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Genetic Diversity of Kemp's Ridley (Lepidochelys kempii) Sea Turtles on South Padre Island, Texas

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GENETIC DIVERSITY OF KEMP'S RIDLEY (*LEPIDOCHELYS KEMPII*) SEA TURTLES ON SOUTH PADRE ISLAND, TEXAS

A Thesis

by

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ABSTRACT

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In 2010, researchers observed an abrupt decline in nesting numbers of the Kemp's ridley following 35 years of positive growth. In wake of this halt, and subsequent lowered nesting averages, evaluation of the genetic diversity of remaining individuals is relevant to investigate the plausibility of a genetic bottleneck, and determine the reproductive stability of the Kemp's ridley.

Here, I evaluate the genetic diversity within a sampling of the Kemp's ridleys on South Padre Island using two methods: targeting the mitochondrial control region and targeting the complete mitochondrial genome. Opportunistic samples were donated from juvenile ridleys on the East coast, and from adult ridleys along the northern Gulf of Mexico. Here, I present the first complete Kemp's ridley mitochondrial genome and discuss the geographic distribution of haplotypes present within the current population. Finally, I compare the depth of analysis achieved via targeting the full genome or by the control region sequence.

DEDICATION

To my parents, MaryAnn and Jesse Frandsen, who have never questioned my dream of working as a sea turtle biologist. Thank you for supporting and encouraging me through the years of scooping poop and filleting fish.

ACKNOWLEDGMENTS

I will be eternally grateful to Dr. Diego Figueroa, my advisor, for his mentoring and advice. From adopting me into his genetics lab, providing a crash course in genetics and sequencing, and assisting with analysis, his patience and optimism have been remarkable. I would like to thank my thesis committee members: Dr. Richard Kline and Dr. Karl Berg. The quality of my research and resulting work was improved greatly by their comments.

I would like to thank Sea Turtle Inc. and SpaceX, who helped fund my graduate assistantship. I would like to thank the numerous STI staff, interns, and volunteers who helped to collect DNA samples during nesting season. Many thanks to Jeff George, Curator of Sea Turtle Inc., for encouraging me to apply for this program and for constantly believing in my future. This project was partly funded by start-up funds from the University of Texas Rio Grande Valley and by the National Academies of Sciences' Early Career Gulf Research Fellowship (NAS 2000007266) to Diego Figueroa. I also extend my gratitude to the Chicago Herpetological Society for monetary support of my research.

This research was largely supported by sample donations from the New England Aquarium, the Georgia Sea Turtle Center, and Jenkinson's Aquarium. Thank you to the veterinarians and staff members who collected the samples from their patients. I would like to thank NOAA for the donation of samples from the 2010 Deepwater Horizon BP oil spill. Without the generosity of the aforementioned individuals and organizations, this research would not have been possible. Thank you.

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CHAPTER I

LITURATURE REVIEW

The Kemp's Ridley Sea Turtle

The Kemp's ridley (*Lepidochelys kempii*, Garman, 1880), arguably the world's most endangered sea turtle species, was originally thought to be a hybrid between loggerheads (*Caretta caretta*) and hawksbills (*Eretmochelys imbricata*), or loggerheads and greens (*Chelonia mydas*) (Bowen, 1991; Garman, 1880). This uncertainty led to the Kemp's being colloquially referred to as the "bastard" sea turtle, until it was given its official name, the Kemp's ridley, after Richard M. Kemp in 1880 (Garman, 1880). Subsequent genetic analysis using a 470 base pair (bp) sequence of the *mt* DNA control region determined that the Kemp's ridley is a unique species, distinct even from its closest relative, the Olive ridley *(Lepidochelys olivacea)*, by a haplotype divergence of 6.9% (Bowen, 1991, 1998). It is estimated that the two species diverged 3 - 6 million years ago, potentially due to geographic isolation by the formation of the Isthmus of Panama (Hendrickson, 1980). The separation of the two species is reflected in their current distribution. The Olive ridley occurs in waters worldwide, while the Kemp's ridley species is restricted primarily to the Gulf of Mexico and parts of the Northern Atlantic (Bowen, 1998).

The location of the primary nesting beach for Kemp's ridleys was published in 1963 after Hildebrand and Carr viewed a film taken by Andres Herrera in 1947 (Caillouet, Carr, 1963; Hildebrand, 1963). The film documented a single nesting event consisting of more than 40,000 Kemp's ridleys on the beach of Barra Coma in Tamaulipas, Mexico (Marquez, 1989). This

species utilizes a mass nesting strategy, termed *arribada*, though numbers of this magnitude are no longer seen. By the establishment of the first turtle camp in 1966, Santuario Playa Rancho Nuevo, the population had fallen to 10% of the original *arribada* (Marquez, 1989), due to harvesting of eggs and adults (Johnson, 1999). From 1978-1985, the population dramatically declined by 30% to fewer than 500 nesting females (Woody, 1985), or about 2% of the original *arribada* (Marquez, 1989). National Marine Fisheries Service later estimated the number to be closer to 300 nesting females during that time (NMFS, 2011). This decline was primarily attributed to heavy incidental capture by Mexican and U.S. shrimp trawls (Woody, 1989), as the main nesting beach in Tamaulipas was actively being protected from poachers by 1978 (Márquez, 1989). By 1986, the Kemp's ridley was designated one of the 12 most critically endangered animals in the world by the International Union for the Conservation of Nature (IUCN) (Burchfield, 2005).

The rapid decline of the species was combated by a combination of domestic and international actions. The harvesting and selling of turtle eggs was prohibited, as was the catch of adult turtles. Rancho Nuevo implemented protection of its beach during the nesting season beginning in 1966, and was declared a Natural Reserve in 1977, subsequently leading to the prohibition of trawling in waters adjacent to the reserve (Marquez, 1989).

In 1963, before federal involvement, Dearl Adams of Brownsville, TX and Illa Fox Loetscher of South Padre Island, TX attempted to establish a second nesting population of Kemp's ridleys on the island by relocating 98 unhatched eggs via plane from Tamaulipas, Mexico (Woody, 1989; Caillouet, 2015b). No hatchlings were produced from this relocation (Caillouet, 2015b). Efforts were continued from 1964-1967, resulting in over 5,000 hatchlings relocated from Rancho Nuevo to South Padre Island (Phillips, 1989; Sizemore, 2002; Burchfield, 2005),

and 1,227 hatchlings successfully released (Zwinenberg, 1977; Phillips 1989; Sizemore 2002; Burchfield 2005). Patrols for signs of nesting females began in 1973 and continued through 1976 (Caillouet, 2015b). Three confirmed nests were documented during this period, and further evidence of sea turtle activity (tracks, strandings, hatchling emergences), confirmed the presence of Kemp's ridleys on the island (Caillouet, 2015b).

In 1978, a bi-national working agreement established between the U.S. and Mexico increased the protection of nesting females and their eggs, and arranged for a donation of 2,000 eggs to the U.S. from Tamaulipas (Marquez, 1989). The egg donation aimed at establishing new nesting colonies in Texas, specifically on Padre Island National Seashore (PAIS), by allowing the newly emerged hatchlings to "imprint" on the Texas beach. Sea turtles exhibit strong affinity for their natal origins, and will travel thousands of miles in their lifetime, only returning to their beach of origin when they are ready to lay eggs. A head-starting program was established in Galveston at the National Marine Fisheries Service's Southeast Fisheries Center (NMFS SEFC) Laboratory, whereby hatchlings imprinted on Padre Island were reared in captivity and allowed to mature for a number of months in hopes of an increased chance of survival after release (Fletcher, 1989). Of the eggs transported from Tamaulipas and incubated at PAIS, 77.1% successfully hatched (Shaver, 2005). In 1996, head-started turtles began to lay egg clutches on PAIS, and were the first demonstrated successful effort at experimentally imprinting sea turtles (Shaver, 2005).

