDER THE AUTHORITY OF CHAPTER 43, SUBCHAPTER C OF THE
TEXAS PARKS AND WILDLIFE CODE

The activities permitted by this document are to be carried out in accordance with the Texas Parks and Wildlife Code, the Rules and Regulations of the Texas Parks and Wildlife Commission, and all of the following provisions:

1. This permit may not be transferred, assigned or conveyed by the holder.

2. The issuance of this permit is not a guarantee that a subsequent permit or renewal of this permit will be granted.

3. Required information and data shall be maintained at the address of the permit holder and shall be available for inspection at the request of personnel of the Texas Parks and Wildlife Department during the active life of the permit.

4. Acceptance of this permit constitutes an acknowledgment and agreement that the holder will comply with all Rules, Regulations, Orders and Proclamations of the Texas Parks and Wildlife Commission issued in accordance with the law and the conditions precedent to the granting of this permit. Failure to comply with any and all provisions of this permit may result in enforcement action, including any criminal penalties authorized by the Parks and Wildlife Code.

5. This permit does not relieve the holder of the responsibility to obey all other local, county, state and federal laws while carrying out the authorized activities.

- Issued by:

March 16, 2015
Amended date

Chris Maldonado
Wildlife Permits Specialist

6. This permit will expire at midnight, **September 24, 2016**.

7. The following individuals may conduct the activities authorized by this permit under the guidance of the permittee:

SUBPERMITTEES: Beth Almaraz, Shantel Swierc, Lindsey Ramirez, Aaron Baxter, Robert Duke

UNPERMITTED ASSISTANTS: A permittee engaging unpermitted assistants shall maintain on file at their office and possess on their person in the field a signed and dated list of all unpermitted persons assisting in permitted activities.

Page 1 of 4

Appendix 1. Continued.

Hill, Erin Marie **Expires 09/24/2016** **Scientific Permit No. SPR-0910-148**

8. The following wildlife species in the specified quantities are authorized by this permit to be:

a. live-capture of terrapins with crab traps and released at capture site unharmed for scientific purposes. Captured terrapins will be tagged, standard measurements collected (weight, carapace width, carapace length, and sex determined). Sub-legal blue crabs will be released at the site of capture. Legal sized blue crabs (>5 inches) will be relocated outside of the study area to prevent the recapture of the same crabs on subsequent days.

Common name	Scientific Name	Quantity
Texas Diamondback Terrapin	*Malaclemys terrapin littoralis*	N.A.
Blue Crab	*Callinectes sapidus*	N.A.

b. ASSESSING POPULATION TRENDS, SALINITY, INDUCED STRESS, AND GENETIC DIFFERENCES IN DIAMONDBACK TERRAPIN POPULATIONS FOUND IN THE TEXAS COASTAL BEND - This permit authorizes the live-capture of native species (crab traps, seine nets, cast nets, fyke nets), take blood sample, mark (scute notch and PIT tag), and release unharmed at capture site for scientific research purposes. All non-target species will be immediately released at site of capture.

Common name	Scientific Name	Quantity
Diamondback terrapin	*Malaclemys terrapin littoralis*	NTE 100

c. IDENTIFYING NESTING HABITATS FOR TEXAS DIAMONDBACK TERRAPIN IN THE NUECES ESTUARY, WITH IMPLICATIONS FOR OTHER TEXAS ESTUARIES: This permit authorizes the live-capture of native speices (modified crab traps, seine nets, cast nets, and fyke nets), portable ultrasound screening of females, attach radio and/or acoustic transmitter to limited quantity of females, and release unharmed at capture site for scientific research purposes. All non-target species will be immediately released at site of capture.

Common name	Scientific Name		Quantity
Diamondback terrapin	*Malaclemys terrapin littoralis*	(catch and release)	N.A.
		(attach transmitter)	NTE 9

9. The following means for taking or capture are authorized by this permit:

a. baited crab traps that will be deployed for no more than 24 hours, or as specified above.

b. ASSESSING POPULATION TRENDS, SALINITY, INDUCED STRESS, AND GENETIC DIFFERENCES IN DIAMONDBACK TERRAPIN POPULATIONS FOUND IN THE TEXAS COASTAL BEND – modified crab traps, seine nets, cast nets, and fyke nets.

c. IDENTIFYING NESTING HABITATS FOR TEXAS DIAMONDBACK TERRAPIN IN THE NUECES ESTUARY, WITH IMPLICATIONS FOR OTHER TEXAS ESTUARIES - Modified crab traps, seine nets, cast nets, and fyke nets.

