A COMPARATIVE ASSESSMENT OF GENETIC VARIATION OF DIAMONDBACK TERRAPIN (*MALACLEMYS TERRAPIN*) IN GALVESTON BAY, TEXAS IN RELATION TO OTHER NORTHERN GULF COAST POPULATIONS

by

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DEDICATION

In loving memory of my uncle,

Paul Dent Hogan,

(October 29, 1946 – July 29, 1993)

whose insatiable curiosity and beloved admiration for all living things
inspired my earliest questions about the natural world.

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ABSTRACT

A COMPARATIVE ASSESSMENT OF GENETIC VARIATION OF DIAMONDBACK TERRAPIN (*MALACLEMYS TERRAPIN*) IN GALVESTON BAY, TEXAS IN RELATION TO OTHER NORTHERN GULF COAST POPULATIONS

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The majority of diamondback terrapin (*Malaclemys terrapin*) genetics studies have focused on Atlantic Coast populations. In contrast, only a few studies have been published examining the genetic structure of Gulf Coast terrapin (Forstner et al. 2000; Hart 2005; Hauswaldt & Glenn 2005; Coleman 2011). Particularly, information is lacking for populations along the northern Gulf Coast of Mexico within the subspecies ranges of the Texas (*M. t. littoralis*) and Mississippi (*M. t. pileata*) diamondback terrapin. Previous to this study, the only northern Gulf Coast populations to have been genetically assessed in published literature were in Nueces Bay, Texas, Cocodrie Bayou, Louisiana, and Mobile Bay, Alabama (Forstner et al. 2000; Hart 2005; Hauswaldt & Glenn 2005; Coleman 2011). To date, no genetic studies have been published on terrapin populations in Galveston Bay, Texas, which is located on the eastern end of the *M.t. littoralis* subspecies range. This study provides the first genetic information for terrapin populations in Galveston Bay and offers a comparison of genetic variation and diversity among other northern Gulf Coast populations utilizing polymorphic microsatellite DNA markers developed by King and Julian (2004). Reference DNA samples were acquired from previously sampled northern

Gulf Coast populations in Nueces, TX, Louisiana, and Alabama, and were compared with Galveston Bay terrapin. Results found in previous studies (Hart 2005; Coleman 2011) were also compared with the results of the reference samples collected in this study, as well as with the genetic diversity found for Galveston Bay. Analyses of molecular variance (AMOVA) were performed to test for genetic differentiation among populations using Wright's F-statistics fixation and differentiation estimator indices. Observed heterozygosities were tested for agreement with Hardy-Weinberg Equilibrium to determine the likelihood of random mating within and among populations. Genetic diversity was assessed based on the number of different alleles observed within each population and compared with results of diversity using Shannon's Information Index. Twenty-one informative alleles on 8 different loci with frequencies of at least 5% were identified for characterizing individuals from northern Gulf Coast terrapin populations and pairs of populations. No significant genetic differentiation was found within Galveston Bay populations. However, with the exception of the Louisiana and Alabama populations, the northern Gulf Coast populations exhibited a significant degree of genetic differentiation among populations and demonstrated a direct, positive correlation with spatial distribution between each pair of populations. Based on the findings of this study, it was concluded that northern Gulf Coast terrapin populations (ranging the coast from Nueces Bay, TX east to Dauphin Island, AL) are distributed within 3 distinct genetic metapopulations, where Louisiana and Alabama terrapin are within a single metapopulation, and the two Texas terrapin populations (Nueces and Galveston) were each within a distinct metapopulation. Additionally, based on the populations sampled in this study, the minimal spatial distance segregating any neighboring pair of genetically distinct northern Gulf Coast metapopulations was found to be approximately 300 kilometers. No significant difference in genetic diversity was found among the northern Gulf Coast populations. The findings of this study emphasize the importance of how additional terrapin population genetics studies in non-sampled areas, in combination with previously collected data, can alter and refine scientific understanding of how species genetic metapopulations interact.

Key words: diamondback terrapin --- *Malaclemys terrapin* --- Galveston Bay terrapin --- northern Gulf Coast terrapin --- population genetics --- genetic variation --- genetic diversity --- microsatellite DNA

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INTRODUCTION

Life History

Due to its unique euryhaline physiological adaptations, the diamondback terrapin (*Malaclemys terrapin*) is a distinct species as it is the only member of the family Emydidae that is strictly estuarine, inhabiting marine estuaries, coastal rivers, and mangrove swamps. Its closest Emydid relative is the map turtle of the genus *Graptemys* (Lamb & Osentoski 1997). The diamondback terrapin is endemic to coastal salt marsh ecosystems in North America ranging across 16 states along the Atlantic Coast and Gulf Coast of Mexico from Cape Cod, Massachusetts to Corpus Christi, TX (Brennessel 2006).

The diamondback terrapin's role in the salt marsh food web is complex, which makes it a valuable subject of study in the conservation of salt marsh ecosystems. Terrapin prey upon snails, crabs and fish and are therefore considered a tertiary consumer within the salt marsh community (Brennessel 2006). Terrapin are thought to be key players in their ecosystems as they prey upon and have a significant, direct influence on the densities of the salt marsh periwinkle, *Littorina irrorata* (Hauswaldt & Glenn 2005). As periwinkle snails impact primary production in saltmarshes by grazing upon algae growing on saltmarsh cordgrass (*Spartina alterniflora*), the extirpation of terrapin from the saltmarsh food web could indirectly cause an overall decline in the productivity of the saltmarsh community (Brennessel 2006).

Historically, terrapin were viewed as an undesirable by-catch species in the 1700's and were a food item only consumed by the underprivileged, such as slaves and servants on plantations and to soldiers in the Continental army (Brennessel 2006; Hart & Lee 2006; Schaffer et al. 2008; Coleman 2011). By the early 1800's, terrapin transitioned into a popular gourmet delicacy for the upper-middle class and elite, known as "terrapin stew" (Coker 1906, 1920; Hildebrand & Hatsel 1926; Hildebrand 1928; Hildebrand 1933; Schaffer et al. 2008).

The late 19th and early 20th century sparked the era of overexploitation of terrapin which led to a major decline in natural stocks (Coker 1906, 1920; Schaffer et al. 2008). In order to alleviate reduction of wild populations while continuing to meet commercial demands, the United

States Bureau of Fisheries established farms in Maryland and North Carolina for commercially harvesting the species (Coker 1906, 1920; Barney 1924, Hildebrand and Hatsel 1926; Hildebrand 1929). Additionally, private farms were established (Hildebrand and Hatsel 1926), which began the uncontrolled anthropogenic alteration of the species gene flow. Terrapin were transferred from various regions of the country, including Texas and Alabama, to the northern Atlantic coast where the terrapin stew dish was most popular (Hildebrand & Hatsel 1926; Hildebrand 1928; Hildebrand 1933; Coleman 2011).

The 1930's brought an end to terrapin farming and commercial trade at the beginning of the Great Depression and prohibition. The dish was no longer affordable to most people and prohibition prevented legal access to sherry, another key ingredient in terrapin stew (Coleman 2010; Brennessel 2006; Hart and Lee 2006; Schaffer et al 2008). The reduction in terrapin farming and harvest during the Great Depression may have benefitted terrapin populations throughout the United States and temporarily allowed natural stocks to recover. However, since that time, populations were never fully able to be restored due to the multitude of other environmental factors that still threaten the species today.

Current Threats and Conservation Concerns

Since its original commercial overexploitation, terrapin have been threatened by multiple threats to their survival and are in dire need of attention regarding the conservation management of the species. The primary threat identified for terrapin since the 1930's is drowning in crab pots (Seigel & Gibbons 1995; Butler et al. 2006). Certain states, such as Delaware, Maryland, and New Jersey, currently have implemented by-catch reduction device (BRD) regulations on crab fishermen in which excluder devices are mandatory on all commercial and recreational crab pots to reduce the amount of terrapin entering traps (Coleman 2011). None of the other states in the range have implemented BRD regulations, though others are currently reviewing the need for these regulations.

Another major threat to terrapin populations is predation on gravid females and their nests by raccoons, which could be considered both a natural and anthropogenically facilitated source of mortality (Butler et al. 2006). The current status and natural history of raccoon populations in the salt marsh is unknown. It is likely, as a result of human facilitation of raccoon populations (i.e. - dietary supplementation via food scraps and waste, surplus shelter provided by houses and sheds), that raccoons have surpassed their carrying capacity in urban areas and are migrating to nearby salt marshes in pursuit of additional habitat and resources. Additionally, the potential loss of natural habitat due to urbanization may also be contributing to this migration. However, it is also possible that raccoons may have always been a major predator on the diamondback terrapin in salt marshes. If this is the case, eradicating them from the marsh could lead to irrevocable damage of not only the terrapin population but the entire community, since their role in this habitat has yet to be fully assessed (Butler et al. 2006).

Other major threats to diamondback terrapin include habitat loss, road mortalities, mortality and injury from boat propellers, and pollution from chemical contaminants. Terrapin have high site fidelity (Gibbons et al. 2001), so the loss of beach nesting habitat from erosion and development has affected populations. Channels dredging changes hydrology and water depth, and is attributed to increasing the rate of erosion of shorelines (Butler et al. 2006; Marion 1986; Roosenburg 1991a; Morreale 1992; Wood and Herlands 1996). Bulk-heading and seawall construction prevent terrapin from accessing nesting beaches, and vegetation planted for erosion control also degrades nesting habitat (Butler et al. 2006; Roosenburg 1991a). Past studies have documented the infiltration of eggs by plant roots (Brennessel 2006). Based on aerial photos from 1950s to 2002 of Galveston Bay, Texas, salt marsh habitat for terrapin has declined and

been redistributed due to subsidence caused by groundwater pumping from the oil industry in Texas City (Brennessel 2006). A substantial amount of marshes that were present in Galveston Bay in the 1950s photographs have since been replaced with tidal flats and open water (Brennessel 2006).

Roosenburg (1991a) conducted a study on boat injuries and mortality rates and found 20% of adult females from Patuxent River, Maryland had propeller scars, while 2% of males had scars. He concluded that because females travel farther offshore to nest, they are more prone than males to boat injuries (Roosenburg 1991a).

Road mortality has also been found to be a significant contributing factor to skewed male-bias sex ratios among populations of many Emydidae species (Steen et al. 2006). Steen et al. (2006) hypothesized that female turtles, regardless of species, are generally more prone to road mortalities than males due to nesting behavior. Steen et al. (2006) tested their hypothesis by compiling data from previous turtle population surveys conducted both on roads (i.e. – surveys of turtles collected alive or dead on or within close proximity of a roadway) and off roads (i.e. – surveys in which turtles were not collected from roadways, biased toward a single sex, and did not analyze museum specimen collections) and compared the sex-ratios found in each type of study. Their results supported their hypothesis that female turtles are more susceptible to road mortalities than males, as they found a significantly higher number of females were reported in on-road studies than off-road studies by a difference of 20% (Steen et al. 2006). They inferred from their findings that turtle populations dwelling or nesting near roadways are likely to eventually become extirpated from those areas as female traffic mortality increases (Steen et al. 2006). The results of a road mortality study conducted on diamondback terrapin in New Jersey also supported the conclusions by Steen et al. (2006) in that they also found female terrapin to be predisposed to higher road mortality as a result of their need to pursue apposite nesting habitat (Szerlag & McRobert 2006).

The natural environmental fluctuations and gradients to which diamondback terrapin are exposed can be extreme and therefore involves a considerable investment of metabolic energy on the animal. As previously discussed, terrapin populations also often face high levels of nest predation, as well as both direct and indirect negative human influences, further reducing the overall reproductive output of the species. Therefore, it is highly likely that these natural and anthropogenic sources of stress to which diamondback terrapin populations are subjected may lead to local extirpations throughout the species range.

Current Status and Protection

Seven subspecies of *Malaclemys terrapin* have been identified and are classified based on their morphological characteristics and geographical distributions (Figure 1, Table 1). The terrapin subspecies distributions, beginning in Cape Cod, Massachusetts extending south along the Atlantic Coast and along the Gulf Coast of Mexico to Laguna Madre, Texas, are as follows: *M.t. terrapin* (northern diamondback), *M.t. centrata* (Carolina terrapin), *M.t. tequesta* (Florida east coast terrapin), *M.t. rhizophorarum* (Mangrove terrapin), *M.t. macrospilota* (Ornate diamondback), *M.t. pileata* (Mississippi diamondback), and *M.t. littoralis* (Texas diamondback).

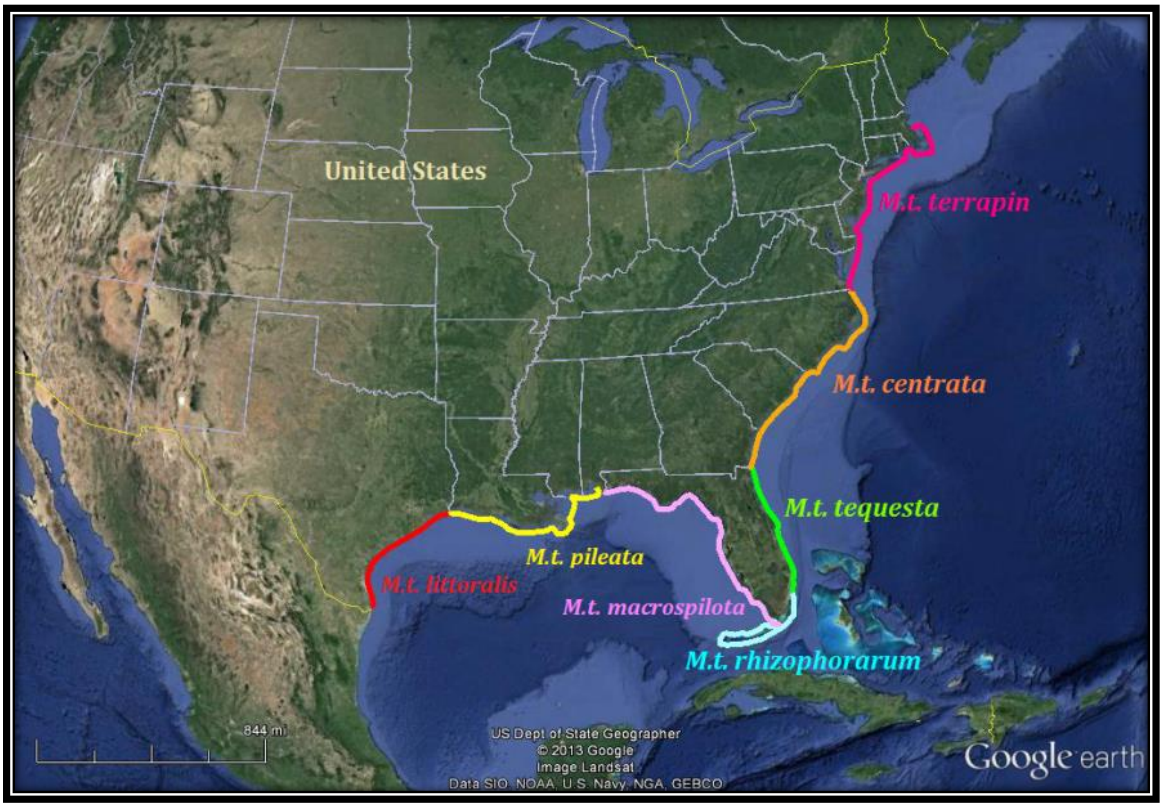

Figure 1 Geographic range and subspecies distribution of *Malaclemys terrapin*.

Table 1 *Malaclemys terrapin* subspecies classifications, morphological characteristics, and geographical ranges (Brennessel 2006).

Subspecies Name	Common Name	Morphological Characteristics	Geographic Range
M.t. terrapin (Schoepff 1793)	Northern diamondback	No knobs on medial keel; Carapace= black to light brown/olive with distinct concentric rings on scutes; Plastron= light-colored, yellow, orange, or greenish gray; Carapace is wider behind bridge	Cape Cod, MA to Cape Hatteras, NC
M.t. centrata (Latreille 1802)	Carolina terrapin	No knobs on median keel; Posterior margins curled upward	Cape Hatteras, NC to northern Florida
M.t. tequesta (Schwartz 1955)	Florida east coast terrapin	Median keel has posterior-facing knobs; Carapace= dark, sometimes tan, with light centers on scutes; No pattern of light concentric circles	Florida east coast
M.t. rhizophorarum (Fowler 1906)	Mangrove terrapin	Median keel has bulbous knobs; Oblong shell; Carapace= brown or black; Plastral scutes outlined in black; Neck and forelimbs= uniform gray with no markings Black striations may be found on hindlimbs	Florida Keys
M.t. macrospilota (Hay 1904)	Ornate diamondback	Median keel has terminal knobs; Carapace scutes have orange or yellow centers	Florida Bay to Florida Panhandle
M.t. pileata (Wied-Neuwied 1865)	Mississippi diamondback	Median keel has terminal tuberculate knobs; Plastron= yellow; Upturned edges of marginals are yellow; Dorsal surfaces of head, neck, and limbs are dark brown or black	Florida Panhandle to western Lousiana
M.t. littoralis (Hay 1904)	Texas diamondback	Deep carapace with terminal knobs on median keel; Plastron= very pale; Dorsal surface of head: white/light color	western Louisiana to western Texas

Although *Malaclemys terrapin* is listed by the 2013 IUCN Red List as a "lower risk/near threatened" species, annotating a need for an update of its current status (Tortoise & Freshwater Turtle Specialist Group 1996), the species is not federally listed under the U.S. Fish and Wildlife Service Endangered Species Act (ESA). Protection offered for the species varies state-by-state due to variation in available population data for each region. Table 2 provides a list of the current status of terrapin within each state. In the northern Gulf Coast states of Texas, Louisiana, Mississippi, and Alabama, it has been recognized as a species of conservation concern.

As a result of numerous studies published on Atlantic Coast terrapin, more information is available for east coast state conservation agencies to make informed management decisions and offer the appropriate protection to this species within their states. However, in many of the Gulf Coast states, (i.e. Texas, Louisiana, Mississippi, and Alabama), comprehensive field and genetic studies have only begun emerging within the past decade, and much information about these populations still remains unpublished and/or unknown. The lack of data available makes it difficult for state agencies and conservation managers in Gulf Coast states to determine the species status and to provide the appropriate protection, if necessary.

Table 2 List of current subspecies status by state.