Beginning in 1987, a final component of the Kemp's recovery included the U.S. shrimp trawl fleet implementing turtle excluder devices (TEDS), which decrease sea turtle mortality from bycatch (TEWG, 2000). The Kemp's ridley primarily utilizes migratory corridors along the nearshore coastal waters of the Gulf of Mexico, travelling laterally within a mean distance of 20

km of land (Shaver, 2016a). These migratory routes coincide with areas of concentrated fishing and human activity, placing the Kemp's at higher risk than other sea turtle species that migrate through oceanic waters (Shaver, 2016a).

Despite the species originally nesting exclusively on a 60 km strip of beach in northern Mexico, being almost completely confined to the Gulf of Mexico, and feeding in a location heavily utilized by shrimp trawlers, the U.S. and Mexico governments were able to mitigate the decline, and positive population growth was seen beginning in the 1980's (NMFS, 2011). The number of nests at Santuario Playa Rancho Nuevo and the nearby beaches grew by 15% annually from 1988-2003 (Heppell, 2005). In 2009, this area documented more than 20,000 nests, representing approximately 8,000 nesting females (NMFS, 2011). Texas also experienced exponential growth of nesting numbers, with nest count increasing from four in 1995, to a record of 209 in 2012 (Frey, 2014).

South Padre Island continues to support a strong nesting colony of approximately 20-70 turtles annually (Jeff George, pers. comm.). Established in 1997, the staff, interns, and volunteers of Sea Turtle Inc. patrol the 64 km of the island's gulf side beaches every summer from March through August. The patrols include four staggered shifts from 6:30am-7:00pm, ensuring that a patroller is never more than 1 hour away from a potential nesting female. Tracks from nesting females are quickly blown away by the wind, and the chances of finding a nest diminish with every passing minute.

Additionally, commercial vehicular traffic is allowed on South Padre Island north of County Beach Access 4. Deep tire ruts form over the course of the summer, further diminishing the visibility of turtle tracks. The island and the lower Laguna Madre host over 1 million visitors annually (City of SPI Convention and Visitors Bureau, 2015), with most visitors arriving during

the summer and peak turtle nesting season. The northern gulf side beachfront resembles a parking lot during this time. Sea Turtle Inc.'s official presence on the beach discourages harassment of nesting females by the public. Furthermore, once the nest cavity has been located, the egg clutch is relocated to secure outdoor corrals, ensuring that eggs do not fall victim to predators, erosion, or human interference. In Texas, all Kemp's ridley nests found by patrol programs are relocated to either an incubation facility or protective corral (Shaver and Caillouet, 2015).

As demonstrated, until recently the species was considered a conservation success. Based on the survival rates at the time, a population model published in 2005 and updated with information from 2009, predicted the population would grow 19% per year from 2010-2020, assuming the current rate of success stayed constant (NMFS, 2011). The population was anticipated to attain at least 10,000 nesting females in a season by 2011, which would have been a key criterion for downlisting the species from critically endangered to endangered (NMFS, 2011). However, in 2010, the positive growth exhibited by Kemp's since the 1980's was interrupted (Caillouet, 2011; Crowder & Heppell, 2011; Gallaway et al., 2013), and annual nesting numbers dropped by 35.4% (Caillouet, 2016). A decline in nesting numbers was evident in 2013 and 2014 (Caillouet, 2014, 2015), and the overall population was predicted to be decreasing by 5% per year (Heppell, 2014).

Past stock assessments have indicated that the interruption of population growth in 2010 was due to a large mortality event during that year (Galloway and Caillouet, 2014; Gallaway and Gazey, 2014). The impact of the 2010 BP Deepwater Horizon oil spill on specific sea turtle stocks is still contested. Modeling has indicated that estimates of Kemp's ridleys present at the site exceeds the publicly available count (Putman, 2014). A more recent study estimates that 51% of the observed oiled sea turtles were Kemp's ridleys, and 99% of the affected ridleys were

from Mexico (Putman, 2017). Though the oil spill may have been a significant variable in the decline of the population, the stock assessment indicating that a large morality event was the main cause of population decline, also predicts that the population should exhibit positive growth starting in 2013 (Galloway and Caillouet, 2014; Galloway and Gazey, 2014). Instead, the Kemp's population declined severely in 2013, prompting the need for a new assessment (Caillouet, 2014).

A recent study evaluated carbon and nitrogen signatures in scute samples taken from 2010-2012, confirming that a large percentage of Kemp's were exposed to oil through 2012 (Reich, 2017). Combined with satellite data, the results show that the Kemp's continued to utilize their main foraging ground in the contaminated northern Gulf of Mexico, and incorporate oil into their system via diet and exposure. The study does not conclude what role this exposure to oil played in the decrease in nesting numbers in 2014.

The most recent studies have alternatively hypothesized that the population, faced with reduced prey levels and a decrease in water temperature in the winter of 2010, led to an increase in the time needed for a female to reach a body condition suitable for migration and nesting (Caillouet, 2014; Gallaway and Gazey, 2014). In turn, this has led to an increasing remigration interval from the standard 2 year interval used in the Bi-national Recovery plan (Gallaway, 2016; Shaver, 2016b). In 2014, the interval was observed at an average of 3.3 years, and in 2015 it increased to an average of 3.5 years (Shaver, 2016b). This hypothesis directs the conservation emphasis to focus on the condition of foraging grounds and prey species, continue with mark-recapture programs, and consistent monitoring for addition of neophytes into the nesting stock.

The plummet in number of nesting females in 2013 and 2014 incited a panic in researchers working with this species, and generated conferences and special sessions highlighting the

need for immediate mediation of this decline (Caillouet, 2015a). The 34th Annual Symposium on Sea Turtle Biology and Conservation held in April 2014 included a session dedicated to Kemp's ridley presentations. Later that year in November, the Second International Kemp's Ridley Sea Turtle Symposium provided a forum for advances in Kemp's specific biology and conservation. In February of 2015, the Oil Spill and Ecosystem Science Conference discussed Gulf of Mexico ecosystem changes, and in the spring of 2016, the Southeast Regional Sea Turtle Meeting held a special session on Kemp's ridley specific presentations. The concern over the recent decrease in nesting numbers was evident across the country.

The 2017 Kemp's ridley nesting season, however, resulted in a record-breaking number of nesting females in Texas. A total of 353 nests were recorded in the state of Texas (Donna Shaver, pers. comm.), far more than the previous record of 209 nests in 2012 (Shaver, 2016b). The overwhelming return of the Kemp's strongly supports the hypothesis that a longer remigration interval is now required for the females to reach peak nesting condition.