10. The following locations for taking or capture are authorized by this permit:

a. Nueces Estuary: San Patricio and Nueces County

Appendix 1. Continued.

b. ASSESSING POPULATION TRENDS, SALINITY, INDUCED STRESS, AND GENETIC DIFFERENCES IN DIAMONDBACK TERRAPIN POPULATIONS FOUND IN THE TEXAS COASTAL BEND – Corpus Christi, Aransas, and San Antonio Bay Systems

c. IDENTIFYING NESTING HABITATS FOR TEXAS DIAMONDBACK TERRAPIN IN THE NUECES ESTUARY, WITH IMPLICATIONS FOR OTHER TEXAS ESTUARIES – Nueces Estuary

11. All specimens taken or salvaged shall be deposited with an appropriate collection, or otherwise disposed of in accordance with paragraph 13d of this permit.

12. **All collection gear left unattended shall be clearly marked with permittee's name and permit number.**

13. **PERMIT HOLDER IS REQUIRED TO:**

a. File a completed report form annually (provided on issuance of this permit), and any reports or publications based on data collected under authority of this permit, with the Texas Parks and Wildlife Department, Wildlife Permits Section, 4200 Smith School Rd., Austin, TX, 78744, **no later than fourteen days following the anniversary date of the permit** (or the expiration date if the permit is due for renewal).
YOUR PERMIT WILL NOT BE VALID UNLESS YOUR REPORT HAS BEEN RECEIVED.

b. Carry a copy of this permit at all times when exercising the provisions of this permit, which shall be subject to inspection by any authorized enforcement officer of the Department upon request.

c. Notify the Texas Parks and Wildlife Department Law Enforcement Office(s) in the region(s) of your field activities by telephone not less than 24 hours nor more than 72 hours prior to collection if collection techniques or devices being used are ordinarily classified as illegal (e.g. using gill nets or electro-shocking devices to collect fish, hunting/collecting along public roads and rights-of-way). **A confirmed response from the local game warden is required prior to collection if the sampling activities being conducted involve methods of capture ordinarily classified as illegal.** To determine appropriate regional office location and/or telephone number, please see http://www.tpwd.state.tx.us/warden/connect/offices. If the regional office(s) or telephone number(s) is unknown, the number(s) may be obtained at any time by calling a Parks and Wildlife Communication Center: Austin - (512) 389-4848; Houston - (281) 842-8100.

d. Dispose of protected wildlife taken under the authority of this permit in only one of the following ways:

(1). Kill and utilize by examination, experimentation, necropsy or dispose of as waste in accordance with state law and city or county regulations (burning is suggested if not in conflict with city, county or state regulations).

(2). Hold permanently for scientific or educational purposes, or donate to another educational display, scientific, or zoological permit holder authorized to receive such specimens, **with required specimen donation form provided by the Department. A copy of the completed form must be submitted with the annual report.**

(3). Release unharmed at collection site.

14. **PERMIT HOLDER IS PROHIBITED FROM:**

a. Selling or bartering specimens collected under the authority of this permit. Specimens may be donated to other permit holders by completing the receipt form enclosed with the permit.

Appendix 1. Continued.

b. Collecting on private premises without prior written consent of the owner or operator of the premises.

c. Collecting in a state park without a separate permit from the Texas Parks and Wildlife Department Natural Resource Program: email: david.riskind@tpwd.texas.gov.

d. Collecting in a wildlife management area without prior written permission from the area manager.

e. Taking species listed by the department as threatened or endangered without express authority in paragraph 8 of this permit.

15. **ADDITIONAL PROVISIONS:**

a. No hunting or fishing license is required for permit holders or individuals listed in paragraph 7 while conducting the activities expressly authorized by this permit. Each listed individual should carry a copy of this permit during collection activities, and a letter of permission from the permittee if working independently.

b. This permit is subject to any applicable federal permit requirements. Where a federal permit is required, the permit holder is cautioned to carry a copy of the federal as well as the state permit during collecting activity. For information on the federal permit contact: U.S. Fish and Wildlife Service, PO Box 709, Albuquerque, NM, 87103-0709; (505)248-7882 or FW2_Birdpermits@fws.gov.

16. PERMIT HOLDER'S ADDRESS FOR RECORDKEEPING PURPOSES:

Erin Marie Hill
Texas A&M University – Corpus Christi
Center for Coastal Studies
6300 Ocean Drive, NRC 3200
Corpus Christi, TX 78412
****

Appendix 2. IACUC Protocol Number 01-15.