State	Subspecies	Status
Alabama	*M.t. pileata*	Species of highest conservation concern: P1
Connecticut	*M.t. terrapin*	Not listed; Illegal to collect or possess in any developmental stage
Delaware	*M.t. terrapin*	Species of greatest conservation need
Florida	*M.t. tequesta* *M.t. rhizophorarum* *M.t. macrospilota*	Not listed
Georgia	*M.t. centrata*	Special concern animal and S3
Louisiana	*M.t. pileata*	Species of special concern
Maryland	*M.t. terrapin*	S4 species
Massachusetts	*M.t. terrapin*	Threatened
Mississippi	*M.t. pileata*	Species of special concern and S2
New Jersey	*M.t. terrapin*	Species of special concern
New York	*M.t. terrapin*	Not listed
North Carolina	*M.t. centrata*	Special concern species and S3
Rhode Island	*M.t. terrapin*	Endangered
South Carolina	*M.t. centrata*	Not listed
Texas	*M.t. littoralis*	Species of concern
Virginia	*M.t. terrapin*	S4 species

Previous Genetics Studies

Although many mark-recapture field studies have been utilized over the years to monitor terrapin population trends, only a limited number of genetics studies have been conducted investigating the genetic structure and diversity of the species. Molecular studies are imperative to understanding species population dynamics, as they provide important complementary information to field studies and are a useful tool for establishing evolutionarily significant management units and assessing the genetic stability of populations (Garza & Williamson 2001).

In an early study, Lamb and Avise (1992) genotyped terrapin mitochondrial DNA (mtDNA) using 18 restriction enzymes and found one restriction enzyme from Cape Canaveral, Florida that resulted in one haplotype ranging northward along the Atlantic Coast and the other ranging along the Gulf Coast to Louisiana. The results of this study suggested Cape Canaveral is an ecological transition zone for diamondback terrapin, where the species is genetically split into two groups: Gulf Coast terrapin and East Coast terrapin. As mitochondrial DNA is useful for evaluating long-term evolutionary trends, other studies using microsatellite DNA were needed to assess short-term population bottle-necks (Garza and Williamson 2001) and genetic variation and structure within the species.

Two studies in the early 2000's were published that developed molecular species-specific microsatellite DNA primers for *Malaclemys terrapin* (Forstner et al. 2000; Hauswaldt & Glenn 2003). Forstner et al. (2000) screened a total of 32 microsatellite loci and found 6 to be polymorphic within the *Malaclemys terrapin* species and 4 that were polymorphic within a given subspecies. The study evaluated genetic structure between samples of *M.t. littoralis* that they collected from Nueces Bay, Texas and *M.t. rhizophorarum* populations in the Florida Keys, using both microsatellite and mtDNA. The results of the mitochondrial DNA sequence analyses found little differentiation within the subspecies but did support the phylogenetic relationship of *Malaclemys terrapin* within the Emydidae family (Forstner et al. 2000). The results of the microsatellite DNA analyses supported the findings of (Lamb & Avise 1992), as well as were consistent with the results of the mark-recapture field studies they performed on the study populations. Interestingly, in the results of their microsatellite analyses, Forstner et al. (2000) found a significant correlation of genetic diversity along a distance gradient among the Gulf Coast subspecies populations sampled. This was the only study that found significant differentiation on a local scale (Forstner et al. 2000). Hauswaldt and Glenn (2003) developed six species-specific microsatellite DNA markers for studying ancestral site fidelity and genetic

structure of diamondback terrapin. The primers were tested on seven other species, but at the time, had not been tested for polymorphism.

In the following year, King and Julian (2004) published a study in which they developed polymorphic microsatellite DNA markers for the bog turtle (*Glyptemys muhlenbergii*) for investigating range-wide population genetic structure. They described 27 markers, tested them for cross-species amplification in the Emydid genera, and inferred a micro-evolutionary rate of non-coding nuclear DNA regions for the Emydid family. The eastern diamondback terrapin was compared in the cross-species amplification and King and Julian (2004) found levels of cross-species amplification successes that were 87% similar or greater than rates for species-specific markers, such as the ones developed by Hauswaldt and Glenn (2003). These results suggested a high degree of sequence conservation in the flanking regions of the microsatellite DNA across the 13 Emydid families analyzed, indicating a wide degree of evolutionary separation among the genera. The results of this cross-species comparison also suggested that microsatellite DNA is subject to different evolutionary constraints than flanking sequences (i.e.- sequences extending on either side of a specific locus or gene) because 70% of the cross-species comparisons yielded polymorphisms (King & Julian 2004).

Hauswaldt and Glenn (2005) utilized their previously developed microsatellite markers (Hauswaldt & Glenn 2003) to investigate hypothesized differences in the genetic structure of terrapin populations within an estuary in Charleston, South Carolina. They were unsuccessful in genetically differentiating the populations within the estuary, so they expanded their study to include a broader geographic scale and sampled from other South Carolina estuaries and other states to gauge the influence of spatial differences on the genetic differentiation of the entire species throughout its range (Hauswaldt & Glenn 2005). They found that East Coast terrapin (from New York to South Carolina) were more genetically similar to the terrapin in Nueces, Texas than to terrapin in Florida, which they speculated was attributed to the translocation of terrapin for farming in the early 1900's (Hildebrand & Hatsel 1926; Hildebrand 1928; Hildebrand 1933). The largest variation in genetic structure was found by grouping Florida and Texas terrapin and comparing with East Coast terrapin (Hauswaldt & Glenn 2005). The results of terrapin genetic structure in Charleston estuary supported independent long-term population monitoring data which documented high site fidelity, with the greatest distance of movement documented at 30 kilometers. Genetic analysis failed to identify any differentiation between local populations collected at this distance. Based on their results, Hauswaldt and Glenn (2005) suggested terrapin demonstrate high nesting site fidelity, which may increase their vulnerability to local extirpation when nesting sites are disturbed or exploited by predators. They also found

that microsatellite markers showed much better resolution than mitochondrial DNA used in previous genetic studies (Hauswaldt & Glenn 2005). Although Hauswaldt and Glenn (2005) sampled terrapin from Florida and Texas, they focused primarily on the East Coast terrapin and a large gap along the Gulf Coast range between Florida Bay and Nueces, Texas was not sampled.

Using the markers developed by King and Julian (2004), Hart (2005) compared Gulf Coast terrapin with other terrapin populations across the range. Based on her results, Hart (2005) argued for six management units that do not coincide with the current subspecies boundaries and found the 3 Gulf Coast subspecies to fall under one management unit. A management unit (MU) is defined as one or more genetically homogenous populations with significantly different nuclear or mitochondrial DNA allele frequencies from other management units, regardless of phylogeny (Moritz 1995). An evolutionarily significance unit (ESU), on the other hand is based on phylogenetic relatedness, and the components that define an ESU are reproductive isolation, genetic distinctness, and ecological distinctness (i.e.- unique environmental adaptations) (Ryder 1986; Waples 1991). In regard to short-term genetic management of a species, MU's are preferred over ESU's because changes in allele frequencies in response to population isolation occurs more rapidly than changes in phylogenetic patterns (Moritz 1995). However, when considering translocation of individuals to locally extirpated neighboring estuaries, ESU's should be taken into account in order to preserve evolutionary processes and unique ecological adaptations of the species (Moritz 1995).

Lester (2007) utilized the findings of Hart (2005) to trace the population origin of diamondback terrapin that were seized from the Asian black market in New York City. Using the same 12 loci as Hart (2005) , Lester (2007) performed assignment tests to determine from which metapopulations and subpopulations each terrapin was poached. She found that the majority of the terrapin came from the Chesapeake Bay in the Maryland subpopulations, and found some that originated from mid-Atlantic and the Carolinas coastal regions (Lester 2007).

Coleman (2011) expanded on Hart's (2005) study and utilized the same technique to evaluate the Mississippi diamondback terrapin (*M.t. pileata*) population in Alabama. The genetic diversity of *M.t. pileata* was compared to a subset of data from Hart (2005) representing other Gulf Coast populations. Coleman (2011) detected lower genetic diversity in all of the sampled Gulf Coast populations, with a mean allelic diversity less than 6 alleles per locus for each population. The decreased genetic variation that Coleman (2011) observed in the populations he sampled indicated that a population bottleneck (Garza & Williamson 2001) had occurred resulting from genetic drift. This was further supported by the observed populations all having

low (< 0.40) M ratios (total number of alleles divided by the allelic range), which is indicative of a recent and severe population decline (Garza & Williamson 2001). Garza and Williamson (2001) defined the threshold M ratio for a bottlenecked population to be 0.68. The M ratios found by Hauswaldt and Glenn (2005) were all above the bottleneck threshold, and the allelic diversity and observed heterozygosities of their study differed from Coleman's (2011) results, despite the fact the populations examined from South Carolina, Florida, and Texas were the same for both studies. The results between these two studies most likely differed due to utilization of different loci. Coleman (2011) found that the populations he sampled were categorized into three groups: a South Carolina group, Florida group, and Northern Gulf of Mexico group.

Since field studies can be often limited in detection probability and incorporate observer bias, the results of genetic analyses may offer a more proximate understanding of the gene flow occurring in a population. If a local population has high heterozygous allele frequencies, these results would suggest that individuals from that population may travel farther distances to neighboring populations to initiate mating and further preserve or enhance the genetic diversity of its home population. This behavior may be caused by small local populations and lack of suitable mating partners, which would result in some members searching other accessible colonies for suitable mates. This external mating outside the local population would help sustain or increase genetic diversity. On the other hand, if a population results in high levels of homozygous allele frequencies, this would indicate an isolated population that is either unable to reach neighboring populations due to geographical or spatial barriers or is currently sustaining itself as a population and has no biological pressure to disperse to other areas. These mechanisms assume a higher degree of genetic variability between semi-isolated populations versus within the same populations.

Another study illustrating the use of microsatellite DNA analysis was conducted by Sheridan et al. (2010) in response to the Hauswaldt and Glenn (2005) study documenting high levels of gene flow, to detect sex-biased dispersal and natal philopatry in a New Jersey terrapin population. Using six highly polymorphic microsatellite loci developed by King and Julian (2004), Sheridan et al. (2010) assessed mating dispersal and site fidelity by comparing the results of a 4-year mark-recapture study with the results of their genetic analyses of New Jersey terrapin. They found the mark-recapture results reflected the majority of individuals to have small home ranges of less than 2 kilometers (Sheridan et al. 2010). Adult females were shown to travel the farthest distances and be the most likely to disperse (Sheridan et al. 2010). The results of their genetic analysis showed no significant sex-biased dispersal when juveniles were included in the analysis, but found a male-biased dispersal when they excluded juveniles and only analyzed

adults (Sheridan et al. 2010). Additionally, Sheridan et al. (2010) found little local genetic structure and high levels of gene flow.

In the most recent study, Drabeck et al. (In Press) completed a genetics study on terrapin in Louisiana investigating the impact of the 2010 BP Deep Horizon oil spill. Since information on Louisiana terrapin populations prior to the oil spill was limited, they utilized molecular techniques as an indirect method to establish an ancestral baseline (Drabeck et al. In Press). Individuals from 4 distinct populations, as well as tissue samples from Louisiana State University's natural history museum collection, were genotyped using microsatellite markers from both the Hauswaldt and Glenn (2003) and King and Julian (2004) studies for comparison (Drabeck et al. In Press). Additionally, they performed contaminants analyses to investigate traces of oil contaminants and the effects of the contaminants on the individuals (Drabeck et al. In Press). Drabeck et al. (In Press) found little to no genetic structure among the Louisiana populations. However, when their results were compared with the range-wide study conducted by Hart (2005), they found the Louisiana terrapin to be most closely related to the Nueces Bay, Texas and North Carolina populations (Drabeck et al. In Press).

Of the studies that have used microsatellite markers to determine genetic differentiation of the diamondback terrapin on a local, regional, and range-wide scale, all detected higher degrees of genetic variation on a range-wide scale and low to moderate variation on a regional scale (Avise 1992; Avise et al. 1992; Lamb & Avise 1992; Forstner et al. 2000; Hart 2005; Hauswaldt & Glenn 2005; Sheridan et al. 2010; Coleman 2011; Drabeck et al. In Press). Hart (2005) was able to detect minimal local variation, while Hauswaldt and Glenn (2005) found no population structure within or among adjacent estuaries. Both of these studies, however, agree that there is less differentiation on a local scale than suggested in mark-recapture studies. Forstner et al. (2000) was the only study that reported any significant local differentiation, as well as concurring genetic and field study results.

Information Needed

Since few genetic studies have been published on *Malaclemys terrapin*, additional genetic studies are warranted in order to better understand the cause of low differentiation in local genetic structure among estuaries. Hauswaldt and Glenn (2005) proposed surveying more dinucleotide loci, establishing a higher sampling regime throughout the entire range, isolating more loci to better assess the phylogeographical genetic structure of the species, establishing more refined management units, and more studies defining the genetic differentiation of the Gulf Coast terrapin. Additionally, Hauswaldt and Glenn (2005) found that only terrapin from Florida could be genetically assigned to their source population as a result of genetic mixing in the terrapin trade. Since female terrapin were traded, mitochondrial DNA could better assess the history of translocated populations and natural populations by addressing gender bias in the gene flow (Hauswaldt & Glenn 2005).

Genetic information on the diamondback terrapin is necessary as the results of microsatellite analysis help infer the impacts of humans on terrapin within the past 200 years of the species' life history, particularly in the translocation of terrapin among sites and regions (Hauswaldt & Glenn 2005). Ecological studies supplemented with molecular genetic analyses offer a more holistic approach to understanding the evolutionary progression of a population (Coleman 2011). Additionally, molecular tools allow for taxonomic definition, hybridization, and individual identification (Haig 1998). Genetic diversity and gene flow rates generated from molecular studies are also useful as a measure of a population's sustainability both ecologically and evolutionarily (Coleman 2011).

The results of this study contribute the first preliminary genetic data available for terrapin populations in Galveston Bay, Texas. Furthermore, the findings of this study refine the results of previous studies in the overall understanding of how northern Gulf Coast terrapin populations are genetically related. However, much information is still needed for Galveston Bay terrapin, as well as for other populations along the Gulf Coast. Particularly, genetic data needs to be attained from *M.t. macrospilota* populations along the Gulf Coast and panhandle of Florida, as well as from western Louisiana and from Matagorda Bay, Texas. Sampling of these areas will provide a more precise indication of how the Gulf Coast populations genetically interact.

OBJECTIVES

Overall Study Objectives

The following were the overall objectives set forth in this study:

1) Determine the level of local genetic differentiation and variation of diamondback terrapin (*Malaclemys terrapin littoralis*) within Galveston Bay, Texas;
2) Determine the genetic differentiation among 4 northern Gulf Coast terrapin populations (Nueces Bay, Texas; Galveston Bay, Texas; southeastern Louisiana; Mobile Bay, Alabama) and distinguish northern Gulf Coast genetic metapopulations;
3) Identify informative alleles for characterizing single and/or pair of populations along the northern Gulf Coast;
4) Compare the genetic diversity of Galveston terrapin in relation to other northern Gulf Coast populations;
5) Compare the results of this study with the results of previous Gulf Coast studies performed by Coleman (2011) and Hart (2005).

Null Hypotheses

The following states the null hypotheses tested to address the objectives of the study:

1) There is no local genetic differentiation among terrapin populations within Galveston Bay;
2) Galveston Bay terrapin are genetically homogenous with other northern Gulf Coast terrapin;
3) Random mating is occurring among and within all populations;
4) There is no difference in the genetic diversity of Galveston Bay terrapin from other northern Gulf Coast terrapin populations.

METHODS

Study Populations and Sample Collection

Blood samples were collected and obtained from diamondback terrapin populations along the Gulf Coast states of Texas, southeastern Louisiana, and Alabama during the period of June 2012 to March 2013. The majority of the samples collected in this study were from Galveston Bay, Texas. Since this was the first genetic data collected on terrapin in Galveston Bay, the larger number of samples allowed for analysis of any local genetic differentiation within the bay system (Objective 1). The other regional systems that were sampled served as reference samples for comparing allelic variation and diversity of neighboring populations along a spatial gradient.

Terrapin sampled in Galveston Bay, Texas were acquired in the field by the Environmental Institute of Houston (EIH) staff and graduate students via hand captures during land surveys and trapping in crab pots modified to prevent terrapin from drowning. A total of 6 sites were sampled in Galveston Bay (Figure 2). A total of 54 terrapin blood samples were collected from 5 sites located in west Galveston Bay. The west bay sites were located at Greens Lake, North and South Deer Islands, the saltmarsh adjacent to Sportsmans Road, and the Sweetwater Lake marsh (Figure 3). Seven terrapin blood samples were collected from east Galveston Bay at Little Pasteur Cove (LPC), located off the Bolivar Peninsula (Figure 4).

Texas terrapin sampled in Nueces Bay were acquired using modified crab pots while accompanying Texas A&M University-Corpus Christi (TAMUCC) Center for Coastal Studies (CCS) research staff in field work for a study on the effectiveness of bycatch reduction devices on the blue crab fisheries in Nueces Bay. The blood samples of Nueces Bay terrapin were collected from a total of 8 individuals on two separate collection efforts in September 2012 and April 2013 (Figure 5). Seven of the terrapin sampled were captured in the Nueces River and one terrapin was captured in the open bay.

Figure 2 Map of Galveston Bay, Texas collection sites. *West*: Greens Lake, North and South Deer Islands, Sportsmans and Sweetwater marshes; *East*: Little Pasteur Cove off Bolivar Peninsula. The red boxes highlight the areas displayed in the aerial images below (Figure 3-Figure **4**).

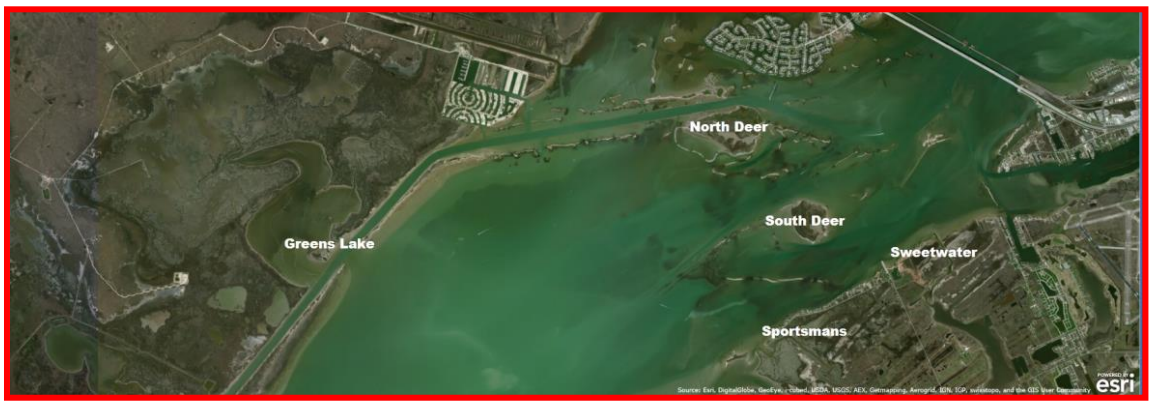

Figure 3 Aerial image of west Galveston Bay sites.

Figure 4 Aerial image of east Galveston Bay site.