CHAPTER II

INTRODUCTION

One way to measure the health of a population is to quantify the genetic variability within the population. Previous studies of Kemp's ridleys have utilized various nuclear markers to determine divergence from Olive ridleys (Bowen, 1991, 1998); determine genetic diversity between nesting colonies (Kichler, 1996); document nesting (Johnson, 1999), and detect multiple paternity in clutches (Kichler, 1999). Male sea turtles opportunistically mate with multiple females throughout the nesting season, thereby establishing a unique nuclear male-mediated gene flow that often differs from the patterns of genetic variation seen when analyzing maternally inherited mitochondrial DNA markers (FitzSimmons, 1997). Through analysis of heterozygosity at microsatellite loci, the original decline of the Kemp's ridley population was determined to not have a significant effect on their genetic diversity by Kichler (1996). However, a later study conducted by Stephens (2003) using microsatellites indicated that the demographic bottleneck led to a measurable loss of genetic variation in the species. The apparent contradictions are potentially resolved if the bottleneck occurred too quickly to be detected by Kichler's (1996) markers. Dutton (2006) used mitochondrial DNA control region sequences to compare haplotype frequencies of nesting females in Texas to haplotype frequencies from females at Rancho Nuevo. The study found six distinct haplotypes, however the results indicated genetic homogeneity between the two populations. This data from a past conference proceeding remains unpublished.

Studies after the 2010 halt in population growth have focused on determining genetic diversity between nesting colonies (Rivera, 2012) and distinguishing individual nesters (Frey, 2014). Microsatellites showed no genotype segregation among rookeries in Tamaulipas (Rivera, 2012). Recent work using mitochondrial DNA concluded that there are at least 2 lineages of females nesting along the Texas coast, and discovered 8 haplotype sequences for Kemp's ridleys (Frey, 2014). Presently, only two partial mitochondrial genomes have been published for Kemp's ridley sea turtles; neither of which could sequence a distinct 117 bp region (Duchene, 2012).

Following the dramatic decrease in nesting numbers in 2010, and lowered nesting averages in 2013-2015, evaluation of the genetic diversity of the individuals within the population is highly relevant to investigate the plausibility of a genetic bottleneck, and determine the reproductive stability of the Kemp's ridley. One method that can be used to determine whether there has been a bottleneck in the Kemp's ridley population is to determine which haplotypes are present within current individuals by analyzing the mitochondrial DNA, and then comparing observed haplotype frequencies to past data. If there was in fact, a large mortality event in 2010 that killed a majority of the adult Kemp's ridley females, then there would be a very obvious decrease in haplotype frequencies compared to those present before 2010. If the haplotype frequencies are similar to those observed before 2010, that would lend support to the alternate hypothesis that Kemp's ridleys are requiring a longer remigration interval.

Haplotypes can be determined by identifying nucleotide differences within DNA. Variation within sea turtle DNA is commonly studied using the control region sequence (~800bp) (Gaos, 2016; Matsuzawa, 2016), a hypervariable region of the mitochondrial genome where most of the variation between individuals can be identified. To date, researchers often use this region, since targeting solely the control region instead of the entire genome provides a relatively

inexpensive method of analyzing samples. The latest study of Kemp's ridley turtles in the Atlantic and Mediterranean used a fragment of the control region to determine their origin (Carreras, 2014).

I evaluated the genetic diversity within a sampling of the Kemp's ridleys in South Padre Island using two methods, one targeting the mitochondrial control region and the other targeting the complete mitochondrial genome. Opportunistic samples were donated from juvenile ridleys on the East coast of the United States, as well as from adult ridleys on the northern Gulf of Mexico. Samples from these areas allowed for a comparison to be made in an attempt to determine whether these ridleys could have potentially originated from Texas.

For the first part of my thesis, I specifically targeted the control region, a 764 bp sequence within the *mt* genome, using standard PCR and Sanger sequencing techniques. The mitochondrial control region was used to accomplish three goals: 1) evaluate the genetic diversity within a sampling of South Padre Island's breeding stock 2) determine whether deceased ridleys that were recovered in the Northern Gulf of Mexico after the BP oil spill potentially originated from South Padre Island and 3) determine whether foraging ridleys on the east coast potentially originated from South Padre Island.

My hypothesis was that I would find low genetic diversity in the nesting females present on South Padre Island, and significant variation between Kemp's ridleys foraging on the East coast and Kemp's ridleys nesting on the Texas coast. I believed South Padre Island would have low levels of diversity as it hosts only a miniscule fraction of the total population of nesting Kemp's ridleys. Additionally, females have a strong affinity to particular beaches, leading to select individuals returning each season. Sampling from the East coast sought to identify new haplotypes not found on the Texas coast. I believed that there would be greater variation between

East Coast and Western Gulf samples, as the distance could have isolated certain genes and new genetic material could have been introduced by rare East coast nesters. However, I hypothesized that the majority of the East coast samples would have originated from South Padre Island. I hypothesized that the samples taken along the northern Gulf of Mexico after the BP oil spill would be indicative of those taken along the Texas coast, indicating that the turtles oiled in the spill could have been from South Padre Island. I believed this would be the resulting pattern as adult ridleys leave their Texas nesting grounds and forage along the northern Gulf coastline.

For the second part of my thesis, I targeted the full mitochondrial (*mt*) genome using next-generation sequencing techniques. Conventionally, standard long-PCR is used for generating *mt* genomes. The entire mitochondrial genome is formed by the amplification of overlapping fragments. These sections are laboriously amplified and sequenced using multiple pairs of primers. The alternative use of next-generation sequencing obtains numerous mitochondrial genomes in a single run. Only two partial *mt* genomes have been published for Kemp's ridley sea turtles using long-PCR followed by sequencing on Roche (454) FLX (Duchene, 2012), a pyrosequencing technology. Complete mitochondrial genomes were used to achieve the following three goals: 1) describe the first complete mitochondrial genome for the Kemp's ridley 2) demonstrate a sophisticated and streamlined method for obtaining complete mitochondrial genomes using modern next generation sequencing technology and 3) compare the genetic resolution of full mitochondrial genomes with that of the control region. I hypothesized that utilizing the full mitochondrial genome versus using solely the control region would result in a fuller resolution of haplotypes present within my samples. Analyzing a sequence of approximately 16,000 base pairs containing numerous protein-coding genes and RNAs should provide more information than only looking at a non-coding sequence of approximately 700 base pairs.

CHAPTER III

METHODOLOGY

Sample Collection

Samples were collected or donated with a sampling permit for that state (Table 1). I collected samples from nesting and stranded ridleys in Texas (Permits TE181752-2 and SPR 0511-130). This involved months of personal patrol effort on the beach via ATVs, looking for stranded sea turtles in the surf, and following tracks left behind by nesting females primarily during the months of March to September. Coordination of the collection of all samples used in the project occurred from October 2015-October 2016.

Samples were representative of four geographic areas around the country (Table 1). Samples representing the Western Gulf originated from nesting females on South Padre Island, stranded Kemp's ridleys that wash ashore on South Padre Island and Boca Chica Beach, and captive held Kemp's ridleys that were collected on the Texas coast. Samples representing the northern Gulf of Mexico originated from Kemp's ridleys stranded on the coasts of Alabama, Louisiana, Mississippi, and Florida. Until recently, these samples had been sequestered by National Oceanic and Atmospheric Administration (NOAA) for analysis regarding sea turtle mortality related to the 2010 BP oil spill. These samples were geographically categorized as BP samples. Samples representing the East coast of the United States originated from necropsied and rehabilitating Kemp's ridleys, mainly found along the coast of Massachusetts.

Nesting and Stranded Sea Turtles

Fourteen DNA samples were obtained from nesting adult Kemp's ridley sea turtles on South Padre Island. Twenty-one samples were collected from deceased Kemp's ridleys stranded on South Padre Island and Boca Chica Beach. Eighteen of the twenty-one stranded samples were able to be used in analysis. All stranded samples were adult sea turtles, except for two hatchlings.