TEXAS A&M UNIVERSITY
CORPUS CHRISTI

OFFICE OF RESEARCH COMPLIANCE
Division of Research, Commercialization and Outreach

6300 Ocean Drive, Unit 5844
Corpus Christi, Texas 78412
O 361.825.2497 • F 361.825.2755

Institutional Animal Care and Use Committee (IACUC)

APPROVAL DATE:	**March 30, 2015**
TO:	**Dr. Paul Zimba**
CC:	**Mr. Aaron Baxter; Ms. Lindsey Ramirez; Ms. Shantel Swierc**
FROM:	**Institutional Animal Care and Use Committee (IACUC) Office of Research Compliance**
SUBJECT:	**Initial Approval**

Protocol Number:	**01-15**
Title:	**Evaluating stress levels and genetic differences in Texas diamondback terrapin populations**
Expiration Date:	**March 30, 2016**
3rd Year Resubmission Due Date: ***(de novo Review)***	**March 30, 2018**

This research project has been approved. As principal investigator, you assume the following responsibilities:

1. **If animals are housed on campus, a copy of this approval letter should be kept in the lab notebook the IACUC will review during its semiannual facility inspections.**
2. **Amendments: Changes to the protocol must be requested by submitting an Amendment Application to the Research Compliance Office for review by the committee. The Amendment must be approved before being implemented.**
3. **Continuations: A continuation must be requested by submitting a Continuation Application to the Research Compliance Officer for review by the committee. The Continuation must be approved or the project cannot continue beyond the approval expiration date.**
4. **Completion Report: Upon completion of the research project (including data analysis and final written papers), a Completion Report must be submitted to the Research Compliance Office.**
5. **Records Retention: Records must be retained for three years beyond the completion date of the study.**

Provisions:

Comments:

Appendix 3. Data collected for body condition score (BCS) and activity level. Notch numbers followed by parentheses indicates that individuals recapture frequency.

Location	Notch	PIT tag	Date	Sex	Weight (g)	BCS	Activity level
Aransas	1	835-835-074	6/14/2015	F	1260	4	3
Aransas	2	835-832-518	6/14/2015	M	370	3	3
Aransas	3	835-854-092	6/14/2015	M	460	3	3
Aransas	4	835-841-513	8/12/2015	F	1370	4	3
Aransas	5	835-832-372	8/12/2015	F	1520	4	3
Aransas	6	835-842-867	8/12/2015	F	1120	4	3
Aransas	7	835-847-281	8/12/2015	F	1020	3	3
Aransas	8	835-840-800	8/12/2015	F	1240	3	2
Aransas	9	835-833-621	8/12/2015	F	1940	4	3
Aransas	10	835-850-605	8/12/2015	F	1600	4	4
Aransas	11	835-838-555	8/12/2015	F	1600	4	4
Aransas	12	835-833-063	9/24/2015	F	1580	4	3
Aransas	13	835-839-637	9/25/2015	F	1220	4	3
Aransas	14	835-844-840	9/25/2015	M	450	4	3
Aransas	15	835-842-366	9/25/2015	M	430	3	3
Aransas	10 (recap.1)	835-850-605	9/25/2015	F	1540	4	3
Aransas	16	835-842-124	9/25/2015	F	1500	4	4
Aransas	11 (recap.1)	835-838-555	10/10/2015	F	1620	4	3
Aransas	17	835-843-061	10/10/2015	F	1520	4	3
Aransas	18	836-531-322	10/10/2015	F	1160	3	3
Aransas	19	836-381-546	10/10/2015	F	1440	4	3
Aransas	20	836-545-025	10/10/2015	M	380	3	3
Aransas	21	836-532-601	10/27/2015	F	1260	3	3
Aransas	22	836-514-610	10/27/2015	F	1820	3	3
Aransas	23	836-541-881	10/27/2015	F	1620	4	3
Aransas	10 (recap.2)	835-850-605	10/27/2015	F	1700	4	4
Aransas	24	836-537-570	10/27/2015	M	430	3	3
Aransas	25	836-354-836	10/27/2015	F	1560	4	3
Aransas	11 (recap.2)	835-838-555	10/27/2015	F	1700	4	3
Aransas	26	836-380-854	10/27/2015	M	455	3	3
Aransas	27	836-382-545	10/27/2015	F	2020	4	3
Aransas	28	836-520-305	10/27/2015	F	1120	4	3
Aransas	29	836-541-551	11/10/2015	M	490	3	3
Aransas	30	836-525-785	11/10/2015	M	400	3	3
Aransas	10 (recap.3)	835-850-605	11/10/2015	F	1620	4	3
Aransas	31 (T0)	836-534-841	7/17/2016	M	410	3	3
Aransas	31 (T1)	836-534-841	7/17/2016	M	410	3	3
Aransas	31 (T2)	836-534-841	7/17/2016	M	410	3	3

Appendix 3. Continued.