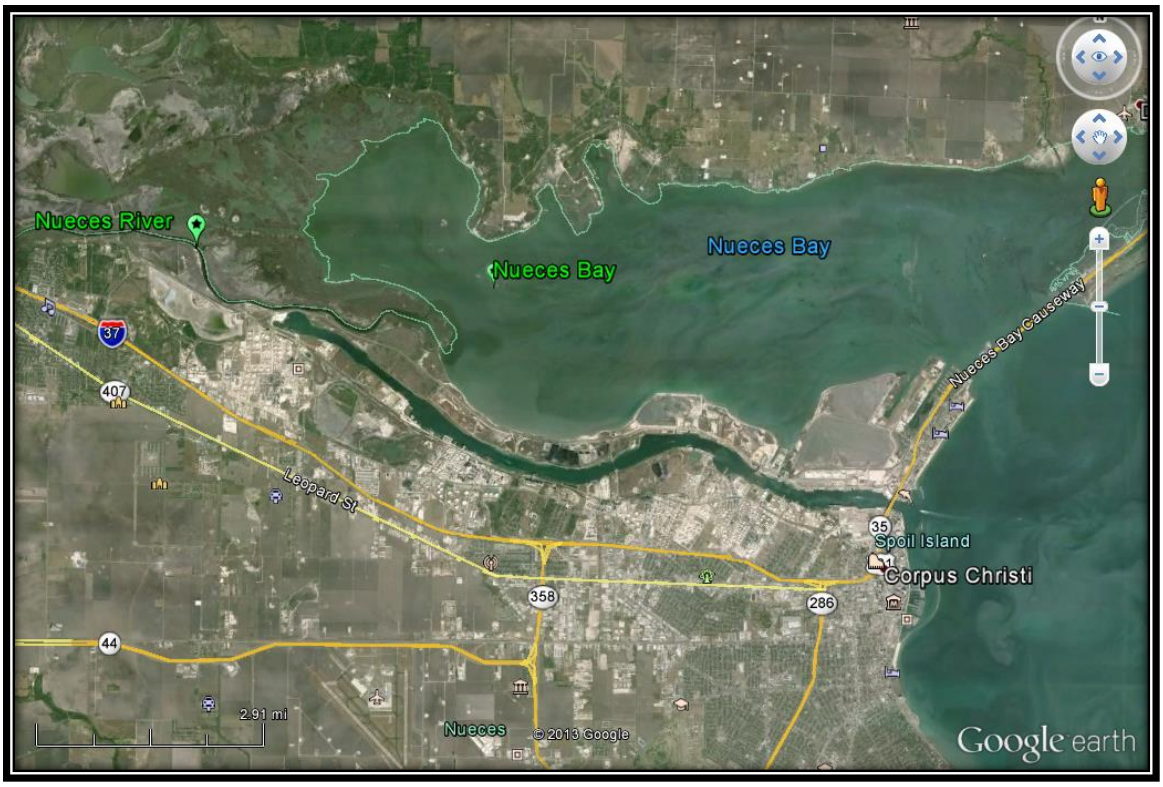

Figure 5 Map displaying sites in Nueces Bay, Texas where terrapin samples were collected for this study.

Louisiana samples were secured from archived samples previously collected by Dr. Cori Richards-Zawacki of Tulane University. The Louisiana samples were excess blood samples that were collected in 2010 as part of a study investigating the effects of the BP Deepwater Horizon oil spill on local Louisiana terrapin populations (Drabeck et al. In Press). Tulane University submitted a total of 12 southeastern Louisiana terrapin samples, composed of 4 individuals per site collected from 3 different sites. The sites from which these samples were collected were Cocodrie Bayou, Lake Michoud, and Shell Beach (Figure 6).

The Alabama blood samples were secured by Dr. Thane Wibbels of the University of Alabama-Birmingham (UAB) from tank-reared juvenile terrapin that are the subjects of a head-start program sponsored by UAB in collaboration with the Dauphin Island Sea Lab (DISL) and the Gulf Coast Diamondback Terrapin Working Group (DTWG). The Alabama samples consisted of 15 whole blood samples from juvenile individuals whose mothers were captured at Cedar Point marsh, located in Mobile Bay north of Dauphin Island (Figure 7).

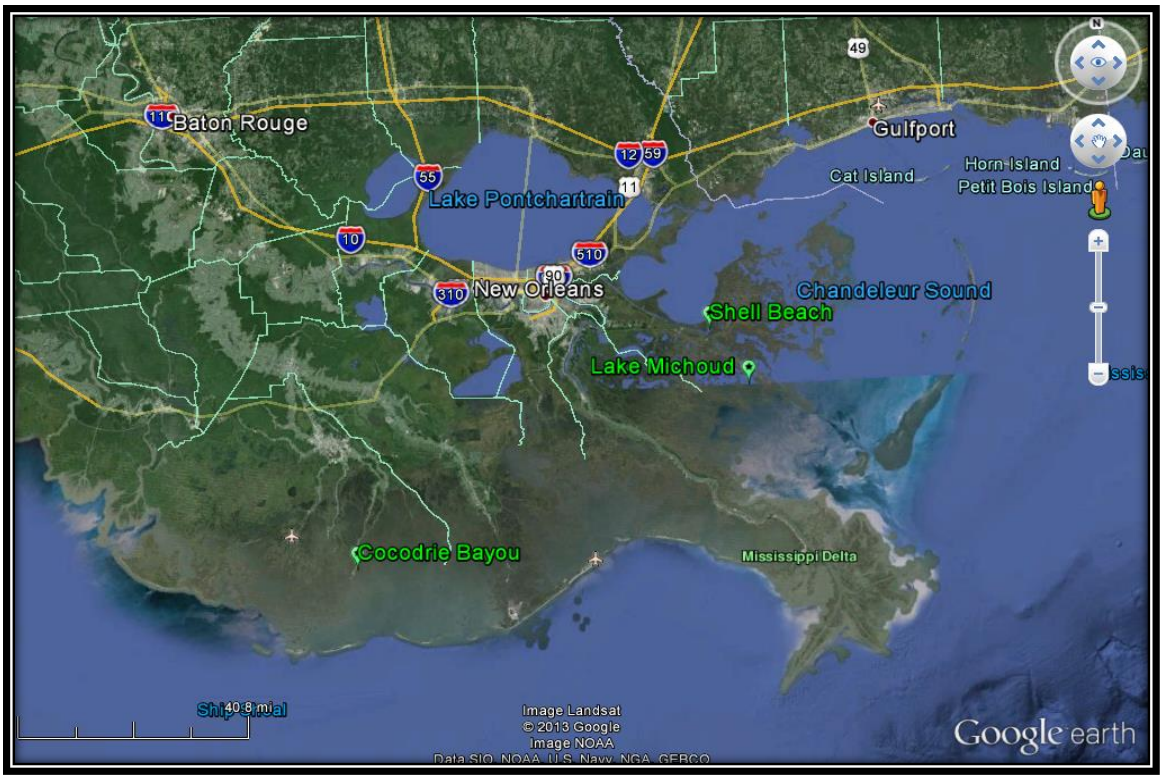

Figure 6 Map displaying sites from where the Louisiana samples were collected by researchers at Tulane University in 2010.

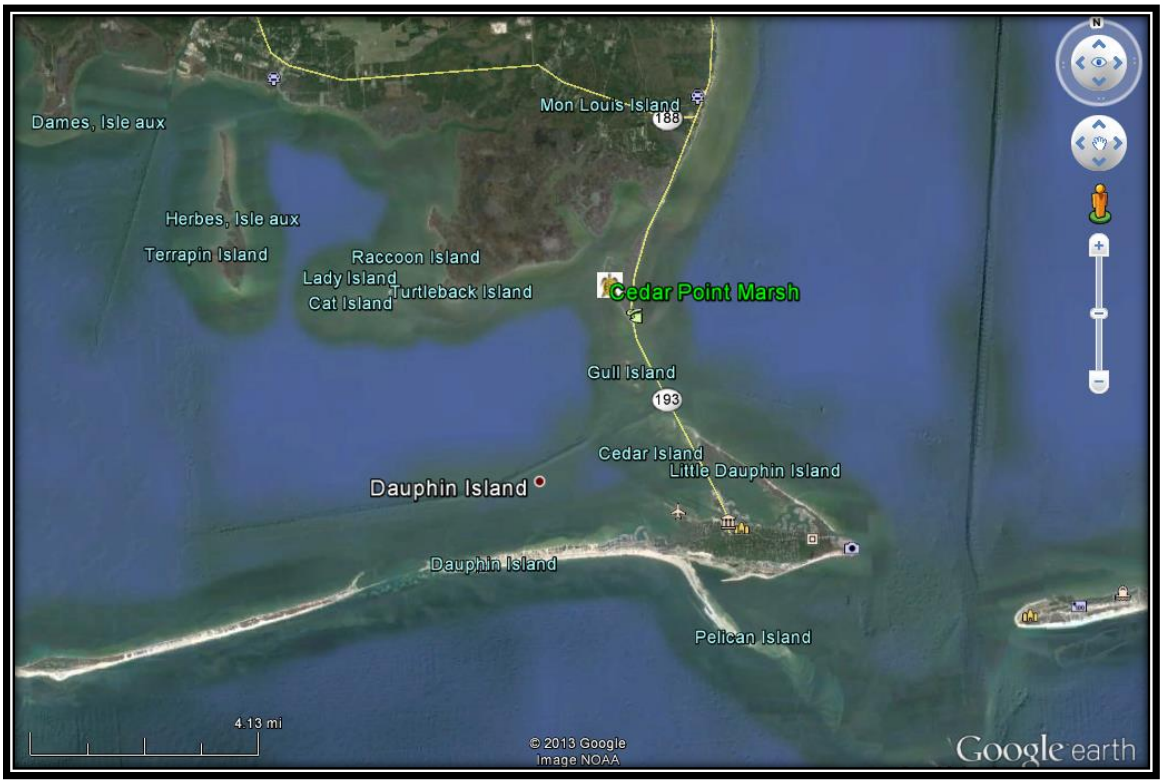

Figure 7 Map displaying Cedar Point Marsh north of Dauphin Island in Mobile Bay, AL which is the site of origin from where the mothers of the Alabama tank-reared juvenile terrapin samples were collected.

A total of 101 individuals were collected across all populations, but only 96 of those samples were genetically analyzed in order to efficiently accommodate the sample array of the standard 96-well plate used in Polymerase Chain Reactions (PCR). The five individuals that were not analyzed were selected based on those with the lowest DNA concentrations from the site with the highest number of individuals collected. The individuals that were not processed were collected from South Deer Island located in west Galveston Bay, Texas. Figure 8 includes a map of the regions sampled illustrating the distance gradient of the collections. Table 3 provides a list of the sites and number of individuals collected for each region under study.

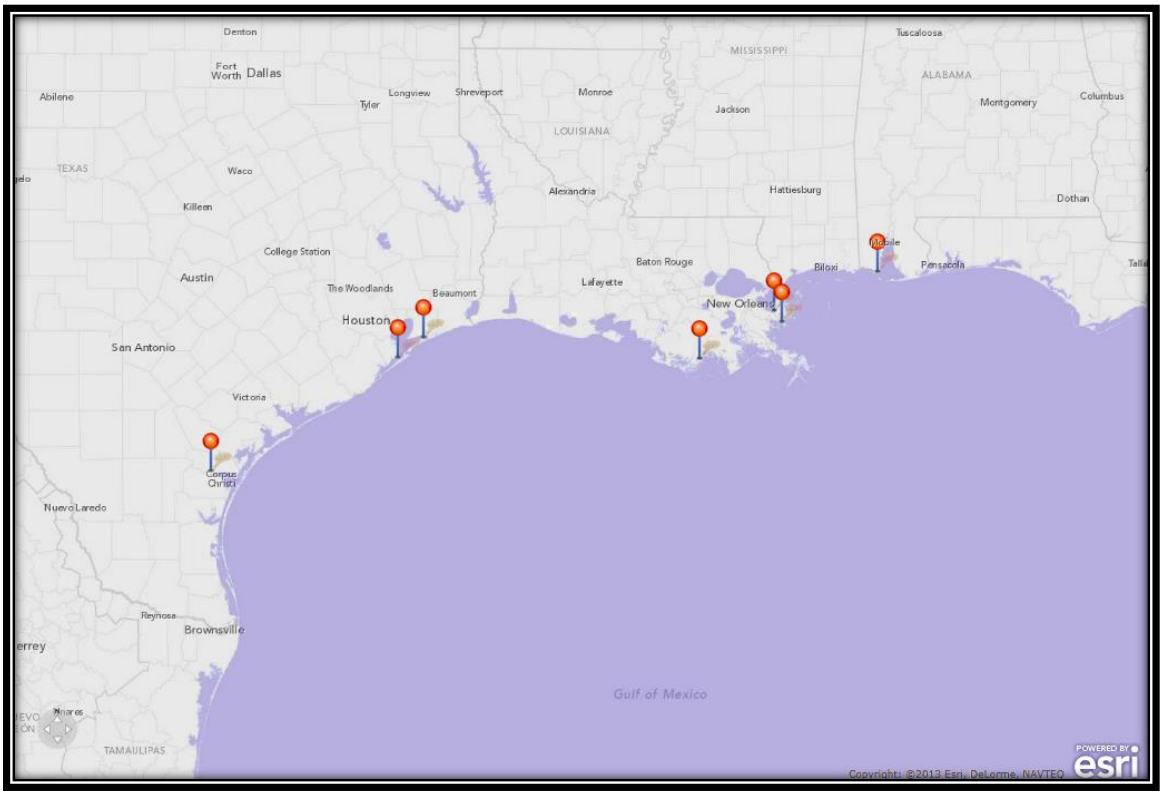

Figure 8 Map of terrapin populations sampled. From west to east: Nueces Bay, TX; west and east Galveston Bay, TX; Cocodrie, LA; Lake Michoud, LA; Shell Beach, LA; Cedar Point marsh north of Dauphin Island in Mobile Bay, AL.

Table 3 List of number of samples collected from each site within each Gulf Coast region sampled.

Gulf Coast Region	Major Water Body	Site	# Samples
Texas - west	Nueces Bay	Nueces River	7
		Nueces Bay	1
		Total:	**8**
Texas - east	West Galveston Bay	Greens Lake	9
		North Deer Island	7
		South Deer Island	26
		Sportsmans Marsh	13
		Sweetwater Marsh	1
	East Galveston Bay	Little Pasteur Cove	7
		Total:	**61**
Louisiana	Lake Quitman	Cocodrie Bayou	4
	Lake Pontchartrain	Lake Michoud	4
	Lake Borgne	Shell Beach	4
		Total:	**12**
Alabama	Mobile Bay	Cedar Point Marsh	15
		Total:	**15**

Blood Tissue Sampling

All blood samples from terrapin collected in Texas were drawn in the field via the subcarapacial sinus vein technique, following the protocol of Hernandez-Divers et al. (2002). All handling and blood collecting procedures were reviewed and conducted under UHCL approved Institutional Animal Care and Use Committee (IACUC) protocol 12.004 R1 (Appendix 1). Dr. Joe Flanagan, Director of Veterinary Services at the Houston Zoo, trained EIH field personnel to perform the method on May 21, 2012 using red-eared sliders (*Trachemys scripta elegans*) captured from the UHCL campus wetlands to demonstrate the method (Figure 9).

The site of venipuncture for the subcarapacial method is located where the dorsal base of the turtle's neck meets beneath the carapace between the two anterior center marginal scutes. This technique was suggested by Dr. Andrew Coleman (Institute for Marine Mammal Studies) and was also the selected method used by Sheridan et al. (2010).

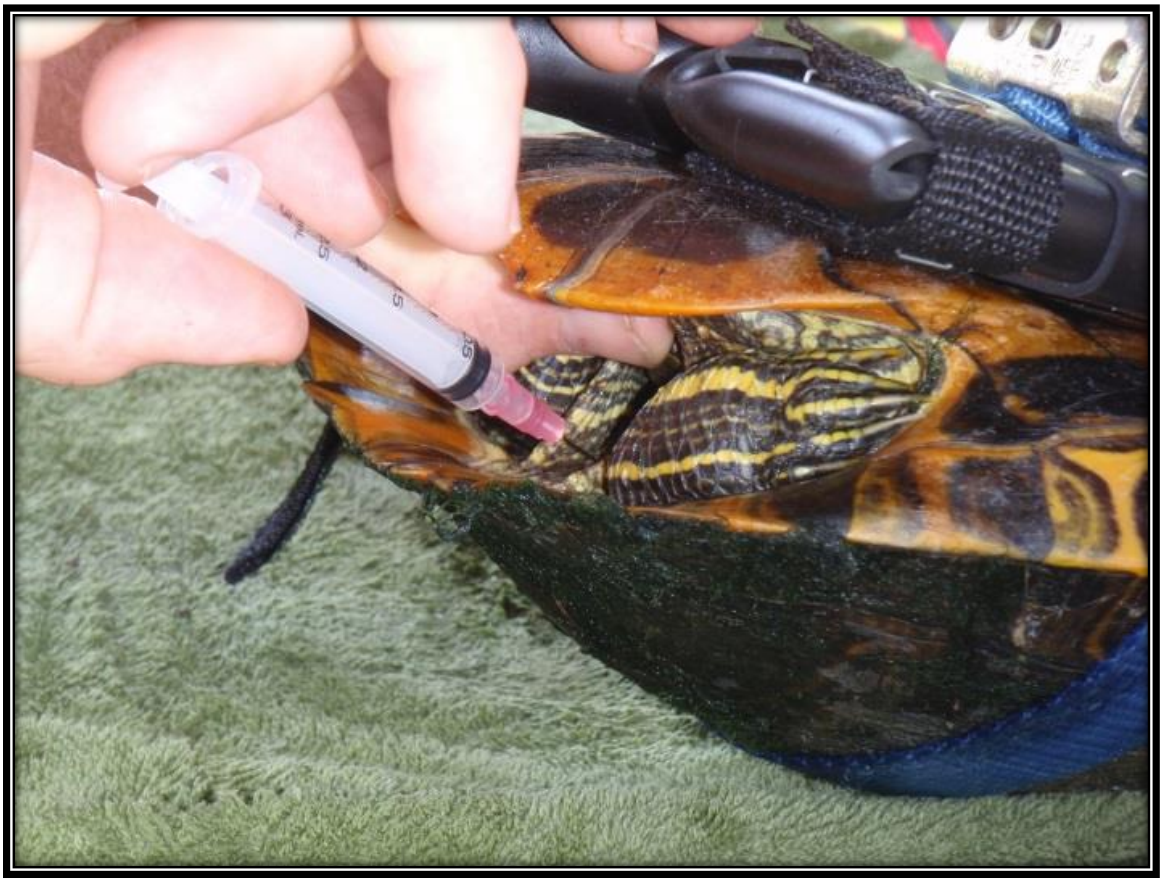

Figure 9 Subcarapacial sinus vein technique for drawing blood from turtles demonstrated using red-eared slider (*Trachemys scripta elegans*) by Dr. Joe Flanagan (Houston Zoo) on May 21, 2012.