Donated Samples

DNA samples were donated from one captive Kemp's ridley at Sea Turtle Inc. in South Padre Island, Texas and one at Jenkinson's Aquarium in Point Pleasant Beach, New Jersey (Table 1). The captive samples used in this study were taken from both juvenile and adult sea turtles. DNA samples donated from the Georgia Sea Turtle Center (Jekyll Island, GA) were from six juvenile Kemp's ridleys initially stranded off the coast of Massachusetts during a cold-stun event. Four of the six samples could be used for analysis. The New England Aquarium (Boston, MA) donated eighty-two tissue samples from necropsied Kemp's ridleys stored in their freezer units. Fifty-four of the samples were used in the study. These samples originated from cold-stunned sea turtles stranded off the coast of Massachusetts between 2013 and 2015. DNA samples collected after the 2010 BP Deepwater Horizon Oil Spill in the Gulf of Mexico were donated from NOAA. These samples were collected from sites off Texas, Louisiana, Mississippi, Alabama, and Florida coasts. Of the thirteen samples from dead Kemp's ridleys stranded in various states of decay, during 2010, eleven were used in the study.

Sample Location	Category	Type of Sample	Preservation Method	Collection Date	Permit	Number sequenced for the control region	Number sequenced for full *mt* genome
South Padre Island, TX Boca Chica Beach,TX	Western Gulf	Tissue	Ethanol	2015-2016	TE181762-2 SPR0511-130	32	8
Brewster, MA Eastham, MA Wellfleet, MA	East Coast	Tissue	Ethanol	2013-2016	TE-697823 29-WJH-16-67	58	2
Mobile, AL Jefferson County, LA Hancock, MS Harrison County, MS Franklin, Florida Nueces County, TX Kenedy County, TX	BP	Tissue	Dry Frozen	2010-2011	DWH NRDA	11	0
South Padre Island, TX Mattituck, NY	Captive	Tissue	Ethanol	2016	TE181762-2 TE-697823	2	0

Table 1. Sampling location, preservation, collection date, and permits for samples used in study.

Experimental Design

Tissue Collection Protocol

DNA tissue samples from Kemp's ridley sea turtles maintained in captive environments were collected using the NOAA procedure for collecting genetic samples from live turtles (NOAA, 2015) (IACUC Permit 2015-003-IACUC). The right rear flippers of live and stranded sea turtles were biopsied using a sterilized biopsy one punch. To prevent infection, the sampled area

was cleaned of any sand and sterilized before and after procedure with Providone-Iodine and alcohol swabsticks. Live sea turtles were manually restrained by trained members of Sea Turtle Inc.'s patrol team and kept calm with a wet towel over the eyes while the biopsy was collected. In the chance of minor bleeding, sterile gauze was applied with pressure to the biopsy site.

Samples from live and stranded turtles were placed into a plastic vial containing 95% ethanol, and were kept chilled until transfer to the -20°C freezer. Samples collected from rehabilitated ridleys at the Georgia Sea Turtle Center were placed into plastic vials containing saturated NaCl with 20% DMSO, and were shipped within 24 hours. The samples were transferred from the saturated NaCl with 20% DMSO to 95% ethanol upon arrival at the laboratory, and then stored at -20°C. Samples collected during the Deepwater Horizon oil spill (DWH) were donated in their original collection containers, including I-Chem jars and aluminum foil within Ziploc bags. In the laboratory, subsamples were taken from the donated muscle tissue using sterilized blades. Subsamples were immediately utilized for DNA extraction. Samples donated from NEAQ arrived as flipper clippings stored individually in small Ziploc bags. When staff NEAQ staff members collected the samples at their facility, gloves and blades were not changed between sea turtle specimens, so subsampling was conducted using sterilized blades. Subsamples were immediately utilized for DNA extraction.

DNA Extraction

DNA (200 ng) were obtained from all samples after standard extraction with Thermo Fisher Scientific's Purelink Genomic DNA extraction kit (model #K1820-01, Thermo Fisher Scientific, USA), following the manufacture's protocol for mouse tissue. Samples were primarily digested overnight before extraction the next day, however, a few samples were only digested for two hours before extraction. Once the DNA was obtained using the Genomic DNA extraction

kit, the concentration of DNA was measured using a Life Technologies Qubit fluorometer (Life Technologies Inc, USA). Thermo Fisher Scientific's Purelink Quick PCR Purification Kit (model #K310001, Thermo Fisher Scientific, USA) was used to purify the PCR product from the NEAQ samples, four deceased stranded samples, and one live stranded sample. Gel electrophoresis in a 2% agarose gel stained with ethidium bromide ensured the extracted DNA of all samples was of high quality molecular weight. Extracted DNA were stored at -20°C.

Polymerase Chain Reaction (PCR).

PCR analysis was conducted at University of Texas Rio Grande Valley in Dr. Figueroa's genetics lab. Two sea turtle specific primers for the control region sequence were used: LCM15382 (5' GCTTAACCCTAAAGCATTGG 3') and H950g (5' GTCTCGGATTTAGGGGTTTG 3') (Abreu-Grobois, 2006; LeRoux, 2012). A 25 µl PCR reaction containing 17.4µl of PCR water, 2.5µl of 10X *Taq* Reaction Buffer, 2.0µl of 10mM dNTPs, 1.0µl of 10µM Forward Primer, 1.0µl of 10µM Reverse Primer, 0.125µl of Dream*Taq* DNA Polymerase, and 1.0 µl of DNA, was run on an Eppendorf Mastercycler pro thermocycler. The following previously tested parameters were used: 1) 2 minutes of initial denaturation at 94°C, 2) 50 seconds of DNA denaturation at 94°C for 36 cycles, 3) 2 minutes of primer annealing at 52°C, 4) 90 seconds of primer extension at 72°C, 5) 5 minutes of primer extension at 72°C (Dutton, 2008). PCR products were purified using Sigma-Aldrich GenElute PCR Clean-Up kit, and Invitrogen Purelink Quick PCR Purification Kit. Each extracted PCR product was sequenced in the forward and reverse direction using the LCM15382 (forward) and H950g (reverse) primers by Eurofins MWG Operon, LLC. A consensus sequence for the control region was created using the LCM15382 (forward) and H950g (reverse) primers with Qiagen CLC Genomics Workbench software.

During the alignment of the forward and reverse sequences for each sample, a manual check was conducted to ensure the quality of the chromatogram reading of nucleotides. Several of the readings differed between the forward and reverse strand, and a few samples only had one readable strand (i.e. just the forward or just the reverse strand). When there was a conflict between forward and reverse sequences, the strand with the clearest chromatogram trace was given priority, and that nucleotide was assigned as the consensus nucleotide. For those samples that only had one readable strand, that reading was used as the consensus sequence, as long as the chromatogram trace was of excellent quality (no double peaks) and with a minimum Phred score of 20.

Next-Generation Sequencing.

DNA extraction was followed directly by indexed library preparation and sequencing at Harvard's Biopolymer facility using the Nextera X2 kit followed by 100bp paired-end multiplexed lane of Illumina HiSeq 2500. The sequences were de-multiplexed according to their indices. De novo assemblies were conducted using the software CLC Genomics Workbench. Default settings were used with reads mapped back to contigs (mismatch cost=2, insertion cost=3, deletion cost=3, length fraction =0.5, similarity fraction=0.8). The join contigs function of the Genome Finishing Module plug-in was then use to combine overlapping contigs. The sequences obtained from the assemblies included the full mitochondrial genome for each specimen. The assembled genome were annotated for ten individuals using Qiagen CLC Genomics Workbench software, referencing the two previously published partial Kemp's ridley genomes on GenBank (www.ncbi.nlm.nih.gov): isolate 68090 and isolate 68091.