Location	Notch	PIT tag	Date	Sex	Weight (g)	BCS	Activity level
Aransas	27 (T0; recap.1)	836-382-545	8/4/2016	F	1920	4	3
Aransas	27 (T1)	836-382-545	8/4/2016	F	1920	4	3
Aransas	27 (T2)	836-382-545	8/4/2016	F	1920	4	3
Aransas	32 (T0)	836-542-310	8/4/2016	F	1540	4	3
Aransas	32 (T1)	836-542-310	8/4/2016	F	1540	4	3
Aransas	32 (T2)	836-542-310	8/4/2016	F	1540	4	3
Nueces	15	835-848-528	4/21/2015	F	1350	3	3
Nueces	17	835-846-330	4/21/2015	M	360	3	3
Nueces	18	835-849-813	4/21/2015	M	340	3	3
Nueces	31	835-842-001	4/22/2015	F	1380	4	4
Nueces	32	835-844-091	4/22/2015	F	1460	4	3
Nueces	33	835-852-581	4/22/2015	F	1450	4	3
Nueces	34	835-839-860	4/22/2015	M	380	3	3
Nueces	35	835-851-285	4/22/2015	F	980	3	3
Nueces	36	835-847-879	4/22/2015	F	1040	4	3
Nueces	37	835-836-117	4/22/2015	M	380	3	3
Nueces	38	835-841-352	4/22/2015	F	1080	3	3
Nueces	39	835-850-819	4/22/2015	M	350	3	3
Nueces	40	835-846-300	4/22/2015	F	850	3	3
Nueces	40 (recap.1)	835-846-300	5/20/2015	F	820	3	3
Nueces	41	835-835-349	6/2/2015	F	1300	3	4
Nueces	20	835-842-528	6/4/2015	F	1155	-	-
Nueces	42	835-843-523	6/11/2015	M	330	4	3
Nueces	20 (recap.1)	835-842-528	6/18/2015	F	1277	4	4
Nueces	43	835-848-587	6/19/2015	F	1500	3	3
Nueces	44	835-802-030	7/7/2015	F	1020	4	3
Nueces	45	835-840-803	7/7/2015	F	1350	4	3
Nueces	46	835-844-326	7/7/2015	M	380	4	3
Nueces	47	835-839-013	7/7/2015	F	1400	4	3
Nueces	48	835-848-351	9/18/2015	F	980	4	3
Nueces	49	835-846-823	9/18/2015	F	1360	4	2
Nueces	50	835-842-832	9/18/2015	F	1200	4	3
Nueces	51	835-849-087	9/18/2015	F	1650	4	3
Nueces	52	835-838-079	9/18/2015	F	500	3	3
Nueces	53	835-846-610	9/18/2015	F	1140	4	2
Nueces	54	835-843-548	9/18/2015	F	1560	3	4
Nueces	55	835-842-520	9/18/2015	F	290	3	3
Nueces	47 (recap.1)	835-839-013	9/18/2015	F	1440	4	3

Appendix 3. Continued.

Location	Notch	PIT tag	Date	Sex	Weight (g)	BCS	Activity level
Nueces	56	835-848-827	9/18/2015	F	1640	4	3
Nueces	57	835-845-301	9/18/2015	F	1220	3	3
Nueces	58	835-853-059	9/18/2015	F	1300	3	3
Nueces	59	835-844-285	9/18/2015	F	1400	4	3
Nueces	60	835-840-274	9/18/2015	F	1060	4	3
Nueces	61	835-839-596	9/18/2015	F	1280	4	2
Nueces	62	835-841-627	9/18/2015	F	1400	3	2
Nueces	63	835-849-561	9/18/2015	M	420	3	3
Nueces	64	836-381-096	10/29/2015	F	1200	4	3
Nueces	65	836-381-322	10/29/2015	F	1480	3	3
Nueces	66	836-513-126	11/12/2015	F	1510	4	2
Nueces	67	836-523-617	6/29/2016	M	240	3	3
Nueces	68	836-538-006	6/30/2016	M	340	3	3
Oso	1	835-837-565	5/29/2015	M	420	3	4
Oso	2	835-843-836	5/29/2015	F	1500	4	2
Oso	3	835-840-793	5/29/2015	M	360	3	3
Oso	4	835-853-283	5/29/2015	M	470	3	3
Oso	5	835-840-861	5/29/2015	M	455	3	3
Oso	3	835-845-065	5/30/2015	M	320	3	3
Oso	7	835-838-313	5/30/2015	M	370	3	3
Oso	8	835-840-297	5/30/2015	M	365	3	3
Oso	9	835-846-267	5/30/2015	M	390	3	3
Oso	10	835-844-616	5/30/2015	M	325	3	3
Oso	11	835-846-556	5/30/2015	M	350	3	3
Oso	12	835-840-817	5/30/2015	M	405	3	3
Oso	13	835-838-341	5/30/2015	M	410	3	3
Oso	14	835-835-073	5/30/2015	M	375	3	3
Oso	15	835-844-262	5/30/2015	M	420	3	3
Oso	16	835-844-517	5/30/2015	M	310	3	3
Oso	17	835-833-106	5/30/2015	M	395	3	3
Oso	18	835-848-360	5/30/2015	M	390	3	3
Oso	19	835-853-356	5/30/2015	M	360	3	3
Oso	20	835-844-546	5/30/2015	M	370	3	3
Oso	21	835-845-364	5/30/2015	M	350	3	3
Oso	22	835-854-518	5/30/2015	M	375	3	3
Oso	23	835-845-557	5/30/2015	M	350	3	3
Oso	25	835-841-635	6/28/2015	M	380	3	3
Oso	24	835-853-000	6/27/2015	F	1150	4	3