The benefits to the subcarapacial sinus method is that it can be performed by a single person efficiently in a field setting without the aid of others, and it is less prone to causing infection to the individual than some of the other techniques which result in larger punctures exposing an opening for external bacteria and parasites to enter the blood stream (Hernandez-Divers et al. 2002). One of the disadvantages to collecting blood from this site is that because it is a blind spot, it is common to extract lymphatic fluid with the sample. The lymphatic fluid mixes with the blood as the syringe creates a vacuum drawing out the sample, which can make it difficult to determine exactly how much blood was drawn into the sample. It is therefore necessary to centrifuge the blood samples in the lab before extracting DNA for achieving a higher yield. When lymphatic fluid appeared in the syringe while drawing blood, the needle was carefully readjusted until blood could be seen filling the syringe. Appropriately concentrated blood samples contained dark, rich blood and little to no lymph.

Blood was drawn using a disposable 25-gauge ½-inch long needle attached to a 1-cc syringe. For stability during the process, the turtle was turned upside down with its head facing away and posterior end held securely against the collector's body (Figure 10). Taking one finger above and behind the terrapin's head and then quickly placing it forward on the top of the head before the animal could react, the collector was able to gently guide the turtle's head inside the carapace and hold it steady above the rostrum until the procedure was completed. While securing the terrapin's head with one finger and stabilizing the body with the same hand, the other hand was used to swab the venipuncture site with 70% isopropyl alcohol for 30 seconds before inserting the needle. Figure 11, Figure 12, and Figure 13 illustrate the processes of insertion, adjusting the needle, and drawing blood.

Figure 10 Photograph of blood sample being drawn from terrapin in field via subcarapacial sinus vein technique.

Figure 11 Insertion of needle at venipuncture site.

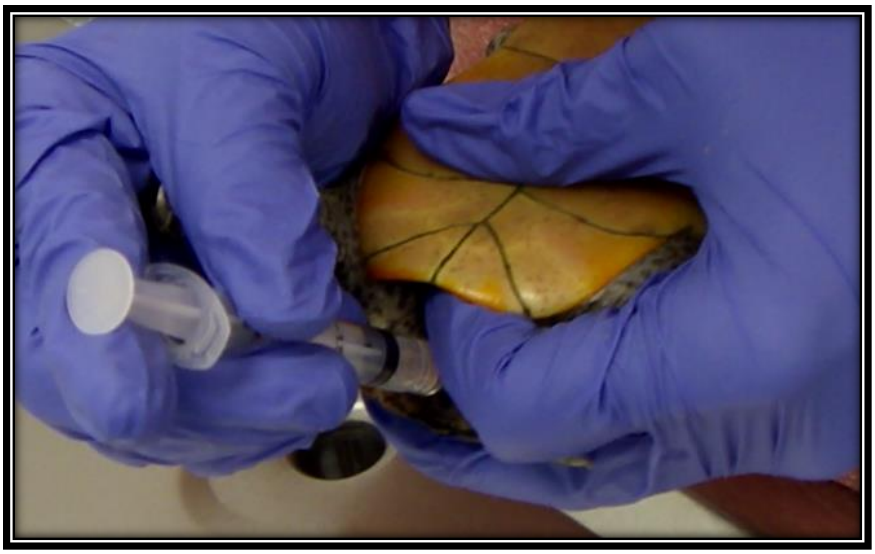

Figure 12 Adjusting needle parallel with the plastron midline.

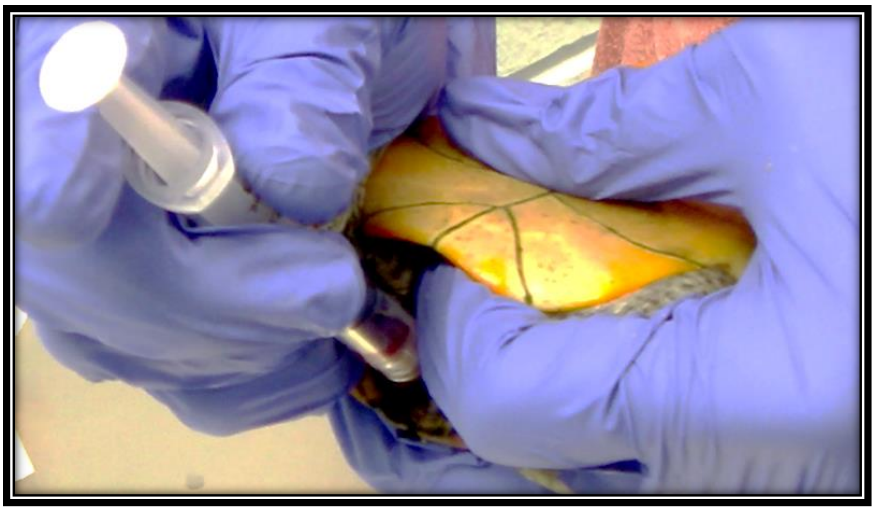

Figure 13 After gently creating a vacuum with syringe, blood begins to flow.

Immediately after blood samples were drawn, the samples were transferred to 1 mL BD® sodium heparin vials (Figure 14) to prevent the blood from coagulating. The samples were preserved temporarily on ice in the field and then were stored at -80° C in the lab freezer immediately upon returning from the field until they could be later processed.

Figure 14 1 mL BD sodium heparin vial used to store blood samples collected in field.

The total amount of blood that can be safely drawn from a reptile or amphibian is dependent on the animal's size and health status, and varies among species. As a generalization, approximately 5 to 8 percent of the animal's total body weight can be safely drawn in one sample (Beaupre et al. 2004). The guideline set for this study in the IACUC protocol was six percent. Based on historical data collection, the average body mass for terrapin in Galveston Bay is 726 grams, ranging between 80 g to 4.45 kg. Thus, blood volumes of Galveston terrapin average 44 mL and range between 5 mL to 267 mL. Healthy reptiles are able to lose up to 10% of their blood volume without any detrimental consequences (Beaupre et al. 2004). From a terrapin in the 80 g to 4.45 kg size range, 0.5 mL to 26.7 mL of blood can be withdrawn safely, with an average of 5 mL. Since the amount of blood required for microsatellite DNA analysis was less than 1 mL, no more than 600 µL of blood was drawn from any of the terrapin sampled in this study. Furthermore, by weighing each individual in the field, it was ensured that no individual weighed less than 175 g, as was also specified in the requirements of this study's IACUC protocol.

Associated Data Collected

Individual terrapin captured in Galveston Bay, Texas were collected as part of a long-term mark-recapture field monitoring study of the local populations. Therefore, along with the blood samples collected from Galveston terrapin, associated physical, morphometric, temporal, and spatial information was also recorded for each individual sampled by EIH field personnel. Additionally, the carapace, plastron, anterior (head), posterior (tail), and right and left profiles of the individual's body were photographed. For each individual collected, the following associative information was recorded: the names of the collector, recorder, and processor; date and times of capture and release; GPS location and name of site; notch and PIT-tag identification numbers; whether the individual was a new capture or recapture; the method of capture; the catch-per-unit effort (CPUE) search/trap time; descriptions of physical traits, injuries, and abnormalities; habitat description; morphometric measurements of carapace, plastron, head width, and body mass; number of growth rings; gender; fecundity of females based on palpation and/or ultrasound screening; behavioral responses of individual before, during, and after handling and processing; vegetation cover; and environmental quality/climate data for the sampling event.

Upon initial capture, each new individual was assigned the next available consecutive notch number in the list of total captures throughout the monitoring project as an external identifier. The notching system used was originally developed by Cagle (1939) and modified in 2009 by former UHCL graduate student, Kelli Haskett, and Dr. Joe Flanagan, Director of Veterinary Services at the Houston Zoo (Haskett 2011). Using a triangular file, a unique identification number was notched into the marginal scutes of each terrapin's carapace that was captured throughout the course of the monitoring study (Figure 15). As an additional form of identification, the posterior left leg of each individual was also tagged with an AVID® PIT-tag using a 12-gauge needle. Each PIT-tag is assigned a distinct 9 digit identification number that is used to scan recaptures as a safeguard against misidentifying and/or double-counting individuals.

Morphometric dimensions of each individual were measured in millimeter units using large tree calipers for head width of females and small vernier calipers for head width of males. Head width was measured at the widest part of the jaw. Carapacial measurements were taken of the midline length, maximum length, maximum width along the shell, and width taken at the suture line between the second and third keels. Measurements of the plastron were taken of the midline length, maximum length, width of bridge at the rear legs, and width of plastron at the second suture line where the plastron is joined with the carapace. The maximum depth of the shell was taken from the top of the second keel, and a second depth was measured between the second and

third keels, so that keel height can be calculated. Finally, body mass was measured to the nearest 0.01 kilogram using a standard forestry hanging scale.

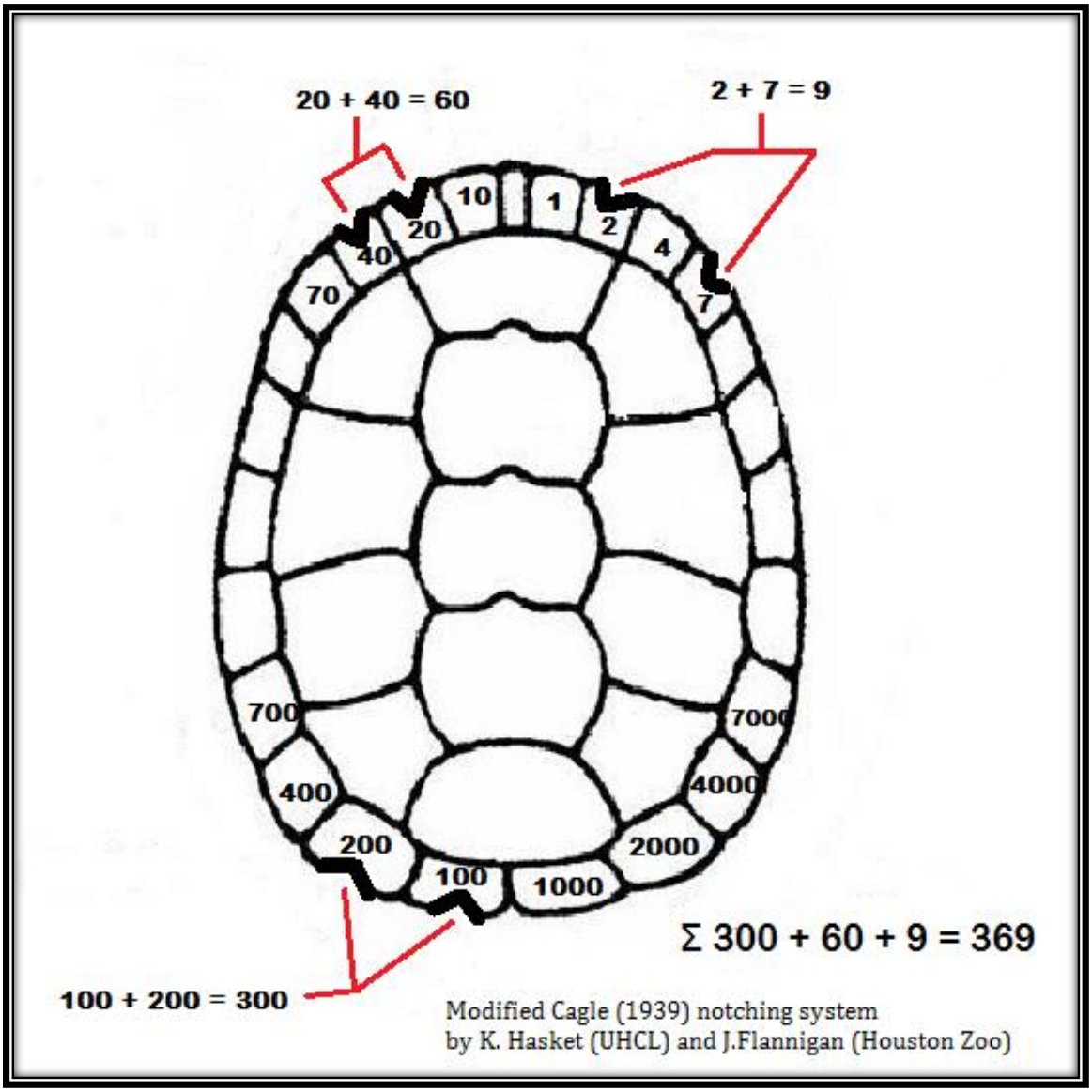

Figure 15 Diagram of modified Cagle (1939) notching system developed in 2009 by Kelli Haskett (UHCL) and Dr. Joe Flanagan (Houston Zoo) for long-term monitoring of Galveston Bay, TX terrapin populations (Haskett 2011). The example above illustrates how the sum of the notches is the identifying number for each individual captured. The marginal scutes of the carapace are notched using a triangular file. The turtle in the figure would be identified as notch #369, indicating it is the 369th individual captured in the study.

DNA Extraction and Quantitation

All blood samples collected in the field and those obtained from researchers were stored at -80°C in the lab freezer until time for processing. Prior to extracting DNA from the samples, whole blood cells were separated from the lymphatic serum via centrifuge. Each blood sample was transferred to a 1.5 mL centrifuge tube and spun at a maximum of 3500 RPM for 5-10 minutes until a pellet formed at the bottom. The lymphatic fluid was then carefully siphoned from above the pellet and transferred to a separate centrifuge tube. The remaining centrifuge tube with the whole blood pellet was used for extracting DNA. The purpose of separating the whole blood cells from the serum was to optimize the DNA yield in the following extraction process. This process was performed on all but the Alabama samples, which were received as spun-down whole blood pellets.

DNA was extracted from the whole blood samples using the Qiagen DNeasy® Blood and Tissue Kit following the steps for nucleated blood provided in the Qiagen® Quick-Start Protocol (Qiagen 2011). The only modification made to the protocol was under step number 8, where instead of eluting the DNA with 200 µL of Buffer AE, 100 µL was used and the step was repeated three times for increased yield, resulting in a final volume of 300 µL of concentrated DNA sample.

Following the extraction process, the DNA extract concentrations were then quantified using the Quant-iT™ PicoGreen® dsDNA reagent and quantitation kit (Molecular Probes 2008). The PicoGreen® reagent works by staining the double-stranded DNA with fluorescent nucleic acid that can be detected by a standard spectrafluorometer, which uses fluorescein excitation and emission wavelengths to determine the absorbance of nucleic acid at 260 nm (A_{260}). The PicoGreen® assay was selected for use because of its high range of detectability, ranging from 25 pg/mL to 1,000 ng/mL, which allows for the detection of DNA even in the presence of preparation compound contaminants, such as ethanol and detergents used in the extraction process (Molecular Probes 2008). The spectrafluorometer instrument used to detect the absorbance of the PicoGreen® samples was the Tecan Infinite® M200 Spectrafluor Plus. Magellan™ Data Analysis software was used to retrieve the results of absorbance, calculate the quantity of DNA in 2 ng/µL, and export the DNA quantity values to a Microsoft Office® Excel spreadsheet.

Using Microsoft Excel, the values of the DNA quantities were divided by 2 µL to calculate the concentration of DNA in each sample. Since all of the samples must have an

equivalent DNA concentration for PCR amplification, each sample was diluted to 1ng/µL. The diluted concentrations for each sample were calculated in Excel based on a 100 µL total volume. Each DNA extract was individually diluted with deionized water for PCR and stored at -20°C in the lab freezer.

Primer Selection

Twelve of the 27 loci developed by King and Julian (2004) were selected for microsatellite analysis in this study. Although these primers were developed for use in bog turtles, the results of cross-species amplification tests reflected high levels of polymorphism in terrapin. Other studies (Forstner et al. 2000; Hauswaldt & Glenn 2003) have developed species-specific markers for terrapin, but neither study resulted in polymorphic levels as high as those found by King and Julian (2004). For this reason, the previous Gulf Coast studies (Hart 2005; Coleman 2011; Drabeck et al. In Press) chose to use the primers developed by King and Julian (2004). In order for the results of this study to be more comparable with the previous Gulf Coast studies, the same 12 SSR markers were used: *Gmu*A18, *Gmu*B08, *Gmu*B67, *Gmu*B91, *Gmu*D21, *Gmu*D55, *Gmu*D62, *Gmu*D87, *Gmu*D90, *Gmu*D93, *Gmu*D114, and *Gmu*D121. Table 4 contains a list of the markers along with supplementary information.

The selected SSR markers for this study were manufactured by Sigma® Life Sciences (Sigma-Aldrich Co. LLC. 2013). The primers arrived with forward and reverse oligonucleotide sequences in 100µM concentrations. Each forward and reverse primer was diluted with deionized water to 10 µM working stock solutions. The forward and reverse primers were diluted once more to 0.45 µM and 1.35 µM concentrations, respectively, to be used in PCR.

Table 4 List of loci used in study to perform microsatellite DNA fragment analysis of *Malaclemys terrapin*. Included is a list of GenBank accession numbers for reference of sequences, ranges in number of basepairs of the DNA fragment sizes of alleles, the number of microsatellite basepair repeats between different alleles, the fluorescence-labeled M13-tailed primer that was combined with the SSR marker in multiplex PCR, and the annealing temperature settings used in PCR. Annealing conditions were based on the results of PCR temperature gradient.

SSR Primer (i.e.- Locus)	GenBank Accession #	Allele Fragment Size Range (# bp)	Basepair (bp) Repeat	Fluorescence-labeled M13 Primer	PCR Annealing Temp. (°C)
***Gmu* A18**	AF337648	101-139	$(GT)_{14}$	FAM (blue)	56 °C
***Gmu* B08**	AF517229	193-264	$(TAC)_{10}$	FAM (blue)	58 °C
***Gmu* B67**	AF517233	140-162	$(TAC)_{13}$	NED (yellow)	58 °C
***Gmu* B91**	AF517234	115-150	$(TAC)_{6}$	VIC (green)	58 °C
***Gmu* D21**	AF517236	145-159	$(ATCT)_{15}$	NED (yellow)	56 °C
***Gmu* D55**	AF517240	153-220	$(ATCT)_{10}$	PET (red)	58 °C
***Gmu* D62**	AF517241	125-185	$(ATCT)_{11}$	VIC (green)	56 °C
***Gmu* D87**	AF517244	212-292	$(ATCT)_{22}$	FAM (blue)	59 °C
***Gmu* D90**	AF517247	106-165	$(ATCT)_{9}$	PET (red)	56 °C
***Gmu* D93**	AF517248	113-196	$(ATCT)_{18}$	NED (yellow)	59 °C
***Gmu* D114**	AF517251	85-122	$(ATCT)_{13}$	VIC (green)	59 °C
***Gmu* D121**	AF517252	120-190	$(ATCT)_{8}$	PET (red)	59 °C

Primer Annealing Optimization

Prior to Polymerase Chain Reaction (PCR) of the samples, a PCR temperature gradient was performed to determine the optimal annealing temperatures for each primer. The PCR gradient was run on a 96-well plate containing all 12 primers across one genomic DNA sample from Nueces Bay, TX, which had the highest DNA concentration of all the terrapin samples. A cocktail mixture (110 µL of 10X Titanium buffer; 110 µL of 10 mM Promega dNTPs; 8.8 µL of 50X Titanium Taq polymerase; 55 µL of undiluted genomic DNA from a single terrapin; 596 µL of deionized water) was arrayed across a 96-well PCR plate. Each of the 12 columns on the 96-well plate contained a different primer, where 1 µL of each the 10 µM forward and reverse primers were added to each well in the column containing the cocktail mix.