Analysis.

The Control Region

Haplotypes were defined with DnaSP software (Rozas, 2009) using the control regions of all samples and of all sequences found on GenBank (Table 2). Minimum-spanning haplotype networks based on geographic location were created using PopArt (Population Analysis with Reticulate Trees) software (Leigh, 2015). The percentage of haplotypes found within each geographic region were calculated and graphed using Excel.

Phylogenetic analyses were performed with MEGA7 (Kumar, 2016) using maximum-likelihood (ML) methods with bootstrap values from 10,000 replicates. The Tamura 3-parameter model (Tamura, 1992) with uniform rates was selected by MEGA7 as the best fitting model of molecular evolution based on the Akaike Information Criterion (AIC). The tree was rooted using two Olive ridley (*Lepidochelys olivacea*) genomes, GenBank accession numbers AM258984.1 and DQ486893.1 (Table 2). Arlequin v3.5.1.2 (Excoffier & Lischer, 2010) was used to make pairwise fixation index (*ΦST*) comparisons among all sampling groups using default settings. The statistical significance of the fixation indices was assessed under the null hypothesis of panmixia by performing 10,000 permutations of the original data set by random reallocation of individuals to each population.

Sequence ID	Sequenced Region	Citation	GenBank Accession Number
Haplotype 1	Control Region	This Study	Accession # pending
Haplotype 2	Control Region	This Study	Accession # pending
Haplotype 3	Control Region	This Study	Accession # pending
Haplotype 4	Control Region	This Study	Accession # pending
Haplotype 5	Control Region	This Study	Accession # pending
Haplotype 6	Control Region	This Study	Accession # pending
Haplotype 7	Control Region	This Study	Accession # pending
Haplotype 8	Control Region	This Study	Accession # pending
Haplotype 9	Control Region	This Study	Accession # pending
Haplotype 10	Control Region	This Study	Accession # pending
SPI Nest 1	Full mitochondrial genome	This Study	Accession # pending
SPI Nest 3	Full mitochondrial genome	This Study	Accession # pending
SPI Nest 6	Full mitochondrial genome	This Study	Accession # pending
SPI Nest 13	Full mitochondrial genome	This Study	Accession # pending
SPI Nest 14	Full mitochondrial genome	This Study	Accession # pending
SPI Nest 16	Full mitochondrial genome	This Study	Accession # pending
SPI Nest 23	Full mitochondrial genome	This Study	Accession # pending
SPI Nest 27	Full mitochondrial genome	This Study	Accession # pending
East Coast 1	Full mitochondrial genome	This Study	Accession # pending
East Coast 2	Full mitochondrial genome	This Study	Accession # pending
LK1.1	Control Region	(Frey, 2014)	KF385935
LK2.1	Control Region	(Frey, 2014)	KF385936
LK3.1	Control Region	(Frey, 2014)	KF385937
LK4.1	Control Region	(Frey, 2014)	KF385938
LK5.1	Control Region	(Frey, 2014)	KF385939
LK6.1	Control Region	(Frey, 2014)	KF385940
LK6.2	Control Region	(Frey, 2014)	KF385941
LK7.1	Control Region	(Frey, 2014)	KF385942
L. kempii isolate 68090	Partial mitochondrial genome	(Duchene, 2012)	JX454981
L. kempii isolate 68091	Partial mitochondrial genome	(Duchene, 2012)	JX454982
L. olivacea 1	Full mitochondrial genome	(Tandon, unpub.)	AM258984.1
L. olivacea 2	Full mitochondrial genome	(Tandon, unpub.)	DQ486893.1

Table 2. Sequence IDs, sequenced regions, and GenBank accession numbers of *Lepidochelys kempii* and *Lepidochelys olivacea* samples used in study.

Full Mitochondrial Genomes

Ten mitochondrial genomes were used in a partitioned phylogenetic analysis using Partition-Finder v1.1.1 (Lanfear, 2014) and RAxML v8.0.0 (Stamatakis, 2014). The gene and codon posi-

tions of the 12 protein-coding genes were used to create data blocks (Table 3). PartitionFinder divided the data into 8 partitions and selected General Time Reversible plus Gamma (GTR+G) as the best evolutionary model (Table 4). Within the RAxML program, 20 independent searches of 1,000 bootstrap replicates delivered the best maximum-likelihood (ML) tree.

Region	**Codon Positions**		
	1	**2**	**3**
ND1	2602-3574	2603-3574	2604-3574
ND2	3575-4616	3576-4616	3577-4616
Cox1	4617-6169	4618-6169	4619-6169
Cox2	6170-6862	6171-6862	6172-6862
Atp8	6863-7048	6864-7048	6865-7048
Atp6	7049-7734	7050-7734	7051-7734
Cox3	7735-8521	7736-8521	7737-8521
ND3	8522-8871	8523-8871	8524-8871
ND4L	8872-9172	8873-9172	8874-9172
ND4	9173-10553	9174-10553	9175-10553
ND5	10554-12361	10555-12361	10556-12361
Cytb	12362-13507	12363-13507	12364-13507
ND6	13508-14033	13509-14033	13510-14033
12s	1-975		
16s	976-2601		
Control	14034-14813		

Table 3. Data block arrangement for 12 protein-coding genes in the *Lepidochelys kempii* genome.

Subset	Best Model	Subset Partitions	Subset Sites
1	GTR+G	12s, 16s, ND2[1]	1-975; 976-2601; 3575-4616
2	GTR+G	Cox2[3], Cox3[1], Cytb[2], ND1[1], ND4L[1], ND5[2]	2602-3574; 6172-6862; 7735-8521; 8872-9172; 10555-12361; 12363-13507
3	GTR+G	Cox1[1], ND1[2], ND3[2], ND4[1], ND4L[2], ND5[3]	2603-3574; 4617-6169; 8523-8871; 8873-9172; 9173-10553; 10556-12361
4	GTR+G	Atp8[2], Atp8[3], Cox1[3], Cox2[2], Cox3[3], Cytb[1], ND1[3], ND2[2], ND3[1], ND3[3], ND4[2], ND4L[3], ND5[1]	2604-3574; 3576-4616; 4619-6169; 6171-6862; 6864-7048; 6865-7048; 7737-8521; 8522-8871; 8524-8871; 8874-9172; 9174-10553; 10554-12361; 12362-13507
5	GTR+G	Atp8[1], ND2[3], ND4[3]	3577-4616, 6863-7048, 9175-10553
6	GTR+G	Cox1[2], Cox2[1], Cox3[2], Cytb[3]	4618-6169, 6170-6862, 7726-8521, 12364-13507
7	GTR+G	Atp6[1], Atp6[2], Atp6[3]	7049-7734, 7050-7734, 7050-7734, 7051-7734
8	GTR+G	ND6[1], ND6[2], ND6[3]	13508-14033, 13509-14033, 13510-14033

Table 4. 8 PartitionFinder partitions of *Lepidochelys kempii* genome data. Superscript numbers refer to codon position 1, 2 or 3.