Appendix 3. Continued.

Location	Notch	PIT tag	Date	Sex	Weight (g)	BCS	Activity level
Oso	26	836-541-563	10/16/2015	F	1580	4	3
Oso	27	836-554-349	10/16/2015	M	345	3	3

Appendix 4. Linear discriminant functions.

Linear discriminant functions for each location.

1) Aransas Bay = -1129 + 0.0239*salinity + -0.96843*air.temp + -0.01040* water.temp + -2.8472E-7*weight + 0.51337*CL + 2.1336E-7*CW + 1.46252E-7*CH + 4.72846E-7*PL + -2.2581E-6*PW + 5.38406*CORT + 2.95797*ALDOST + -7.67952*PRL + 1.47216E-7*glucose + -1.3452E-6* sodium + 2.34951E-7*potassium + 1.27425E-9*chloride + 3.46549E-7*CO_2 + 1.00236E-8*GLU + -1.6126E-6*BUN + 4.73983E-7*UA + 0.0000849*CREA + 4.39797E-7*ALB + 3.38265E-7*TPRO + 40.43472*TBILI.

2) Nueces Bay = -868.930 + 0.02050*salinity + -0.66910*air.temp + -0.00738* water.temp + -2.2626E-7*weight + 0.42799*CL + 6.82169E-7*CW + 6.72772E-8*CH + -2.3535E-6*PL + -1.277E-6*PW + 4.57120*CORT + 2.48970*ALDOST + -6.33611*PRL + 8.877E-8*glucose + -6.1096E-7* sodium + -4.97E-7*potassium + -1.9829E-7*chloride + 3.08099E-7*CO_2 + 3.74657E-7*GLU + -1.3455E-6*BUN + -3.2424E-7*UA + 0.0002125*CREA + 8.6296E-7*ALB + 6.51735E-7*TPRO + 33.58372*TBILI.

3) Oso Bay = -1552 + 0.02920*salinity + -1.97788*air.temp + -0.02034* water.temp + -3.563E-7*weight + 0.67373*CL + 5.29207E-7*CW + 1.93988E-7*CH + 3.48183E-7*PL + -2.7322E-6*PW + 7.33553*CORT + 4.04436*ALDOST + -12.11087*PRL + 2.20189E-7*glucose + -2.0476E-6* sodium + 3.68751E-7*potassium + -4.1616E-7*chloride + 5.18772E-7*CO_2 + -2.3423E-8*GLU + -2.1193E-6*BUN + 6.56493E-7*UA + 0.0000876CREA + 6.9575E-7*ALB + 5.38935E-7*TPRO + 64.38036*TBILI.

Appendix 4. Continued.

Linear discriminant functions for each sex.

1) Females = -200.99518 + 0.00612*salinity + -5.5249E-6*air.temp + 0.02797*water.temp + -3.7827E-6*weight + 0.21007*CORT + 0.41387*ALDOST + 0.80649*PRL + -3.7001E-7*glucose + 0.0000215*sodium + -2.46E-6*potassium + -2.7492E-6*chloride + -1.7098E-6*CO_2 + 7.4022E-7*GLU + -3.641E-7*BUN + -6.765E-6*UA + 0.0003381*CREA + 4.69801E-6*ALB + 3.7484E-6*TPRO + -2.98114*TBILI.