The Bio-Rad® C1000 Touch™ Thermal Cycler (96-well) was the equipment used in performing the reactions. PCR conditions set for the temperature gradient were as follows: 94°C initial denaturation for 3 minutes; 40 cycles of 94°C denaturation for 30 seconds, 70°C to 52°C annealing gradient for 30 seconds, 72°C extension for 1 minute; 72°C final extension for 20 minutes.

The PCR product was tested for amplification via gel electrophoresis at 9 volts across a 2% ethidium bromide agrose gel. Based on the results of the temperature gradient, primers were combined in three groups of 4 by the most similar optimal annealing temperatures. The 3 primer groups annealing temperatures were 56°C, 58°C, and 59°C. Refer to Table 4 above for a complete list of which primers were amplified together and the corresponding M13 labels used in the following multiplex PCR.

Multiplex Polymerase Chain Reaction (PCR)

Multiplex PCR was utilized in amplifying 96 genomic DNA samples across the 12 SSR primers, using four M13-tailed fluorescence-labeled primers (FAM, NED, VIC, and PET). PCR products were first amplified on a 384-well plate using the Bio-Rad® C1000 Touch™ Thermal Cycler (384-well) prior to being multiplexed to in a 96-well plate and analyzed in the ABI 3500xL Genetic Analyzer (Applied Biosystems). Each well of the 384-well PCR plate contained a total volume of 7.0 µL consisting of: 2 µL of 1 ng/µL genomic DNA; 0.7 µL of 10X Titanium buffer; 0.8 µL of 10 mM Promega dNTPs; 0.08 µL of 50X Titanium Taq polymerase; 1.2 µL of deionized water; 0.225 µL of 5µM M13 labeled primer stock (FAM, NED, VIC, or PET); 0.9 µL of 0.45 µM unlabeled forward SSR primer; 1.1 µL of 1.35 µM unlabeled reverse SSR primer. The following PCR conditions were set for each primer grouping, with the exception of the annealing temperatures (specified above in Table 4): 94°C initial denaturation for 3 minutes; 40 cycles of 94°C denaturation for 30 seconds, 56, 58, or 59°C annealing for 30 seconds, 72°C extension for 30 seconds; 72°C final extension for 30 minutes.

Twenty-four subsamples of product from each of the 4 primers on the 384-well plate were tested for amplification post-PCR via gel electrophoresis. Electrophoresis was performed on a 1.5% ethidium bromide agrose gel and set to run at 9 volts. Upon verification of amplification, the PCR products were multiplexed from the 384-well PCR plate combining the 4 primers of each sample into a single 96-well half-skirted plate. Two diluted plates (1:20 and 1:40) were prepared from the concentrated multiplexed PCR product. From each of the diluted multiplexed PCR plates, 1 µL of product was combined with 0.1 µL of ABI GeneScan™ 600 LIZ® Size Standard and 9.0 µL of ABI Hi-Di™ Formamid in each well of two separate 96-well plates compatible with the ABI 3500xL Genetic Analyzer (Applied Biosystems). The diluted multiplexed plates mixed with the size standard were denatured at 95°C for 5 minutes and then immediately chilled on ice for 5 minutes prior to performing capillary electrophoresis in the ABI 3500xL Genetic Analyzer (Applied Biosystems).

Figure 16 provides a workflow diagram illustrating the entire laboratory processes performed in this study from the collection of blood samples to the retrieval of microsatellite data.

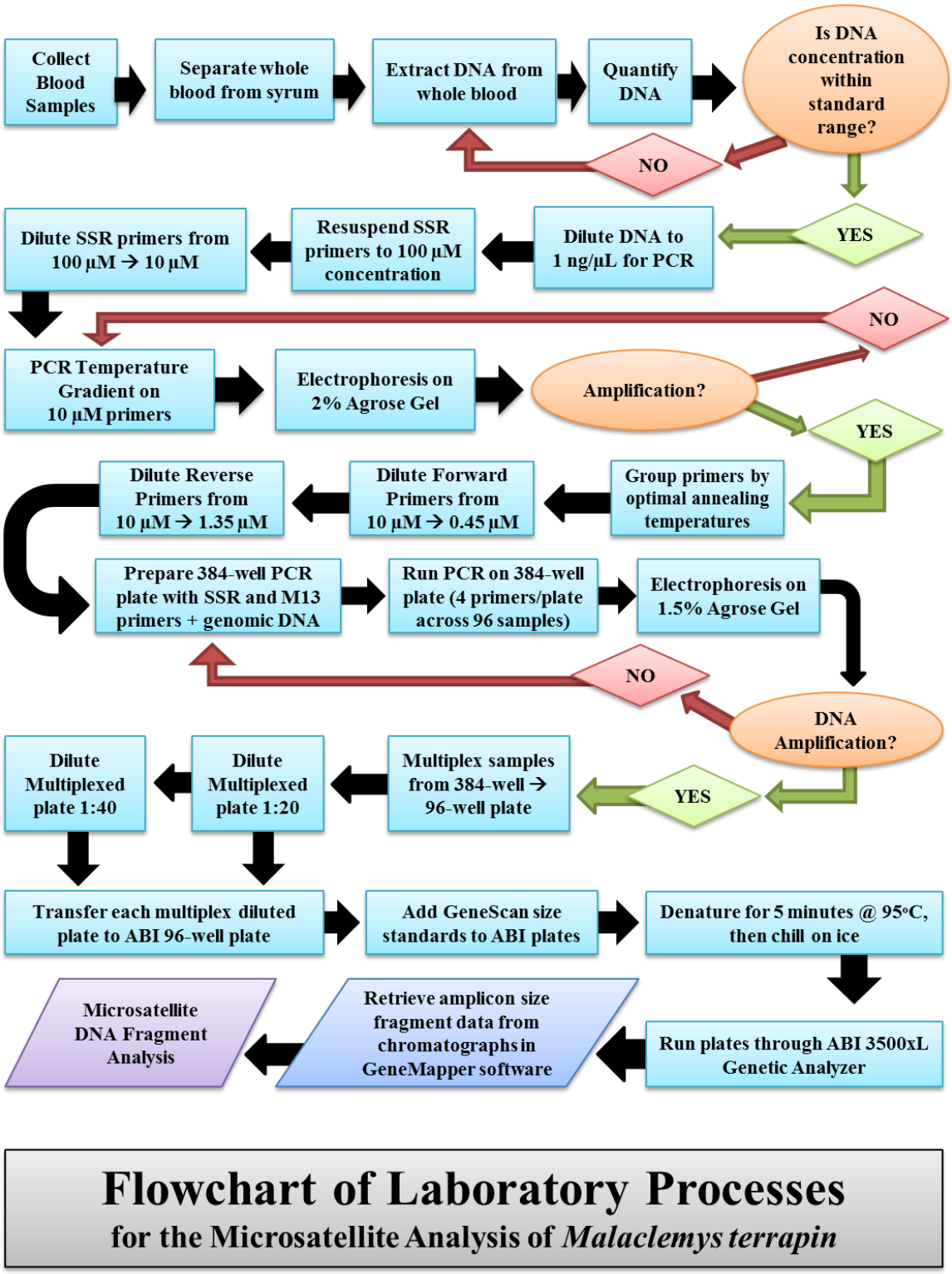

Figure 16 Flowchart of laboratory processes illustrating a summary of the steps that were performed in the collection of microsatellite DNA fragment size data for the individuals sampled in this study.

Genetic Analyses

Allele fragment size data was generated using GeneMapper® software version 4.1(Applied Biosystems 2009). A microsatellite DNA analysis was also performed in GeneMapper® v. 4.1 (Applied Biosystems 2009) in which fragment sizes were scored, binned, and genotypes were assigned for each individual. Results of allele sizes called in the microsatellite analysis were manually validated by examining the amplicon peaks displayed in electropherogram plots in GeneMapper®.

All statistical genetic analyses, with the exception of the test for Hardy-Weinberg Equilibrium (HWE), were performed in Microsoft Excel® 2010 (Microsoft Corporation) using *GenAIEx*®: *Genetic Analysis in Excel* software version 6.5 (Peakall & Smouse 2006, 2012), a population genetic software written in Visual Basic for Applications (VBA) within Excel® for use in teaching and research. The data input format used to perform all tests was two columns per locus codominant fragment size data. The test for HWE was performed using the software Arlequin version 3.1 (Excoffier et al. 2005).

Analyses of Molecular Variance (AMOVA) were performed to test for genetic variation among populations by estimating Wright's F-statistics (Fst) (Wright 1965), which is used to assess population genetic structure by allowing the hierarchical partitioning of genetic diversity (i.e.-heterozygosity) within and among populations (Peakall & Smouse 2012). The AMOVA parameters were set to run 1,000 standard permutations calculating the allelic distance F-statistics among samples from codominant allele size matrix input data. An AMOVA summary table was generated along with pairwise matrices of Fst and standardized F'st values for comparing genetic distances between pairs of populations. Both Fst and F'st values were reported and used in analyzing differentiation among the study populations. While probability (P) estimates were calculated for unstandardized Fst values, the standardized F'st values served only as a comparative measure for distinguishing genetic differences between populations and do not rely on P-values (Meirmans 2006).

For all statistical tests performed, only 11 of the 12 genotyped loci were included in the analyses. While the AMOVA is able to adjust for error with some missing data within loci, the results are less reliable as the proportion of missing data increases. Since the locus *Gmu* D21 was missing allele fragment size data for 30% of the individuals sampled, it was excluded from all genetic analyses.

A preliminary AMOVA was performed only on individuals from Galveston Bay in order to determine any local genetic population structure for appropriate grouping in further

comparison with the other northern Gulf Coast populations. A pairwise Euclidean distance matrix between each of the 6 Galveston sites was generated in *GenAIEx*®. Based on the distances among the sites, the west Galveston Bay sites were assumed to be one genetic population, as the maximum distance between any two neighboring sites was 6.5 kilometers between Greens Lake and North Deer Island. Additionally, the west bay sites are distributed in an open orientation lacking any apparent physical evidence of inhibition to gene flow. Furthermore, extensive long-term mark-recapture monitoring of these sites by EIH researchers supports this assumption based on observations of terrapin recaptured and/or radio-tracked at sites different from the location where they were originally tagged, as well as reports of tagged terrapin received from local residents (EIH, unpublished data).

East Galveston Bay terrapin populations were expected to be genetically isolated from the west bay terrapin by factors of both spatial and anthropogenic physical barriers. The one east bay site, Little Pasteur Cove (located off the Bolivar Peninsula), is approximately 45 kilometers from the nearest neighboring west bay sites, the Deer Islands. Additionally, the Houston Ship channel presents a physical barrier inhibiting gene flow between east and west bay populations. The entrance to the Houston Ship Channel is maintained at minimum depth and width of 14 and 162 meters, respectively, and is one of the busiest waterways in the world, hosting the passage of over 50 ships and 300 barges per day (Houston-Galveston Navigation Safety Advisory Committee 2006). From 2010 to date, the U.S. House of Representatives has allocated a total of $123.2 million toward the maintenance dredging and expansion of the Houston Ship Channel in order to accommodate the increasing volume of cargo carrier traffic (Perez 2012). Based on the heavy vessel traffic and large distances, it is unlikely that terrapin would survive swimming across this waterway and therefore little, if any, gene flow would naturally occur between east and west bay populations. Therefore, in the preliminary AMOVA, the Galveston Bay terrapin were grouped as 2 separate populations, east and west bay, and analyzed for genetic differentiation.

Upon determination of genetic variation in Galveston Bay populations, an AMOVA following the above-mentioned parametric settings was performed to test the genetic variance among Galveston in relation to the other northern Gulf Coast terrapin populations sampled in the study. The samples were grouped in the analysis and run as 4 populations: Nueces Bay, Galveston Bay, Louisiana, and Alabama. With the exception of the Louisiana population, it was assumed that the sets of samples from the other 3 bay systems were each from a single gene pool. Louisiana samples, however, were most likely from 3 different genetic populations as the nearest neighboring sites were a distance of approximately 30 kilometers apart and the third site,

Cocodrie Bayou, was roughly 60 kilometers from its nearest neighbor. Because there were only 12 Louisiana samples, 4 from each of the 3 assumed genetically independent sites, grouping such low sample sizes as single populations would have introduced bias and skewed results in the statistical tests. Furthermore, the Louisiana terrapin samples were a subset of the same individuals included in the Drabeck et al. (In Press) study, and using the same molecular markers in their analysis, they found little to no local differentiation among the sites. Based on their findings and as a consequence of small sample size, Louisiana samples were grouped as a single population in the AMOVA comparing northern Gulf Coast populations.

In addition to the AMOVA test for genetic differentiation among northern Gulf Coast populations, standard genetic population parameters were also assessed. It was advised in Appendix 1 of the *GenAIEx*® software manual (Peakall & Smouse 2006, 2012) that the Hardy-Weinberg Equilibrium (HWE) test following Hedrick (2000) be performed in the GenAIEx software primarily for the purposes of teaching and data exploration, rather than for research. For the purpose of research, it was suggested that the software program Arlequin (Excoffier et al. 2005) be used to statistically test for random mating of populations because they offer exact permutation tests of HWE via Markov Chain and dememorisation steps, which allows for observed data to be randomly shuffled and recalculated for a set number of times, resulting in more precise estimates of linkage-disequilibrium (i.e.- random mating). Additionally, Hedrick (2000) warns that any sample size less than 50 used to calculate the Chi-squared values in testing for deviation from the HWE should be interpreted with caution.

Since the sample sizes in this study were all below 50 for each population, Arlequin v. 3.1 (Excoffier et al. 2005) was used to perform exact permutation tests of HWE following the methods of Guo and Thompson (1992) and Levene (1949) to test for random mating within each of the northern Gulf Coast populations. The HWE test type was set as locus-to-locus for 1,000,000 Markov chain randomization steps, followed by 100,000 dememorisation steps. The overall mean observed and expected heterozygosities for each population were reported in column bar graphs displaying error bars representing the 95% confidence interval (CI) of each mean.

For all column bar graphs, the error bars of the reported mean values represent the 95% confidence interval of the mean, which was used in interpreting statistically significant differences among population means. The 95% CI of each mean was calculated from the standard error of the mean ($\pm 1\ SE\bar{\mu}$) reported in *GenAIEx*® using the following formula:

$$95\%\ \mathrm{CI}\ \approx (1.96) \times (\pm 1\ \mathrm{SE}\bar{\mu}\ )$$

To evaluate informative alleles for characterizing populations, a summary list of the alleles that occurred at each locus within each population was generated, along with a list of private alleles that were unique to a single population. A list of informative alleles was first created by selecting alleles that only occurred in 50% or less of the populations. The list was then narrowed down to the most informative alleles based on those that were locally common (i.e. - resulted in frequencies over 5% in one or more populations).

The frequency function was used to assess the genetic diversity of each population by calculating the mean number of different alleles over all loci (Na), mean number of locally common alleles over loci with frequencies over 5% that occur in 50% or less of the populations, and Shannon's Information Index (I) of relative allelic diversity. Results of these values were reported by their overall means over all loci for each population in column bar graphs, and included error bars representing the 95% confidence interval of each mean for visual comparison of genetic diversity among populations.

The mean results for observed heterozygosities and mean number of different alleles within each sampled population were also reported in column bar graphs, displaying error bars representing the 95% confidence interval of each mean, alongside the mean values and 95% CI published for Nueces Bay, TX and Louisiana by Hart (2005) and for Alabama by Coleman (2011). These combined graphs served as a two-fold function. First, much like how the addition of a missing piece to the middle of a jigsaw puzzle enables one to better infer what the overall image will be, the combined graphs enable easy visualization of how the addition of genetic information of Galveston Bay terrapin provides a more clearly refined understanding of the genetic relationships among the northern Gulf Coast terrapin populations. Second, the combined results of this study's reference sample data for Nueces, Louisiana, and Alabama displayed in a side-by-side visual comparison with the previous studies' results of the same populations and loci functioned as a relative index for this study in evaluating the reliability of statistical results, as well as served as a valid mechanism in careful interpretation of significance values.

RESULTS

AMOVA Results for Galveston Bay

The results of the AMOVA for Galveston Bay found no significant genetic differentiation between east and west bay populations (F_{ST} = 0.004; P (F_{ST} > 0) = 0.289), but did find significant genetic variation both among individuals (F_{IS} = 0.209; P (F_{IS} > 0) = 0.001) and within individuals (F_{IT} = 0.212; P (F_{IT} > 0) = 0.001) from each population (Table 5). The standardized F'_{ST} value calculated for genetic differentiation between the east and west bay populations supported the results of the unstandardized F_{ST} estimate, as it was close to zero (F'_{ST} = 0.008), indicating little genetic deviation (Table 5). Since no significant genetic difference was found between the east and west Galveston Bay terrapin populations, the null hypothesis that populations within Galveston are genetically homogenous was not rejected. Thus, all individuals sampled from Galveston Bay were grouped as one population in the following AMOVA test for genetic partitioning among the northern Gulf Coast populations sampled in this study.

Table 5 Results of AMOVA test for local genetic differentiation within Galveston Bay. F_{ST} estimates genetic variation among populations; F_{IS} estimates the genetic variation among individuals; F_{IT} estimates genetic variance within individuals; standardized F'_{ST} is a relative index based on a [0, 1] scale, where differentiation among populations increases as the value approaches 1, and does not test use probability to test for significance. Significant F-stat values [P (F > 0) $\leq$ 0.050] are highlighted in bold.

AMOVA Results for Populations within Galveston Bay					
Fixation Indices:	F_{ST}	F_{IS}	F_{IT}	F_{STmax}	F'_{ST}
F-Stat Values:	0.004	**0.209**	**0.212**	0.497	0.008
Probability (F > 0) =	0.289	**0.001**	**0.001**	-----	-----

AMOVA Results for Northern Gulf Coast Populations

The results of the second AMOVA tested for genetic variance among the four northern Gulf Coast terrapin populations in Nueces Bay, TX, Galveston Bay, TX, southeastern Louisiana, and Mobile Bay, AL. All of the F-statistics results of the AMOVA significantly deviated from zero, indicating a significant level of genetic differentiation among populations (F_{ST} = 0.069; P (F_{ST} > 0) = 0.001), among individuals (F_{IS} = 0.192; P (F_{IS} > 0) = 0.001), and within individuals (F_{IT} = 0.248; P (F_{IT} > 0) = 0.001) (Table 6). The overall estimated standardized F'_{ST} value (F'_{ST} = 0.137) supported the F_{ST} estimate, as it was higher than zero (Table 6).

Table 6 Results of AMOVA test for genetic differentiation among northern Gulf Coast terrapin populations sampled in study. The fixation and differentiation indices are listed for genetic partitioning among populations, among individuals, and within individuals. Significant F-stat values [P (F > 0) ≤ 0.050] are highlighted in bold.