CHAPTER IV

RESULTS

Phase I: Control Region Sequences

The control region was sequenced for 113 samples, resulting in ten unique haplotypes within the dataset. Eight of these haplotypes matched Lk 1.1, 2.1, 3.1, 4.1, 5.1, 6.1, 6.2, and 7.1 (Frey, 2014). Individuals with haplotype 1 were highly abundant, comprising 49.6% of all samples (Fig 1). Haplotype 2 comprised 25.7% of all samples, manifesting strongly in the Western Gulf, East coast, and BP samples (Fig 1). Haplotypes 5 and 10 were more closely related to Haplotype 1, while the remaining haplotypes 3, 4, 6, 7, 8, and 9 were more closely related to haplotype 2 (Fig 2). The maximum parsimony tree of the haplotypes supports the minimum-spanning haplotype network. Haplotypes 5 and 10 branch from Haplotype 1, which is predominately related to Haplotype 2 (Fig 3). The remaining haplotypes deviate by small increments from Haplotype 2 (Fig 3).

Approximately 79% of the haplotypes found on the Texas coast were Haplotypes 1 and 2 (Lk 4.1 and 6.1) (Fig 2). Samples taken after the BP oil spill reflect a similar frequency of 82%, but are predominantly Haplotype 2 (Fig 2). Samples collected from the East coast have a 80% frequency for Haplotypes 1 and 2, but are predominantly Haplotype 1 (Fig 2). Two previously undefined haplotypes were found: Haplotype 3 from a nesting female and Haplotype 8 from a sample collected on the East coast (Table 5).

The phylogenetic reconstruction based on the ten haplotypes of the control region does not resolve the relationship between these haplotypes, with most branches collapsed due to weak support (Fig 3). Only Haplotypes 1, 5 and 10 are grouped in a strongly supported clade with Haplotype 1 basal to Haplotypes 5 and 10.

Haplotype	(Frey, 2014) Correlation	Western Gulf	BP	East Coast	Captive
Haplotype 1	Lk4.1	20	3	32	1
Haplotype 2	Lk6.1	13	6	15	1
Haplotype 3		1	0	0	0
Haplotype 4	Lk5.1	1	0	0	0
Haplotype 5	Lk3.1	1	0	6	0
Haplotype 6	Lk2.1	3	0	2	0
Haplotype 7	Lk1.1	1	2	2	0
Haplotype 8		0	0	1	0
Haplotype 9	Lk6.2	1	0	0	0
Haplotype 10	Lk7.1	1	0	0	0

Table 5. Number of individuals of each haplotype found in each region, and relation to haplotypes described in Frey, 2014.

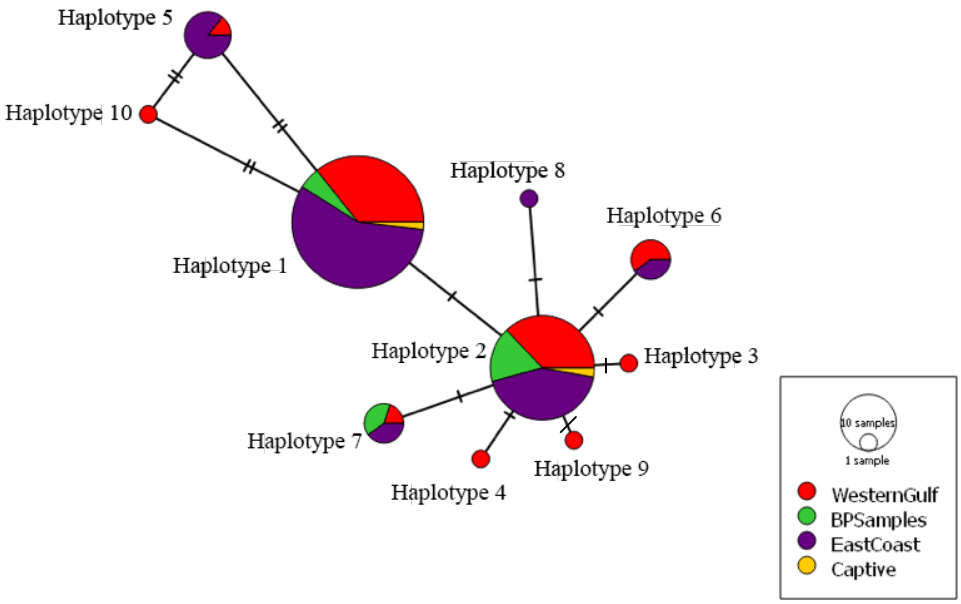

Figure 1. Minimum-spanning haplotype network of *Lepidochelys kempii* control region sequences. Circles represent the ten unique haplotypes found within the samples. Size of the circle is proportional to the number of samples belonging to that haplotype. Colors represent the group designation of the samples: Red- Western Gulf of Mexico, Green- BP samples, Purple- East Coast, and Yellow- Captive samples. The lines connect similar haplotypes, while notch marks on the lines represent mutational steps between haplotypes.

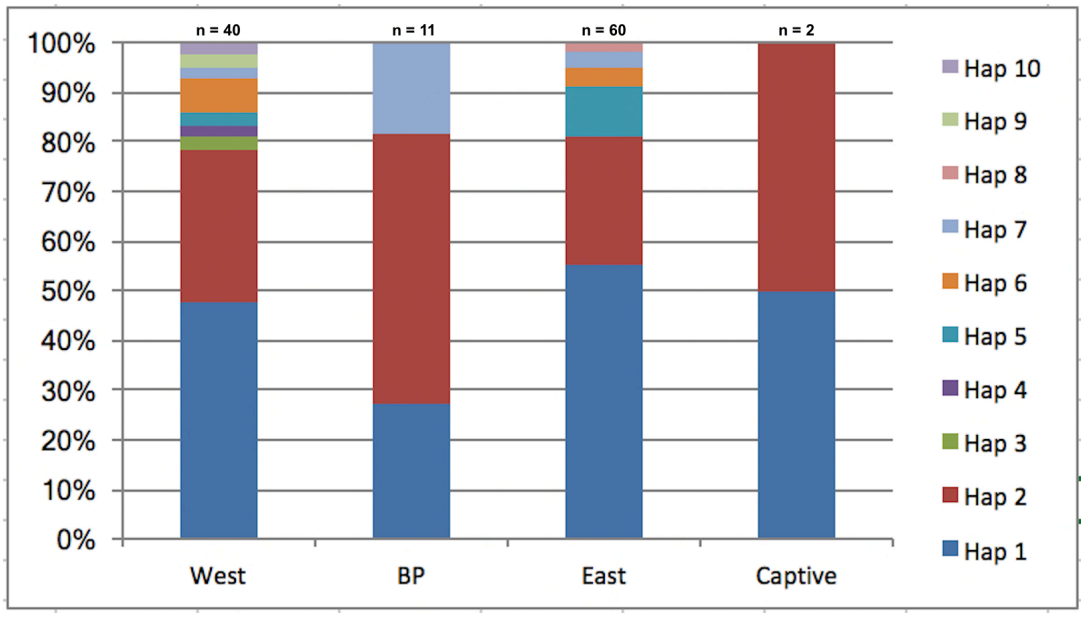

Figure 2. Percentage of haplotypes found within each group.