2) Males = -188.61417 + 0.00666*salinity + -4.78E-6*air.temp + 0.02579*water.temp + -3.3897E-6*weight + 0.18965*CORT + 0.44995*ALDOST + 0.73416*PRL + -3.089E-7*glucose + 0.0000184*sodium + -2.0924E-6*potassium + -2.3359E-6*chloride + -1.4555E-6*CO_2 + 6.35371E-7*GLU + -3.0441E-7*BUN + -5.7628E-6*UA + 0.0002907*CREA + 4.02859E-6*ALB + 3.19493E-6*TPRO + -2.68374*TBILI.

Linear discriminant functions for females for each location.

1) Aransas Bay = -245.74340 + 0.00375*salinity + -4.3936E-6*air.temp + 0.07162*water.temp + -1.5566E-6*weight + -2.0223E-6*CL + -4.7602E-6*CW + -7.3474E-7*CH + -1.7021E-6*PL + -1.6728E-6*PW + 0.06662*CORT + 0.28059*ALDOST + 1.28788*PRL + -8.9601E-8*glucose + 0.0000233*sodium + -1.6496E-6*potassium + -1.8324E-6*chloride + -5.0896E-7*CO_2 + 8.19807E-7*GLU + 3.74709E-7*BUN + -2.8901E-6*UA + 0.0004003*CREA + 5.27478E-6*ALB + 4.15328E-6*TPRO + 0*TBILI.

2) Nueces Bay = -238.94480 + 0.00342*salinity + -3.9151E-6*air.temp + 0.07136*water.temp + -1.5108E-6*weight + -1.5797E-6*CL + -4.3447E-6*CW + -6.6428E-7*CH + -1.6648E-6*PL + -1.6244E-6*PW + 0.18199*CORT + 0.17001*ALDOST + 1.14174*PRL + -5.6358E-8*glucose + 0.0000210*sodium + -1.492E-6*potassium + -1.785E-6*chloride + -1.2466E-7*CO_2 + 7.60239E-7*GLU + 2.94217E-7*BUN + -2.742E-6*UA + 0.0003724*CREA + 4.87438E-6*ALB + 3.83408E-6*TPRO + 0*TBILI.

3) Oso Bay = -292.88997 + 0.00422*salinity + -5.3723E-6*air.temp + 0.07855*water.temp + -2.2288E-6*weight + -2.1411E-6*CL + -5.9114E-6*CW + -8.9805E-7*CH + -2.2285E-6*PL + -2.3417E-6*PW + 0.24233*CORT + 0.25364*ALDOST + 0.98063*PRL + -7.606E-8*glucose + 0.0000285*sodium + -2.0184E-6*potassium + -2.5539E-6*chloride + -1.159E-7*CO_2 + 1.04255E-6*GLU + 4.26E-7*BUN + -3.6539E-6*UA + 0.0005120*CREA + 6.66621E-6*ALB + 5.24237E-6*TPRO + 0*TBILI.

Appendix 5. Comparison of stress hormones and blood biochemistry in chelonians. Values in bold represent variable concentrations that overlap with concentrations presented in this study for diamondback terrapins. All values are ± SE unless stated otherwise.

Terrestrial	CORT (ng/mL)	ALDOST (ng/mL)	PRL (ng/mL)	Na+ (mEq/L)	K+ (mEq/L)	Cl- (mEq/L)	CO_2 (mEq/L)	References
Radiated tortoise *Geochelone radiata*				121.0-132.0	5.1-5.8	92.0-99.0	24.0-29.0	12
Desert tortoise *Gopherus agassizii*	0.20-4.08 (F)[13]; **0.58-6.45 (M)[13]**			**130-157[12]**	2.2-4.5[12]			12, 13
Aldabra tortoise *Geochelone giganteus*				133 (129-136)	**4.7 (3.2-6.1)**	93 (87-107)		12
Gopher tortoise *Gopherus polyphemus*				**138 (127-148)**	**5.0 (2.9-7.0)**	**102 (35-128)**		12
Mediterranean tortoise *Testudo hermanni*	32 ± 2.5[19]	0.083 ± 0.01[19]		130-144[12]; 132.0 ± 2.0[19]	4.5-5.0[12]; 4.9 ± 0.1[19]	**96-115[12]**		12, 19
Leopard tortoise *Geochelone pardalis*				128-145	3.8-5.1			12
Pancake tortoise *Kinixys erosa*				111-146	4.2-6.1	**83-116**		12
Common box turtles *Terrapene carolina*				**144 (138-149)**	**5.6 (3-9.7)**	106 (101-112)		12
Reference tortoise profile								14

Appendix 5. Continued.