AMOVA Results for Northern Gulf Coast Populations					
Fixation Indices:	F_{ST}	F_{IS}	F_{IT}	F_{STmax}	F'_{ST}
F-Stat Values:	**0.069**	**0.192**	**0.248**	0.505	0.137
Probability (F > 0) =	**0.001**	**0.001**	**0.001**	-----	-----

The results of the AMOVA pairwise F_{ST} and standardized F'_{ST} comparisons between each pair of northern Gulf Coast populations sampled showed significant genetic differentiation between all but one pair of populations (Table 7). Louisiana and Alabama was the only pair of populations that did not result in significant genetic differentiation (F_{ST} = 0.014; P (F_{ST} > 0) = 0.122; F'_{ST} = 0.028). Distances between population pairs were calculated to the nearest kilometer in *GenAIEx*® (Peakall & Smouse 2006, 2012) in a Euclidean distance matrix generated from latitudinal and longitudinal coordinates in decimal-degrees that were estimated for each bay system using Google Earth® software (Google Inc. 2013). Of all the pairwise combinations tested, Louisiana and Alabama were also within the closest geographical proximity (171 km) to each other.

The standardized F'_{ST} values also supported the F_{ST} values as they were consistently in directional agreement, and the relative values of both F_{ST} and F'_{ST} indices among all population pairs resulted in a direct, positive correlation with spatial distribution. Table 7 lists the pairwise values results between each pair of population. The table includes the geographical distances between each pair of populations, which serves to illustrate the observed correlation of genetic differentiation among the populations occurring along a spatial distance gradient. To better visualize the proportion of genetic differentiation among populations pairs, Figure 17 provides a pie chart illustrating the relative standardized F'_{ST} values for each population pair over the total sum of F'_{ST} values for all pairs of populations sampled.

Table 7 Results of pairwise F_{ST} and F'_{ST} comparisons between pairs of northern Gulf Coast populations. Values highlighted in bold indicate significant Fst values [P (F > 0) $\leq$ 0.050]. Standardized F'st is interpreted on a relative [0, 1]-scaled basis, where differentiation occurs as the value approaches 1.

Pairwise F_{ST} and F'_{ST} Comparisons between Northern Gulf Coast Populations

Highest Divergence	Population 1	Population 2	Distance (km)	F_{ST}	Prob. (F_{ST} > 0)	F'_{ST}
	Nueces, Texas	Alabama	953	**0.121**	**0.001**	0.219
	Galveston, Texas	Alabama	654	**0.102**	**0.001**	0.202
	Nueces, Texas	Louisiana	789	**0.079**	**0.001**	0.151
	Galveston, Texas	Louisiana	498	**0.055**	**0.001**	0.114
	Nueces, Texas	Galveston, Texas	312	**0.028**	**0.011**	0.053
Most similar	Louisiana	Alabama	171	0.014	0.122	0.028

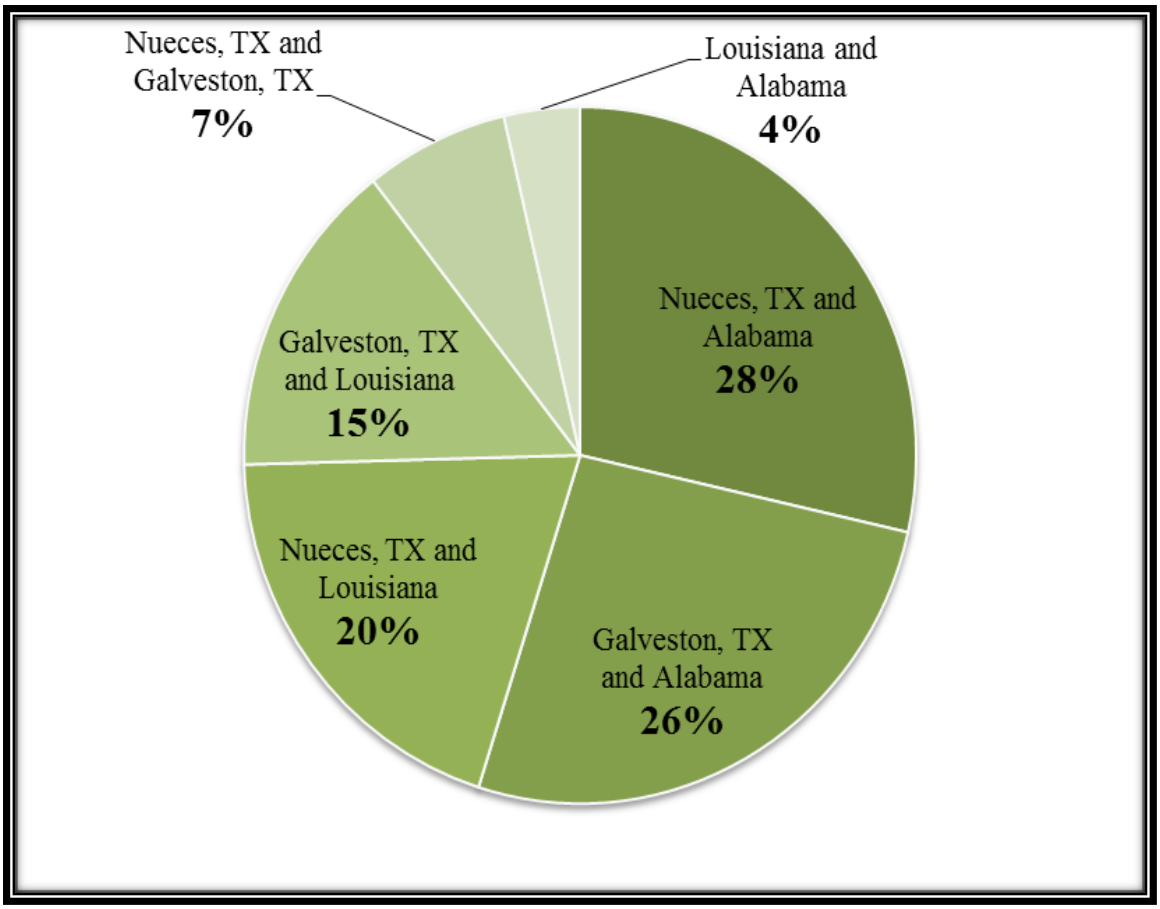

Figure 17 Pie chart illustrating the relative proportion of genetic differentiation between each pair of populations. Proportions are calculated as the pairwise F'st value of each population pair over the total sum of the pairwise F'st values for all populations.

Results of Hardy-Weinberg Equilibrium (HWE) Tests for Random Mating

The overall mean observed heterozygosities across all loci for each population did not significantly differ from the mean expected heterozygosities for any of the northern Gulf Coast populations (Figure 18). Therefore, the null hypothesis that random mating is occurring within each population was not rejected. In addition, none of the observed heterozygosities for any of the populations significantly differed from the mean heterozygosities observed in other populations, so the null hypothesis that random mating is occurring among populations was also not rejected (Figure 18). Though the mean observed heterozygosities among all of the populations were not statistically different, Louisiana appeared to have the highest level of mean observed heterozygosity (H_O = 0.539) and Alabama appeared to have the lowest (H_O = 0.389) (Figure 18). The overall mean heterozygosities for Nueces (H_O = 0.407) and Galveston (H_O = 0.430) appeared to be similar.

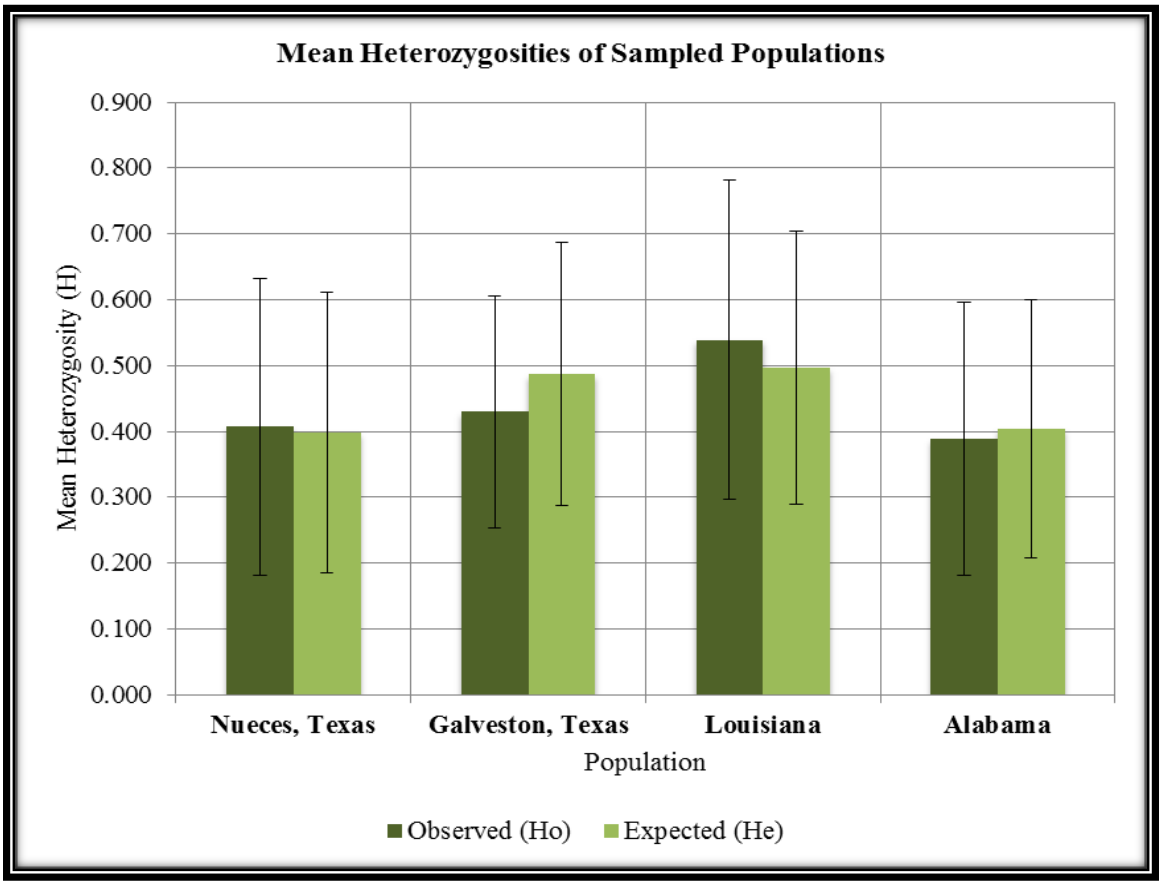

Figure 18 The overall mean observed and expected heterozygosities over all loci for northern Gulf Coast populations sampled in study. Error bars represent 95% confidence interval of the mean.

The observed and expected values for each locus within each population did result in some significant deviations from Hardy-Weinberg equilibrium (HWE) for some loci in all of the populations, except for Nueces Bay (Table 8). In the Galveston Bay population, the observed heterozygosities of 3 out of the 11 loci significantly deviated from HWE at loci *Gmu* A18, *Gmu* D62, and *Gmu* D121(Table 8).The observed heterozygosity of locus *Gmu* D93 was the only locus that significantly deviated from HWE for the Louisiana population. Two loci significantly differed from HWE in Alabama at loci *Gmu* A18 and *Gmu* D121. Both deviated loci in Alabama also were deviated in Galveston (Table 8). In all of the sampled populations, loci *Gmu* B91 and *Gmu* D114 were monomorphic, therefore were not tested in HWE and considered non-informative. Locus *Gmu* D55 was monomorphic for both Nueces and Alabama populations, and locus *Gmu* B67 was monomorphic only for the Nueces Bay population.

Table 8 Results of HWE test for each locus within each population. Observed heterozygosities of loci that significantly deviated from the expected values [P ($H_O \neq He$) ≤ 0.050)] within a population are highlighted in yellow and P-values are highlighted in bold.

Population	Locus	$H_{(OBSERVED.)}$	$H_{(EXPECTED)}$	P ($H_O \neq He$)
Nueces, TX	*Gmu* A18	0.875	0.742	1.0000
Nueces, TX	*Gmu* B08	0.875	0.800	0.9624
Nueces, TX	*Gmu* B67	***Monomorphic***		
Nueces, TX	*Gmu* B91	***Monomorphic***		
Nueces, TX	*Gmu* D55	***Monomorphic***		
Nueces, TX	*Gmu* D62	0.857	0.802	0.2477
Nueces, TX	*Gmu* D87	0.750	0.792	0.6174
Nueces, TX	*Gmu* D90	0.375	0.592	0.1621
Nueces, TX	*Gmu* D93	0.500	0.425	1.0000
Nueces, TX	*Gmu* D114	***Monomorphic***		
Nueces, TX	*Gmu* D121	0.250	0.233	1.0000
Galveston, TX	*Gmu* A18	0.538	0.739	**0.0016**
Galveston, TX	*Gmu* B08	0.717	0.714	0.3459
Galveston, TX	*Gmu* B67	0.193	0.204	0.5371
Galveston, TX	*Gmu* B91	***Monomorphic***		
Galveston, TX	*Gmu* D55	0.098	0.096	1.0000
Galveston, TX	*Gmu* D62	0.650	0.750	**0.0425**
Galveston, TX	*Gmu* D87	0.820	0.875	0.0854
Galveston, TX	*Gmu* D90	0.610	0.691	0.2753
Galveston, TX	*Gmu* D93	0.590	0.564	0.3510
Galveston, TX	*Gmu* D114	***Monomorphic***		
Galveston, TX	*Gmu* D121	0.508	0.718	**0.0004**
Louisiana	*Gmu* A18	0.917	0.750	0.3584
Louisiana	*Gmu* B08	0.636	0.749	0.6346
Louisiana	*Gmu* B67	0.091	0.091	1.0000
Louisiana	*Gmu* B91	***Monomorphic***		
Louisiana	*Gmu* D55	0.200	0.189	1.0000
Louisiana	*Gmu* D62	0.750	0.783	0.9086
Louisiana	*Gmu* D87	0.917	0.891	0.9774
Louisiana	*Gmu* D90	1.000	0.786	1.0000
Louisiana	*Gmu* D93	1.000	0.598	**0.0041**
Louisiana	*Gmu* D114	***Monomorphic***		
Louisiana	*Gmu* D121	0.417	0.627	0.0628
Alabama	*Gmu* A18	0.467	0.782	**0.0038**
Alabama	*Gmu* B08	0.733	0.701	0.8338
Alabama	*Gmu* B67	0.077	0.077	1.0000
Alabama	*Gmu* B91	***Monomorphic***		
Alabama	*Gmu* D55	***Monomorphic***		
Alabama	*Gmu* D62	0.667	0.623	1.0000
Alabama	*Gmu* D87	1.000	0.862	0.9870
Alabama	*Gmu* D90	0.600	0.511	1.0000
Alabama	*Gmu* D93	0.533	0.480	1.0000
Alabama	*Gmu* D114	***Monomorphic***		
Alabama	*Gmu* D121	0.200	0.411	**0.0095**

Informative Alleles for northern Gulf Coast Populations

Galveston and Louisiana were the only populations that exhibited private alleles unique to each single population. Louisiana resulted in 2 private alleles at separate loci, *Gmu* D55 and *Gmu* D62. Galveston had a much larger number of 14 private alleles at 6 different loci. The total numbers of private alleles for each locus in Galveston were: *Gmu* B08= 1; *Gmu* D55= 3; *Gmu* D62= 4; *Gmu* D90= 3; *Gmu* D93= 1; and *Gmu* D121= 2 (Figure 19).

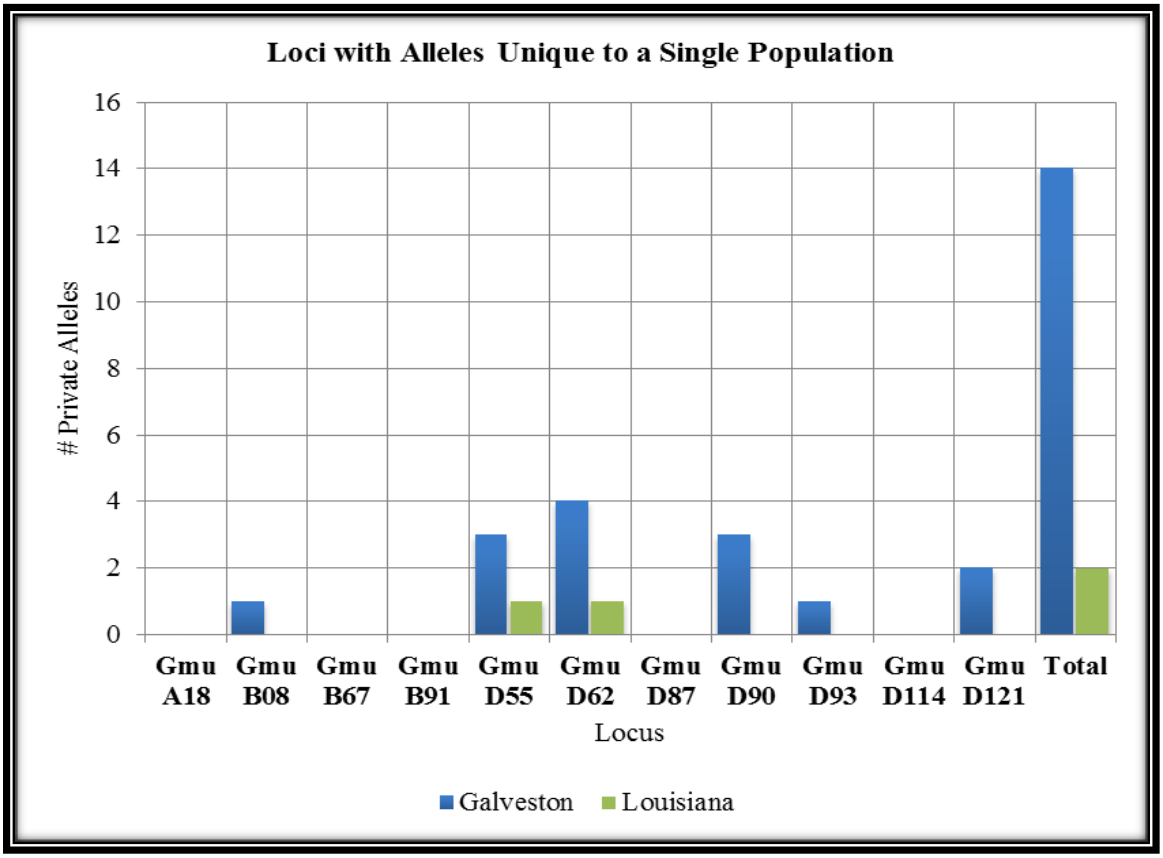

Figure 19 Distribution of private alleles unique to Galveston and Louisiana populations among all loci.

Although no private alleles were observed for Nueces and Alabama populations, there were a number of alleles that were unique simply by their absence in one population and presence in others (Table 9). Six different alleles were present in all of the populations except for Nueces. Louisiana also lacked an allele that was present in all of the other populations. Informative alleles for characterizing each population and pairs of populations were determined based on alleles that were either present or absent in 50% or less of the populations (Table 9).