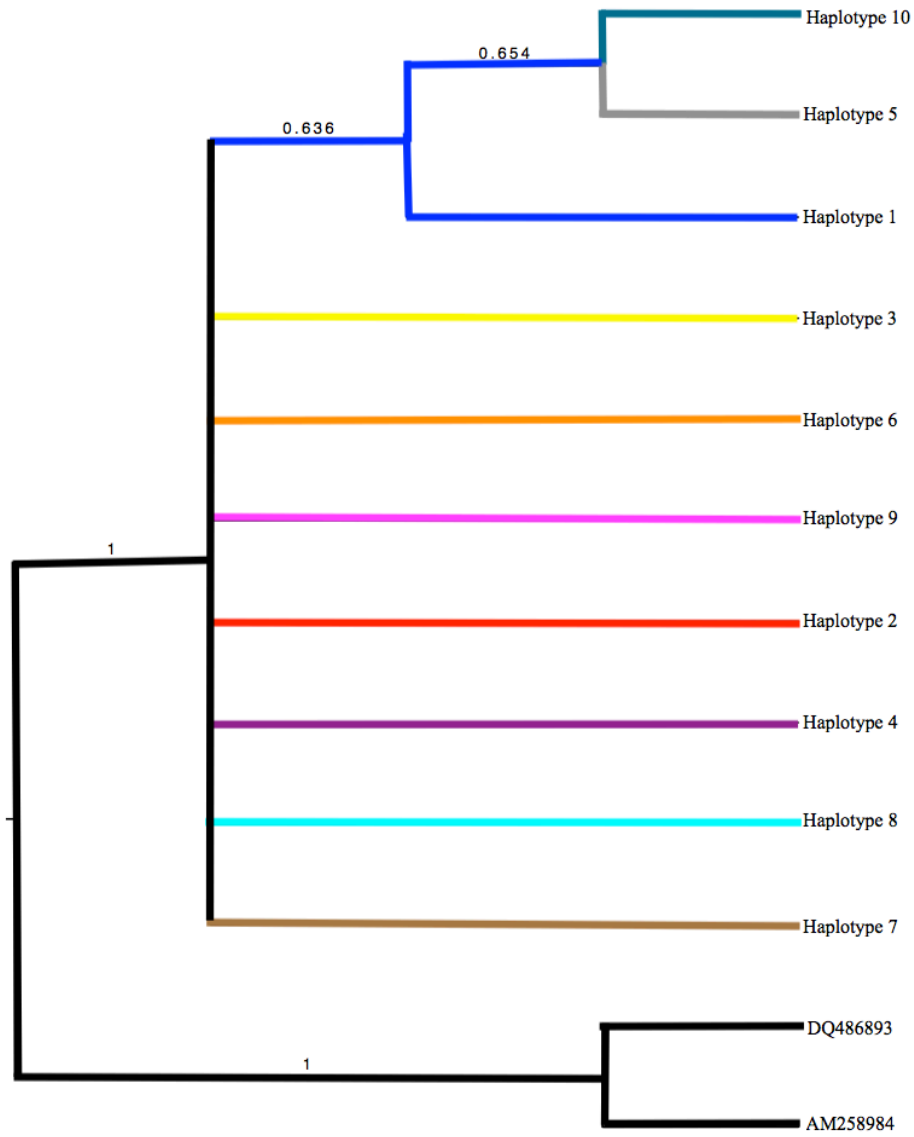

Figure 3. Maximum Likelihood (ML) tree of control region sequences with Frey sequences. Bootstrap support numbers indicate the strength of the branch. Corresponding haplotypes are listed to the side. The tree is rooted with DQ486893 and AM258984, *Lepidochelys olivacea* (Pacific ridley) sequences.

Sample Comparison	*ΦST*	P Values
BP v. Western Gulf	0.0369	0.17686±-.0067
East Coast v. Western Gulf	-0.00621	0.52562±0.0083

Table 6. Pairwise fixation index (*ΦST*) comparisons between sample regions. A *ΦST* close to zero indicates samples are homogenous. A p-value greater than 0.05 indicates that there is not a significant difference between samples.

Part II: Mitochondrial Genomes

The genomes of ten individuals were successfully sequenced and compared to two partial genomes present on GenBank (Accession JX454981, JX454982). The ten genomes range in length between 16372 and 16483 bp. The Kemp's ridley *mt* genome consists of 12 protein-coding genes: ND(1-6,4L), ATP6 and ATP8, COX(1-3), 22 TRNAs, 2 RNAs, and a large spacer segment containing the "control region" (Fig 4). In the complete genome, eight out of twelve protein-coding genes are separated by a tRNA sequence (Fig 4). The two partial genomes have these 12 protein-coding genes, however they contain several stand-in "N"s within each gene, representing uncertainty as to which base pair is present at that point within the gene.

The ten individuals completely sequenced matched three of the ten previously defined haplotypes (based on the control region) from the literature: Lk 2.1, 4.1, 6.1 (Frey, 2014). One sample collected from a nesting female on South Padre Island (Nest 1) matched Haplotype 6 (Lk 2.1). Four nesting samples (Nest 3, 13, 16, 23) two GSTC samples (GTSC-1 and GTSC-2), and GenBank JX45981 matched Haplotype 1 (Lk 4.1). Three samples from nesting females (Nest 14, 16, 23), as well as GenBank JX454982, matched Haplotype 2 (Lk 6.1). Individual nesters had a high calculated percentage of identical alignment between sequences. Individuals ranged from having genome sequences 99.12% to 99.90% identical to each other (Fig 5). The number of differences in alignment positions ranged from 2 to 144 (Fig 5).

The resulting phylogenetic tree is colored according to these haplotypes as defined by the control region. Haplotype 1 (LK 4.1) is blue, Haplotype 2 (LK 6.1) is red, and Haplotype 6 (Lk 2.1) is orange. GenBank JX454982 (Haplotype 2) forms the basal branch. This is followed by Nests 14, 16 and 23 (all Haplotype 2) as unresolved branches, forming a polytomy with a strongly supported clade of specimens belonging to Haplotype 1. This Haplotype 1 clade is made up of a polytomy of 3 unresolved branches (Nests 6 and 13 and GTSC-1), and two supported clades: one containing Nest 27 and Genbank JX454981 and the other containing Nest 3 and GTSC-2 (Fig 6).

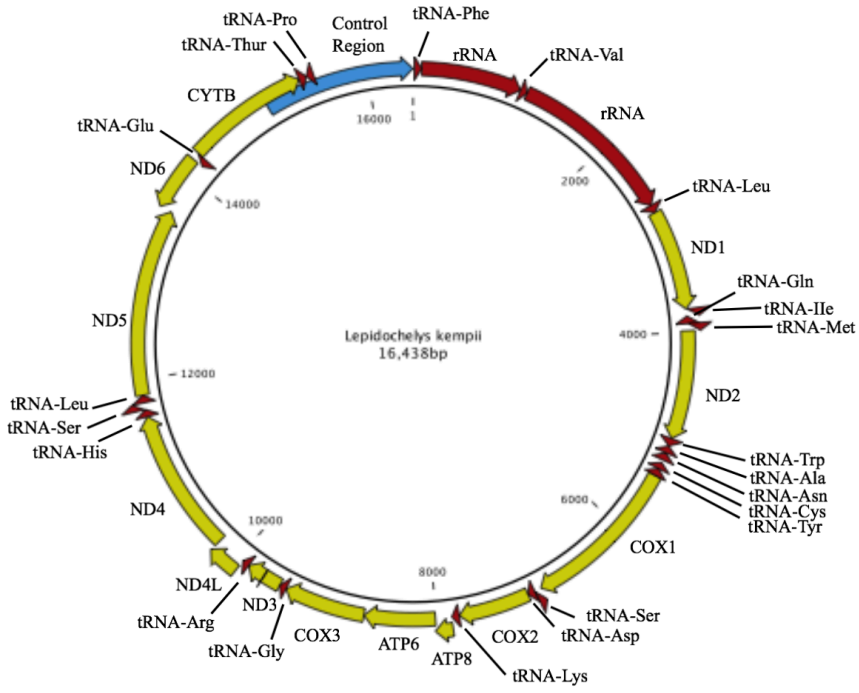

Figure 4. Mitochondrial gene arrangement of the Kemp's ridley *(Lepidochelys kempii)* sea turtle