Terrestrial Continued	GLU (mg/dL)	BUN (mg/dL)	UA (mg/dL)	CREA (mg/dL)	ALB (g/dL)	TPRO (g/dL)	TBILI (mg/dL)	References
Radiated tortoise *Geochelone radiata*	**46.2-92.8**		**0.0-0.6**		0.8-1.3	3.2-5.0		12
Desert tortoise *Gopherus agassizii*	**30-150**[12]		2.2-9.2[12]			**2.2-5.0**[12]		12, 13
Aldabra tortoise *Geochelone giganteus*	50 ± 16		**1.6 (0-4.9)**	0.1 (0.1-0.2)	**1.5 (0.3-2.6)**	**4.1 (0.6-6.2)**	**0.2 (0-0.3)**	12
Gopher tortoise *Gopherus polyphemus*	**75 ± 4.5 (55-128)**	**30.1 ± 8.7 (1.0-130.0)**	**3.5 (0.9-8.5)**	**0.3 (0.1-0.4)**	**1.5 (0.5-2.6)**	**3.1 (1.3-4.6)**	**0.02 (0-0.1)**	18
Mediterranean tortoise *Testudo hermanni*			2.1-9.8[12]	< **0.3**[12]		3.1-5.4[12]		12, 19
Leopard tortoise *Geochelone pardalis*			**1.2-1.6**	0.9-2.5	**1.2-2.2**	3.2-4.9		12
Pancake tortoise *Kinixys erosa*			**0.9-9.2**	0.1-0.3	**1.2-2.1**	**2.4-4.1**		12
Common box turtles *Terrapene carolina*	**84 (33-155)**		**1.6 (0.5-3.1)**	0.4	**2.2 (1.2-3.2)**	**5.6 (2.7-7.5)**	**0.5 (0.1-1)**	12
Reference tortoise profile	10 (mmol/L) post-hibern.; 1.4 (mmol/L) pre-hibern.		10.0-100 (µmol/L)	20.0-150 (mmol/L)	25.0-41.0 (g/L)	55.0-75.0 (g/L)		14

Appendix 5. Continued.

Freshwater	CORT (ng/mL)	ALDOST (ng/mL)	PRL (ng/mL)	Na+ (mEq/L)	K+ (mEq/L)	Cl- (mEq/L)	CO_2 (mEq/L)	References
Red-eared slider *Trachemys scripta*	**0-10[2]**	0.34 ± 0.02[11]		137 (133-140)[12]; 125.6 ± 0.85 (mmol/L)[11]	6.3 (4.3-8.3)[12]; 4.16 ± 0.12 (mmol/L)[11]	102 (97-107)[12]; 96.13 ± 2.32 (mmol/L)[11]		2, 11, 12
Giant Snake-Necked Turtle *Chelodina expansa*				117.3 ± 2.2 (mmol/L)	3.25 ± 0.04 (mmol/L)	81.3 ± 2.3 (mmol/L)		1
Murray River Turtle Emydura macquarii				127.8 ± 1.2 (mmol/L)	3.34 ± 0.05 (mmol/L)	92.1 ± 1.7 (mmol/L)		1
Mediterranean turtle *Mauremys caspica leprosa*				117.6 ± 14.3 summer; 123.98 ± 7.02 autumn	3.10 ± 0.43 summer; 3.58 ± 1.13 autumn			17
Eastern mud turtle *Kinosternon subrubrum*				121 (mmol/L)	42 (mmol/L)	98 (mmol/L)		3
Common map turtle *Graptemys geographica*				124 (mmol/L)	24 (mmol/L)	87 (mmol/L)		3
Giant South American turtle *Podocnemis expansa*	**5.49 ± 3.96 SD (0.70-26.2; F)[7]; 4.48 ± 4.03 SD (0.80-25.2; M)[7]**		**0.81 ± 0.52 SD (0.12-3.37; F)[7]; 0.99 ± 0.61 SD (0.10-3.58; M)[7]**					7, 15

Appendix 5. Continued.