Table 9 List of informative alleles based on presence or absence in ≤ 50% of the sampled populations that could potentially be used in characterizing single and pairs of northern Gulf Coast terrapin populations. **no evidence:* indicates the absence of an allele from a population. **Private allele*: indicates an allele unique to a single population.

Locus	Allele (#bp)	Nueces, Texas	Galveston, Texas	Louisiana	Alabama
***Gmu* A18**	**123**	***no evidence**	present	present	present
***Gmu* B08**	**220**	present	present	***no evidence**	***no evidence**
	223	absent	***Private allele**	absent	absent
	238	***no evidence**	present	present	present
***Gmu* B67**	**149**	***no evidence**	present	present	present
***Gmu* B91**	*****Monomorphic: non-informative marker for study populations*****				
***Gmu* D55**	**177**	absent	absent	***Private allele**	absent
	181	absent	***Private allele**	absent	absent
	185	absent	***Private allele**	absent	absent
	193	absent	***Private allele**	absent	absent
***Gmu* D62**	**125**	absent	***Private allele**	absent	absent
	133	absent	***Private allele**	absent	absent
	145	***no evidence**	present	present	***no evidence**
	149	present	present	***no evidence**	present
	153	present	present	***no evidence**	***no evidence**
	157	***no evidence**	present	present	***no evidence**
	161	absent	***Private allele**	absent	absent
	165	***no evidence**	present	present	***no evidence**
	169	absent	***Private allele**	absent	absent
	181	absent	absent	***Private allele**	absent
***Gmu* D87**	**228**	***no evidence**	present	present	present
	232	***no evidence**	present	present	present
	248	***no evidence**	present	present	present
	260	present	present	***no evidence**	***no evidence**
	264	present	present	***no evidence**	present
	268	***no evidence**	present	present	present
	272	***no evidence**	present	present	***no evidence**
***Gmu* D90**	**122**	present	present	***no evidence**	***no evidence**
	130	***no evidence**	present	present	***no evidence**
	138	***no evidence**	present	present	present
	142	present	present	***no evidence**	***no evidence**
	146	absent	***Private allele**	absent	absent
	150	absent	***Private allele**	absent	absent
	154	absent	***Private allele**	absent	absent
***Gmu* D93**	**153**	absent	***Private allele**	absent	absent
***Gmu* D114**	*****Monomorphic: non-informative marker for study populations*****				
***Gmu* D121**	**132**	absent	***Private allele**	absent	absent
	136	***no evidence**	present	present	present
	148	***no evidence**	present	present	present
	152	absent	***Private allele**	absent	absent
	156	***no evidence**	***no evidence**	present	present

The list of informative alleles in Table 9 (above) was further narrowed down to a list of the most informative locally common alleles based on alleles observed in 50% or less of the populations that also resulted in frequencies of 5% or greater in at least one of the populations sampled in this study (Table 10). The list of the most informative locally common alleles resulted in a total of 21 alleles among 8 different loci.

Table 10 List of most informative locally common alleles for characterizing individuals from specific northern Gulf Coast terrapin populations or pairs of populations that resulted in frequencies ≥ 5% in ≤ 50% of the populations sampled in this study. The fragment size of the allele for each locus is listed in number of DNA base pairs. Informative alleles characterizing a population by their "absence" indicates that an allele (frequency ≥ 5%) appeared in all but one of the northern Gulf Coast populations. Alleles (freq. ≥ 5%) that appeared only in a single population or pair of populations are characterized by their "presence."

Characteristic Population(s)	Locus	Allele size (#bp)	Characterized by:
Nueces, Texas	*Gmu* A18	123	absence
	Gmu B67	149	absence
	Gmu D87	228	absence
	Gmu D87	248	absence
	Gmu D87	268	absence
	Gmu D90	138	absence
	Gmu D121	136	absence
	Gmu D121	148	absence
Galveston, Texas	*Gmu* D121	132	presence
Louisiana	*Gmu* D55	177	presence
	Gmu D62	149	absence
	Gmu D87	264	absence
***M.t. littoralis* (Nueces + Galveston, TX)**	*Gmu* B08	220	presence
	Gmu D62	153	presence
	Gmu D87	260	presence
	Gmu D90	122	presence
	Gmu D90	142	presence
***M.t. pileata* (Louisiana + Alabama)**	*Gmu* D121	156	presence
Galveston, TX + Louisiana	*Gmu* D62	145	presence
	Gmu D62	157	presence
	Gmu D90	130	presence

Results of Genetic Diversity among Northern Gulf Coast Populations

The overall genetic diversity of the northern Gulf Coast populations did not statistically differ among populations in any of the metrics used to evaluate allelic diversity. The metrics that were used to assess overall genetic diversity among populations were: the mean number of different alleles over all loci (Na); the mean number of different alleles over loci with frequencies greater than or equal to 5% (Na Freq. $\geq$ 5%); and the mean number of locally common alleles with at least 5% frequency or greater that occurred within 50% or less populations (No. LComm Alleles [$\leq$ 50%]) (Figure 20). The only statistical difference that was observed in the measures for genetic diversity was the overall mean number of locally common alleles was significantly lower than the mean number of different alleles and mean number of different alleles with $\geq$ 5% frequencies in all of the populations (Figure 20). In addition, the overall observed mean Shannon's Information Index (I) values used as a supplementary measure for relative genetic diversity did not statistically differ among the sampled populations (Figure 21).

However, in comparison to other northern Gulf Coast populations, Galveston appeared to result in the highest allelic diversity for all 3 matrices (Na = 5.727; Na Freq. $\geq$ 5% = 3.636; No. LComm Alleles [$\leq$ 50%] = 0.636) (Figure 20), as well as appeared to have the highest value for Shannon's Information Index of relative genetic diversity (I = 1.042). Alternatively, the overall mean allelic diversity and Shannon's Index of relative diversity that was observed for Nueces appeared to consistently result in the lowest values in comparison to the diversity observed in the other populations (Figure 20 Figure 21).

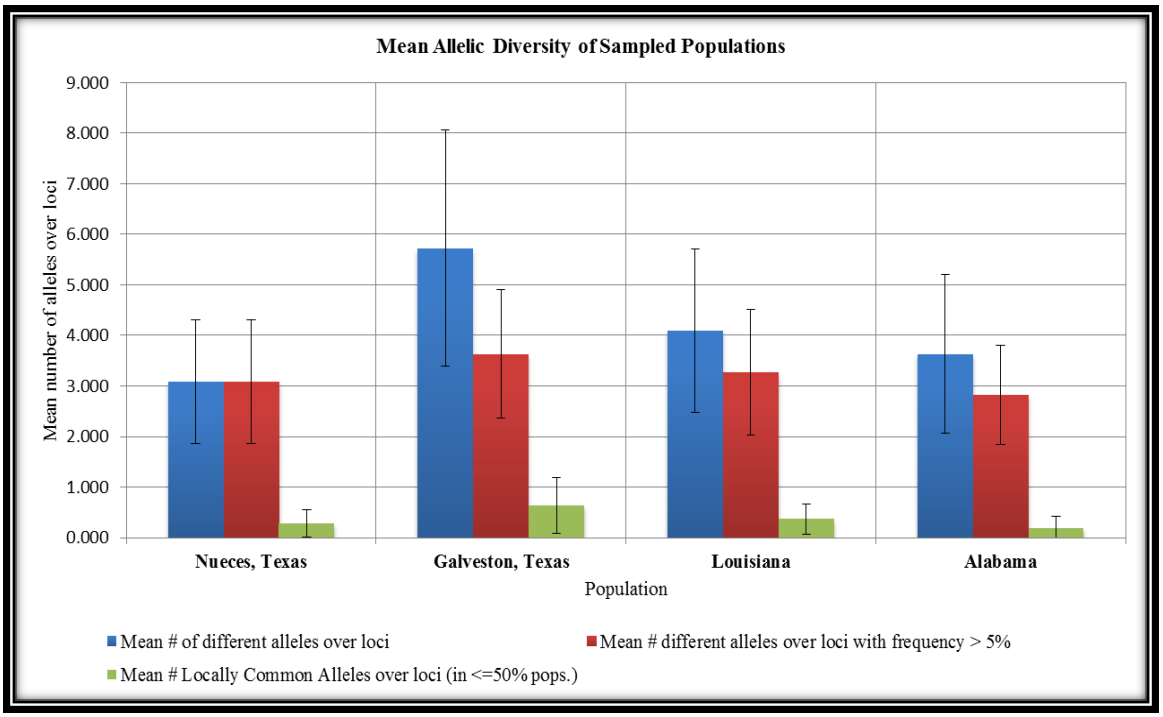

Figure 20 Overall mean allelic diversity of sampled populations based on the mean number of different alleles over all loci, mean number of different alleles with frequencies ≥5%, and mean number of locally common alleles with a frequency of ≥5% in ≤ 50% of the populations. Error bars represent the 95% confidence interval of the mean.

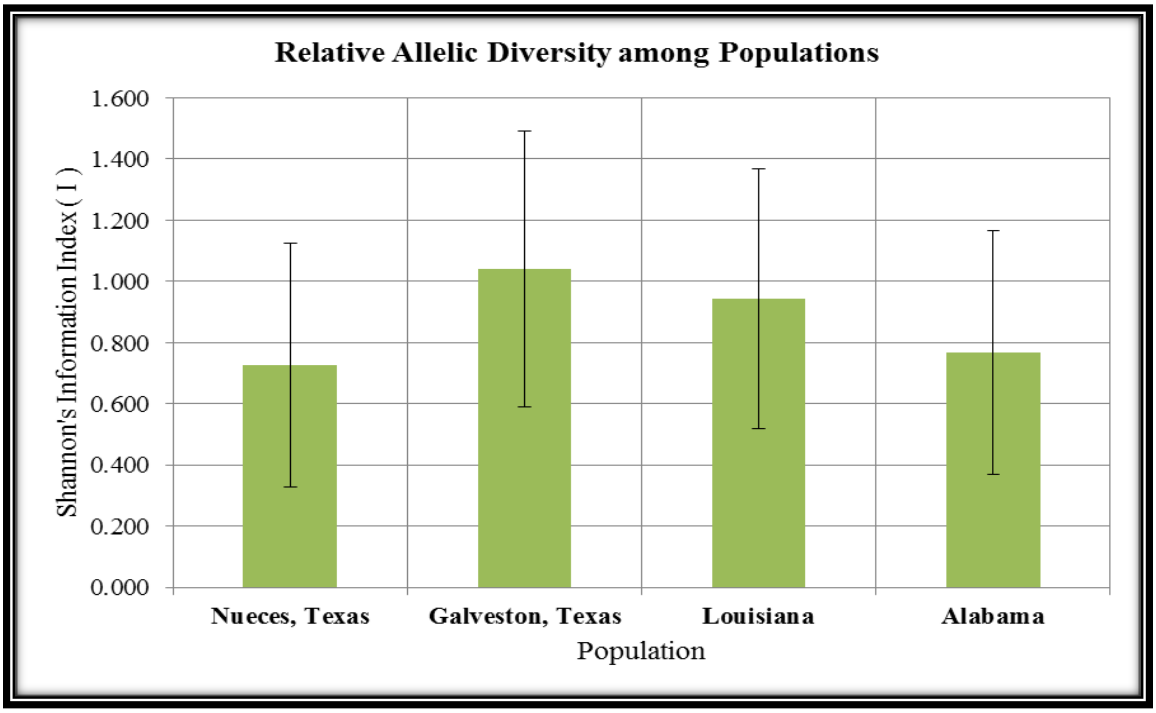

Figure 21 Overall genetic diversity among sampled populations based on Shannon's Information Index (I) for relative diversity. Error bars represent 95% confidence interval of the mean.

Comparison of Results with Previous Gulf Coast Studies

The results of the mean number of different alleles found in this study's populations was compared with the results for the mean number of different alleles found in previous studies for Nueces and Louisiana by Hart (2005) and for Alabama by Coleman (2011) (Figure 22). In comparison with the other studies, no statistical differences in allelic diversity were found between the results of allelic diversity observed for Galveston in this study with the results observed for the other northern Gulf Coast populations in the previous studies (Figure 22). Furthermore, no statistical differences in the observed allelic diversities of the other northern Gulf Coast populations were found among any of the studies, current and previous (Figure 22).

Although no statistical difference was found in comparing the allelic diversity of Galveston with the diversities found in the previous studies, the results for Louisiana observed by Hart (2005) and for Alabama observed by Coleman (2011) appeared to be similar, while the results of both this study and previous for Nueces (Hart 2005) both appeared to be similar, as well as both appeared lower than Galveston (Figure 22). Also, the mean number of different alleles observed for Nueces, Louisiana, and Alabama in the previous studies appeared to be higher than the mean number of different alleles observed for those populations in this study.

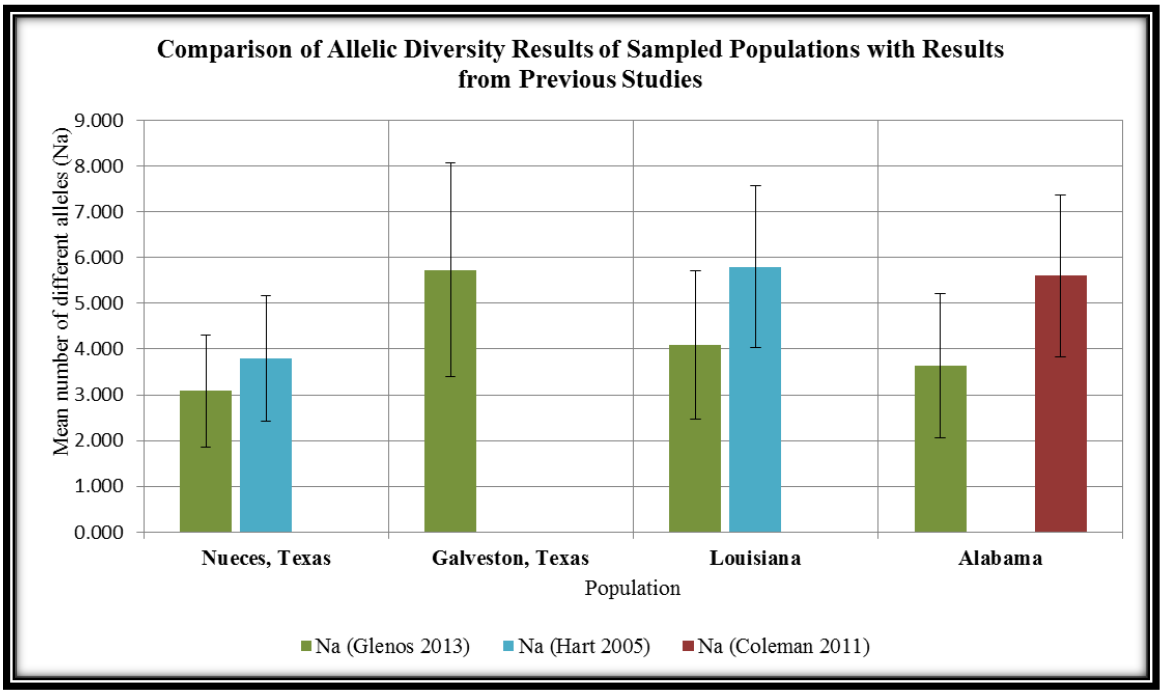

Figure 22 Comparison of allelic diversity results with previous studies by Hart (2005) and Coleman (2011). Error bars represent the 95% confidence interval of the mean.

In comparison of the results of observed heterozygosities of sampled populations within this study with the results of previous studies, there was no significant difference in values found in this study with any of the values found by Hart (2005) or Coleman (2011) (Figure 23). Although the mean heterozygosities were not statistically different between studies, the values reported by Hart (2005) and the values found in this study both appeared to be higher for Louisiana than Nueces. However, the mean heterozygosity observed by Hart (2005) for Louisiana appeared to be slightly lower than the mean value found in this study. On the contrary, the value reported for Alabama by Coleman (2011) appeared to be slightly higher than the mean heterozygosity found for Alabama in the current study. The value for Galveston found in this study also appeared to be slightly lower than the mean heterozygosities for the other northern Gulf Coast populations reported in the previous studies, but in comparison with the results of the other populations found in this study appeared to be similar with Nueces and Alabama and slightly lower than Louisiana, although not significantly different (Figure 23).

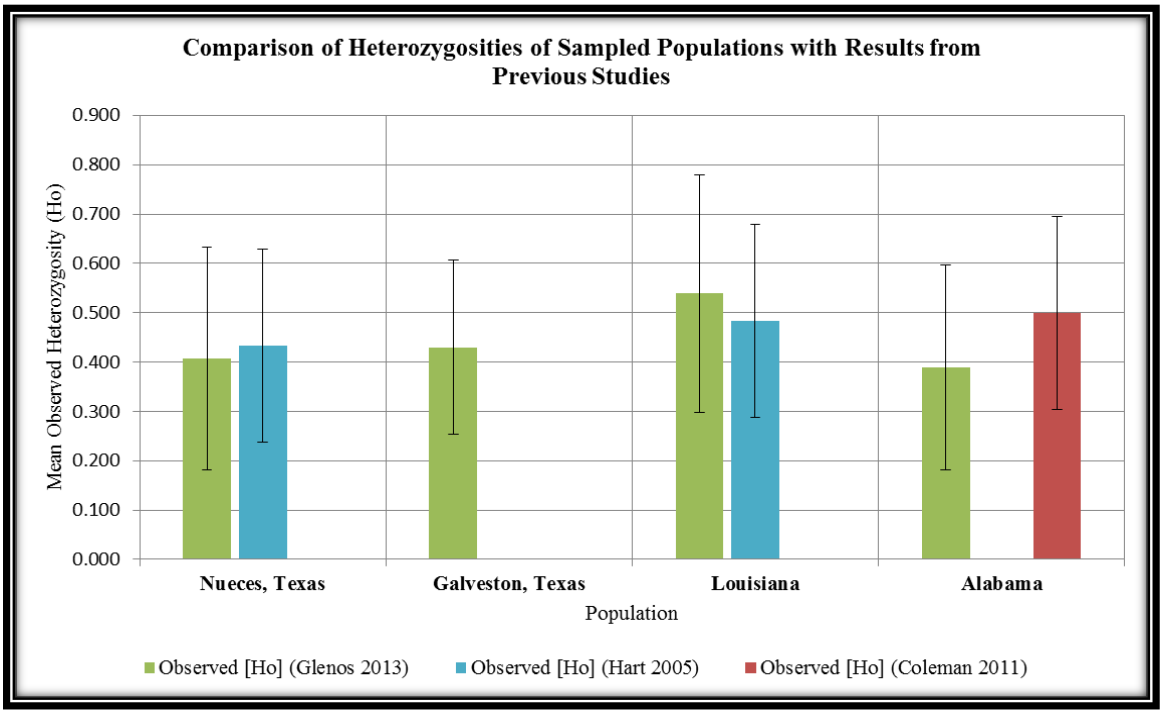

Figure 23 Comparison of observed heterozygosities found in this study with results from previous studies by Hart (2005) and Coleman (2011). Error bars represent the 95% confidence interval of the mean.