Freshwater Continued	GLU (mg/dL)	BUN (mg/dL)	UA (mg/dL)	CREA (mg/dL)	ALB (g/dL)	TPRO (g/dL)	TBILI (mg/dL)	References
Red-eared slider *Trachemys scripta*	**67 (20-113)[12];** 3.43 ± 0.23 (mmol/L)[11]		**1.2 ± 0.7[12];** 59.7 ± 4.8 (µmol/L)[11]	0.3 ± 0.1[12]	1.8 ± 0.5[12]	4.5 ± 1.1[12]	0.3 ± 0.3[12]	2, 11, 12
Giant snake-necked turtle *Chelodina expansa*			38.7 ± 2.6 (µmol/L)					1
Murray river turtle *Emydura macquarii*			68.9 ± 9.6 (µmol/L)					1
Mediterranean turtle *Mauremys caspica leprosa*	86.8 ± 16.9 summer; 143.7 ± 14.6 autumn		5.0 ± 1.8 summer; 4.5 ± 0.8 autumn		0.89 ± 0.08 summer; 1.25 ± 0.01 autumn	3.29 ± 1.27 summer; 4.15 ± 0.41 autumn		17
Eastern mud turtle *Kinosternon subrubrum*								3
Common map turtle *Graptemys geographica*								3
Giant South American turtle *Podocnemis expansa*	91.3 ± 17.7 (84.9-97.7)[15]					3.5 ± 1.3 (3.0-4.0)[15]		7, 15

Appendix 5. Continued.

Marine	CORT (ng/mL)	ALDOST (ng/mL)	PRL (ng/mL)	Na+ (mEq/L)	K+ (mEq/L)	Cl- (mEq/L)	CO_2 (mEq/L)	References
Green sea turtles *Chelonia mydas*								5
Loggerhead sea turtles *Caretta caretta*	**4.71 ± 0.96 (small)[8];** 1.19 ± 0.08 (large)[8]			**147.0–154.0 (mmol/L)[4]**	**3.2–5.2 (mmol/L)[4]**			4, 8
Kemp's Ridley sea turtle *Lepidochelys kempi*	0.09 ± 0.02[16]; **0.03-82.87[9]**	0.35 ± 0.03[16]		153 ± 2[16]	4.3 ± 0.4[16]	123 ± 1[16]		9, 16

Marine Continued	GLU (mg/dL)	BUN (mg/dL)	UA (mg/dL)	CREA (mg/dL)	ALB (g/dL)	TPRO (g/dL)	TBILI (mg/dL)	References
Green sea turtles *Chelonia mydas*	108.5	16.65	**1.38**	0.3	2.32			5
Loggerhead sea turtles *Caretta caretta*	**60.0–109.0[4];**		**0.5–1.70[4]**		1.2-1.7[4]	4.1–5.6[4]		4, 8
Kemp's Ridley sea turtle *Lepidochelys kempi*	4.4 ± 0.3 (mmol/L)[16]; **41.3-175.1[9]**							9, 16

Appendix 5. Continued.

Brackish Water	CORT (ng/mL)	ALDOST (ng/mL)	PRL (ng/mL)	Na+ (mEq/L)	K+ (mEq/L)	Cl- (mEq/L)	CO_2 (mEq/L)	References
Diamondback Terrapin *Malaclemys terrapin*	0.76 ± 0.58 SD (enclosure)[10]; 0.55 ± 0.45 SD (free-ranging)[10]; 0.08-1.35 (PCB 126 exposed)[6]			155.7 ± 2.9 Jan; 155.1 ± 2.1 Feb; 144.3 ± 1.7 Mar (mmol/L)[10]	2.9 ± 0.1 Jan; 2.8 ± 0.1 Feb; 3.1 ± 0.1 Mar (mmol/L)[10]	110.1 ± 1.9 Jan; 107.7 ± 3.8 Feb; 104.3 ± 1.2 Mar (mmol/L)[10]		6, 10
Diamondback Terrapin *Malaclemys terrapin*	4.66 ± 1.01	0.13 ± 0.02	2.53 ± 0.17	147.1 ± 0.9 (mmol/L)	3.78 ± 0.04 (mmol/L)	113.5 ± 1.5 (mmol/L)	23.2 ± 0.6 (mmol/L)	Present Study

Brackish Water Continued	GLU (mg/dL)	BUN (mg/dL)	UA (mg/dL)	CREA (mg/dL)	ALB (g/dL)	TPRO (g/dL)	TBILI (mg/dL)	References
Diamondback Terrapin *Malaclemys terrapin*	2.6 ± 0.5 Jan; 2.1 ± 0.2 Feb; 3.3 ± 0.4 Mar (mmol/L)[10]		234.2 ± 34.9 Jan; 261.7 ± 12.6 Feb; 134.8 ± 37.0 Mar (µmol/L)[10]					6, 10
Diamondback Terrapin *Malaclemys terrapin*	72.2 ± 3.2	57.8 ± 3.2	1.6 ± 0.2	0.0 ± 0.0	2.1 ± 0.1	2.7 ± 0.1	0.05 ± 0.01	Present Study

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