DISCUSSION

Genetic Differentiation within Galveston Bay

The results of the AMOVA test found little to no genetic differentiation within Galveston Bay terrapin populations. The F_{ST} value for variation among populations did not significantly differ (F_{ST} = 0.004; P-value = 0.289), so the null hypothesis that there is no genetic differentiation between the populations of terrapin in east and west Galveston Bay was not rejected. Moreover, the standardized F'_{ST} value for Galveston (F'_{ST} = 0.008) further agreed with the F_{ST} value, as it was close to zero, indicating little to no genetic partitioning of populations. As recommended by Wright (1978), F_{ST} values can best be interpreted as follows: 0.00-0.05 = little to no genetic differentiation; 0.05-0.15 = moderate genetic differentiation; 0.15-0.25 = great genetic differentiation; > 0.25 = very great genetic differentiation. Therefore, according to the suggestions of Wright (1978), the genetic differentiation found within Galveston Bay is minimal. However, the values for genetic differentiation both among individuals (F_{IS} = 0.209; P-value = 0.001) and within individuals (F_{IT} = 0.212; P-value = 0.001) were significantly different, indicating the presence of genetic subpopulations within the bay.

The results of the AMOVA among populations make little biological sense as to why the east and west Galveston Bay terrapin would not be genetically differentiated considering the physical barrier of the Houston Ship Channel that bisects Galveston Bay, as well as the vast spatial difference between the sites. More than likely, the non-differentiated results are an artifact of a small sample size representing the east bay population (n=7). It would be interesting to see if the results were to change if the analysis was performed again with larger, more comparable sample sizes, as well as samples from other sites in Galveston Bay. For instance, in Moses Lake there are known terrapin populations and have been many reported sightings by EIH field staff, by staff working for The Nature Conservancy (TNC) located adjacent to the Texas City Dike, and by local residents who frequently fish in the surrounding marshes of the lake (EIH, unpub. data). This would have been a potentially informative site to have included in the study as it may have

offered key insight to the link in gene flow occurring between east and west bay Galveston. Unfortunately, samples from Moses Lake were unattainable in every search effort performed by the EIH terrapin field crews during the course of this study.

If this analysis is performed again in the future with a more robust data set containing more terrapin sites in different areas of the bay and there is still no genetic differentiation among populations, the most likely biological explanation would be that gene flow among populations is facilitated by other naturally occurring phenomena (e.g. - hurricanes and severe storms; birds of prey) that may function in transporting individuals from one area of the bay to another. In 2008, after Hurricane Ike, juvenile terrapin were apparently transported approximately 300 kilometers along the coast from the Port Aransas area to Galveston Bay (Tony Amos & George Guillen, pers. comm.). Terrapin have also been recovered from the rocks of the Galveston seawall following severe storm surges (George Guillen, pers. comm.). In addition to storms serving as mechanisms of dispersal, birds of prey have also been observed to lose their grasp of terrapin hatchlings and juveniles while flying back to their nests and drop them in areas far from where they were captured. In 2011, a local Houston, TX resident reported that while standing in his driveway, a terrapin hatchling fell out of the sky on the ground in front of him, and when he looked up, he saw the large bird of prey that dropped it flying over (EIH, unpub. data).

Galveston Bay is unique in that it is such a large estuary that is dissected down the middle by one of the world's largest man-made ship channels. It is difficult to fathom how and why a terrapin could or would have reason to cross it. However, it should be noted that terrapin have been reported by local fishermen, as well as observed by EIH field crews, swimming in marshes adjacent to the ship channel on the east end of Galveston Island, so it would not be impossible (EIH, unpub. data). This movement along with dispersal during storm events could explain the gene flow between the east and west sides of the bay.

Genetic Differentiation among Northern Gulf Coast Terrapin Populations

The results of the AMOVA among northern Gulf Coast terrapin populations resulted in significant genetic differentiation for all fixation indices among populations (F_{ST} = 0.069; P (F_{ST} > 0) = 0.001), among individuals (F_{IS} = 0.192; P (F_{IS} > 0) = 0.001), and within individuals (F_{IT} = 0.248; P (F_{IT} > 0) = 0.001). Additionally, the standardized F'_{ST} differentiation index value observed for relative allelic distance among populations (F'_{ST} = 0.137) indicated a moderate degree of genetic differentiation (Wright 1978) among the northern Gulf Coast terrapin populations sampled in this study. Therefore, the null hypothesis that northern Gulf Coast populations are genetically homogenous was rejected, supporting the alternative hypothesis that northern Gulf Coast populations are subject to a significant degree of genetic differentiation. With the exception of the pairwise comparison between Louisiana and Alabama (F_{ST} = 0.014; P (F_{ST} > 0) = 0.122; F'_{ST} = 0.028), all other values generated from the pairwise F_{ST} population comparison tests were significantly greater than zero and pairwise F'_{ST} values were all above 0.05, indicating that Louisiana and Alabama was the only pair of northern Gulf Coast populations sampled in this study that are not genetically different.

Because F_{ST} fixation index is dependent on the amount of genetic variation within populations, high levels of variation have a tendency to cause lower F_{ST} estimates, and small population sizes also tend to effect its reliability of estimating genetic differentiation (Meirmans 2006). It was suggested in the Appendix 1 of the *GenAIEx*® manual (Peakall & Smouse 2006, 2012) and recommended by Bird et al. (2011) that not only should the F_{ST} fixation index values be reported but also the standardized F'_{ST} differentiation index values following the methods of Meirmans (2006). The standardized F'_{ST} differentiation index is treated as a relative value and is not based on probability (Meirmans 2006). Since the standardized F'_{ST} index is an estimate of the proportion of total variance explained by genetic differentiation among populations, relative to the maximum proportion of variance attainable (F_{STmax}), it is independent of variation within populations (Bird et al. 2011). This often yields a smaller estimate, and can sometimes even be negative when population sizes are small (Bird et al. 2011). While no fixation or differentiation index has been found to be all-around superior to other indices for estimating genetic differentiation, the F_{ST} is the best choice recommended for the type of data analyzed in this study (Bird et al. 2011).

An appropriate conclusion can be ensured when the standardized F'_{ST} values are in accordance with the F_{ST} values, but if the results of the two differ, it may be due to a particular

occurrence (Bird et al. 2011). Both sets of pairwise F_{ST} and standardized F'_{ST} results among the northern Gulf Coast populations directionally agreed and resulted in a direct, positive correlation with spatial distance. Based on the results of the populations sampled in this study, the harmonious correlation detected among the fixation and differentiation indices with spatial distance supports a reliable conclusion that northern Gulf Coast terrapin populations separated by a distance of over 300 kilometers are most likely to fall within different genetic pools. Furthermore, of the populations sampled in this study, it may also be reliably concluded that Nueces Bay terrapin, Galveston Bay terrapin, and Louisiana and Alabama terrapin are distributed into 3 distinct genetic metapopulations.

Tests of Random Mating in Northern Gulf Coast Terrapin Populations

The overall results of the Hardy-Weinberg Equilibrium (HWE) tests in this study found no significant deviation in the mean observed heterozygosities from the expected for any of the northern Gulf Coast populations sampled, which supports the null hypothesis that random mating is occurring within each population. Also, the overall mean observed heterozygosities for all of the populations appeared to be similar and did not significantly differ among the current and previous studies. This most likely indicates that within each population, there are no substantial physical or biological barriers inhibiting individuals from naturally dispersing among subpopulations and neighboring sites for mating. It is possible that the biological pressure from small population sizes could be the key driver for terrapin individuals in these northern Gulf Coast populations to expend the effort to disperse longer distances in search of suitable mates. This, in turn, would sustain or enhance the genetic diversity of their subpopulations.

The HWE tests among loci within each population did result in significant differentiation among some loci in all populations, except for Nueces. This indicates that within Galveston, Louisiana, and Alabama populations, certain genes may be selected for by individuals within these populations. Each of the Gulf Coast bay systems sampled in this study have their own unique environmental variations, whether it be salinity, temperature, annual rainfall, levels of pollutants/toxins, etc. It would take an entirely different, highly extensive, multivariate study involving sequencing of the entire terrapin genome to begin to address the specific conditions and adaptations for which these traits are being selected.

However, it is of importance to note that, of the 3 populations that resulted in non-random loci, Galveston and Alabama shared 2 loci, *Gmu* A18 and *Gmu* D121, selected for by individuals in their populations. Coleman (2011) also found significant deviation of locus *Gmu* A18 from HWE, but did not have the same results for any of the other loci that were found to be significantly different from HWE in this study. Most likely, the difference between the studies was due to differences in sample sizes, as his was much higher for Alabama, allowing for more variations in the exact randomization tests performed in the Markov chain permutations. Therefore, it is more likely the HWE test results for Alabama estimated by Coleman (2011) are more reliable than the ones estimated in this study.

Informative Alleles for Characterizing Populations

Of the northern Gulf Coast populations sampled, Galveston resulted by a large degree as having the highest number of private alleles that were unique to its population. Louisiana was the only other population where private alleles were found, in which 2 alleles were observed to be unique to its population. However, of the 2 private alleles observed in the Louisiana terrapin sampled, only one of them occurred at a frequency of greater than 5%.

Private alleles are more informative in characterizing populations, but are not the only measure by which informative alleles can be identified. Both single and pairs of populations can also be characterized by the absence of certain alleles which are observed in other populations. By narrowing down the list of informative alleles to those only found to be locally common (i.e. - observed to have occurred or were found to be absent from 50% or less of the populations sampled and to have resulted in frequencies $\geq$ 5% in at least one or more of the detected populations), a more reliable, selective list of the most informative alleles was generated. In this study, 21 alleles among 8 different loci were identified as the most informative alleles for characterizing individuals from the northern Gulf Coast populations sampled.

It should be noted that the informative alleles listed in this study were identified qualitatively based on the alleles observed from the populations sampled, and have not been statistically tested. While this list of informative alleles can potentially serve as a useful tool for tracing individuals to their source populations, it should be interpreted with caution and further explored with statistical analyses in future studies. Furthermore, it is strongly advised that the most locally common informative alleles listed in Table 10 be used in precedence before consulting the more general informative alleles listed in Table 9.

Genetic Diversity among Northern Gulf Coast Terrapin Populations

Although no significant statistical differences were found among the northern Gulf Coast populations in the overall genetic diversities in this study or previous, Galveston appeared to consistently result in slightly higher values for allelic diversity in comparison with the other populations among the current and previous studies. Likewise, Nueces Bay terrapin appeared to consistently result in slightly lower genetic diversity in comparison with the other populations.

Despite the small sample size for Nueces, the diversity found in this study appeared to be similar to the diversity found by Hart (2005). However, it should be noted that both studies lacked robust sample sizes for Nueces Bay, as her study sampled 15 individuals, while this study sampled only 8. Therefore, the reliability of both estimates for the genetic diversity of Nueces terrapin is questionable and a future reassessment with a more robust dataset would offer more certainty in reaching a statistically reasonable conclusion on the genetic diversity of Nueces Bay terrapin in relation to other northern Gulf Coast populations.

Conservation Implications

While microsatellite DNA analyses can be a useful tool for statistically analyzing the population genetics of multivariate datasets in a way that patterns can more easily be understood and examined, it can also be problematic. For instance, the fixation and differentiation indices used in differentiating populations via AMOVA often overestimate partitioning among populations (Bird et al. 2011) and mutations commonly occur that tend to violate assumptions in stepwise permutation models (Meirmans 2006). Additionally, although standardized indices of differentiation have been developed for handling population datasets with smaller samples sizes of less than 50 individuals (Hedrick 2000; Meirmans 2006), smaller datasets can still be problematic and often overinflate estimates, even when using the standardized indices.

It should not be dismissed, however, the importance of the information that can be inferred from the utilization of microsatellites to analyze populations. By using more than one index of fixation and/or differentiation to relatively assess genetic variation among and within populations, reasonable conclusions can be drawn, as discussed previously. How northern Gulf Coast terrapin populations are genetically related is pertinent information that must be obtained and assessed when considering conservation management decisions for the species. For instance, if managers were interested in establishing terrapin conservation banks from which individuals could be translocated to enhance or attempt to restore a neighboring population in critical decline, the genetic relationship between those populations would need to first be determined along with intensive supplementary habitat suitability studies.

Other ecologically important information that can be used from the results of this study is the list of informative alleles unique to specific populations. Lester (2007) demonstrated how this could be done by using data from the Hart (2005) study to genetically genotype terrapin that were illegally sold in the New York City Asian black market and identify the source populations from which they were taken. Using the informative alleles found in this study, the same approach could be used for northern Gulf Coast populations which would provide a powerful tool for wildlife enforcement officials. Additionally, when terrapin are transported to other areas by hurricanes and severe storms, birds of prey, or by any other natural phenomena, their origin could be identified using population-specific genetic markers.

Future Research

As previously mentioned, the sample sizes used in this study were not ideal. However, this study represents a significant contribution towards addressing the large geographic gap in terrapin genetic information along the northern Gulf Coast. Additionally, it is the first study published on the genetics of terrapin in Galveston Bay, setting the foundation for future studies. Not only should more genetic information on terrapin be gathered from other areas along the Gulf Coast, but many more should be conducted in Galveston Bay. Furthermore, studies should include more robust datasets from a larger number of different sites around Galveston Bay and involve DNA sequencing of individuals for a more clear understanding of individual mating dispersal behavior. By sequencing DNA strands of individuals, more information may be available to determine which terrapin genes are being selected for and which environmental or biological factors are most important in terms of selection pressure. Also, more studies are needed examining the relationship of previously reported terrapin phenotypes for each subspecies and observed population structure determined by genetic studies.

Finally, no statistical differences in the results of genetic diversity were found among northern Gulf Coast terrapin populations or among the current and previous studies. Future studies would benefit in re-evaluating genetic diversity among these populations with larger, more comparable sample sizes. Particularly, a much larger sample size is needed for Nueces Bay in order to reassess the genetic diversity of its terrapin population in relation to other northern Gulf Coast populations. Nueces Bay is not only located at the far western end of the Texas terrapin (*M. t. littoralis*) subspecies range, but also marks the end of the entire *Malaclemys terrapin* species range. Therefore, it would be biologically expected to exhibit somewhat lower levels of genetic diversity relative to the other Gulf Coast populations, as it is assumed to be subject to unilateral gene flow, limiting its genetic exchange to the nearest neighboring populations located northeast of it.

CONCLUSION

Diamondback terrapin (*Malaclemys terrapin*) along the northern Gulf Coast of Mexico within the subspecies ranges of *M. t. littoralis* and *M. t. pileata* in Nueces Bay, TX, Galveston, TX, eastern Louisiana, and Mobile, Alabama were found to have a significant level of genetic differentiation among the populations. The populations were concluded to consist of 3 distinct genetic metapopulations, where Louisiana and Alabama terrapin were within a single homogenous genetic pool. Moreover, a direct and positive correlation with spatial distribution between populations was found, in which a geographical separation of at least 300 kilometers was inferred to be the minimal geographic distance separating northern Gulf Coast terrapin genetic metapopulations.

The previous northern Gulf Coast terrapin genetics studies by Hart (2005) and Coleman (2011) concluded, based on their findings, that the northern Gulf Coast populations could be managed as one evolutionary management unit (EMU), but suggested that more data be attained to better discriminate these differences. The results of this study have refined the results of the previous studies and show that there is a level of distinct genetic differentiation among the northern Gulf Coast group that occurs in accordance with spatial distribution.

Little to no genetic structure was found on a local scale in Galveston Bay and the estuary was considered as one single genetic population. However, a more robust dataset for Galveston Bay terrapin is needed to draw this conclusion with more certainty.

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APPENDICES

Appendix 1 Institutional Animal Care and Use Committee (IACUC) Notice of Approval

University of Houston-Clear Lake
Office of Sponsored Programs
2700 Bay Area Blvd.
Houston, TX 77058-1098
(281) 283-3015 Voice
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Memo

To: Dr. George Guillen

From: Richard Puzdrowski, Chair, Institutional Animal Care and Use Committee

CC: file CL12.004 R1
Dr. Terry Blasdel, UH Main

Date: 8/9/2013

Project Title: Population genetics of the Texas diamondback terrapin

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UHCL ANIMAL CARE AND USE COMMITTEE
REVIEW ACTION

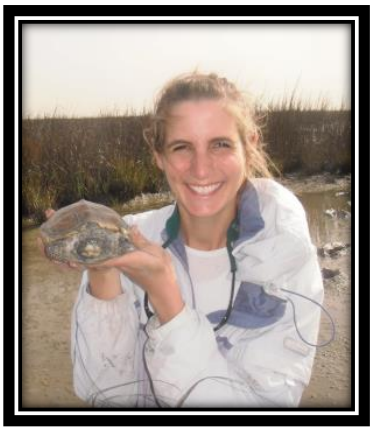
☒ Was given final approval on **8/7/2013** by the IACUC's Designated Member Review according to institutional and federal guidelines.

Either reapplication or renewal is required on an annual basis, or if you change the protocol (i.e. experimental methods, species, etc.).

If you continue to use animals on protocol #12.004 R1 after 8/7/2014, a reapplication must be submitted to the Institutional Animal Care and Use Committee for approval before that time period to prevent interruption of the study.

Richard Puzdrowski, Ph.D.
Chair, UHCL IACUC

ABOUT THE AUTHOR



Sybil Maria Glenos

**Author correspondence*: sybilmg@gmail.com

Sybil Glenos received her B.S. degree in Marine Biology from Auburn University in May 2008. Since Auburn, she has interned in a marine ecosystems lab at the Dauphin Island Sea Lab studying submerged aquatic vegetation, assisted in a diamondback terrapin study in Alabama, performed field surveys of secretive marsh birds in Alabama and Mississippi as part of a national standardized study, and served as project leader surveying gopher tortoise populations in the state of Alabama. Additionally, she collaborated on an international research study examining the effects of canopy structure on sea urchin predation (currently under review for publication). Over the course of her graduate career at UHCL, she has worked as a research assistant for the Environmental Institute of Houston primarily performing research on the monitoring of terrapin populations in Galveston Bay, as well as investigating the effects of commercial blue crab fisheries on terrapin by-catch. Sybil chose to conduct her Masters research on diamondback terrapin because it is her favorite species of turtle. The qualities she most admires about terrapin are their intrinsic beauty, their remarkable physiological capabilities to sustain in such dynamic environments, and the ease of which they can be captured and handled makes them ideal study subjects.

Previous Publication:

Guyer C, Glenos S, Hermann SM, Stober J (2011) The status of gopher tortoises (Gopherus *polyphemus*) in Alabama with special reference to three important public properties. Alabama State Wildlife Grant Final Report. Auburn University Department of Biological Sciences, Auburn, AL. pp. 28.

Reviewed: 7/29/2021