		1	2	3	4	5	6	7	8	9	10	11	12
Nest 23 (Hap 2)	1		97	137	94	89	94	144	96	120	116	129	136
Nest 13 (Hap 1)	2	99.41		44	5	10	5	74	3	41	23	62	66
Nest 27 (Hap 1)	3	99.17	99.73		43	50	45	113	43	81	21	102	104
Nest 16 (Hap 2)	4	99.43	99.97	99.74		7	2	75	4	42	22	59	67
Nest 1 (Hap 6)	5	99.46	99.94	99.70	99.96		7	70	9	37	29	54	62
Nest 14 (Hap 2)	6	99.43	99.97	99.73	99.99	99.96		75	4	42	24	59	67
Moose Tracks (Hap 1)	7	99.12	99.55	99.31	99.54	99.57	99.54		71	35	93	18	12
Nest 3 (Hap 1)	8	99.42	99.98	99.74	99.98	99.95	99.98	99.57		40	22	61	65
Black Raspberry (Hap 1)	9	99.27	99.75	99.51	99.74	99.77	99.74	99.79	99.76		60	23	35
Nest 6 (Hap 1)	10	99.30	99.86	99.87	99.87	99.82	99.85	99.44	99.87	99.64		81	85
JX454982 (Hap 2)	11	99.22	99.62	99.38	99.64	99.67	99.64	99.89	99.63	99.86	99.51		17
JX454981 (Hap 1)	12	99.17	99.60	99.37	99.59	99.62	99.59	99.93	99.60	99.79	99.48	99.90	

Figure 5. Percent Identity and Differences between individuals. The lower half of the figure shows percent identity, while the upper half shows the number of differences within (size of alignment) aligned base pairs that include 12 genes, two RNAs, and the control region of the mitochondrial genome

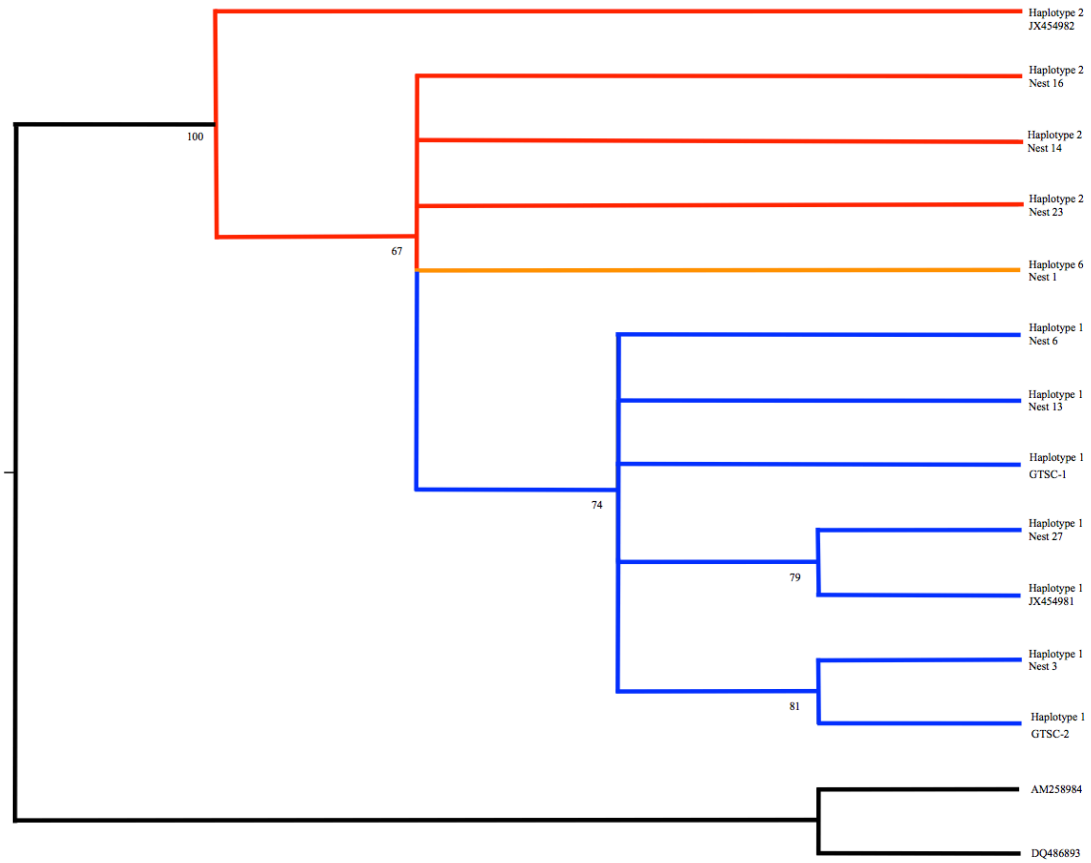

Figure 6. Phylogenetic tree created with full mitochondrial genomes and rooted with *Lepidochelys olivacea*. Branch colors represent haplotypes. Bootstrap support numbers indicate the strength of the branch. The multiple-coding nature of the 12 protein coding genes was taken into account.

CHAPTER V

DISCUSSION

Phase I: Control Region

1) Quantifying the Genetic Diversity of South Padre Island's Breeding Stock

The genetic diversity of the sea turtles sampled on South Padre does not appear to be affected by the previous decline in nesting numbers in 2010, and estimated 5% rate of population decline (Heppell, 2014). All but one haplotype documented by Frey, 2014 (present in 2003 and 2006) were found in this study. The Western Gulf haplotype frequencies are indicative of the 82% frequency of Haplotypes 1 and 2 previously found in Frey, 2014. Their samples were collected starting in 2001, suggesting that the genetic diversity has remained stable for the last 14 years. Two individuals (one nesting female collected in 2016, and one sample on the East coast collected in 2015) returned a haplotype not referenced in Frey (2014). Since Frey's (2014) study spanned 11 years and over 500 samples, this suggests that perhaps new haplotypes are still being introduced into the breeding stock.

A greater number of haplotypes were found on the Western Gulf than on the East coast or within the BP samples. This is consistent with the fact that the majority of the population nests in northern Mexico, with a growing number documented along the Texas coast (Shaver, 2015, 2016b). The turtles sampled on the East coast and after the BP oil spill are likely transient turtles from the Western Gulf, who are migrating between foraging grounds (Shaver, 2016a).

2) Origin of BP Oil Spill Samples

The haplotype frequencies of the Western Gulf and the BP oil spill samples were similar. Haplotype 2 was the dominate of the three haplotypes present in the BP samples, while Haplotype 1 and 2 were almost equivalently present in the Western Gulf samples. This region had haplotype 1 and 2 frequencies of 80-82%, also similar to Frey, 2014. This indicates that there is a chance that all the sampled individuals came from the same area, potentially from the Texas coast. When conducting a population pairwise test, the resulting fixation index (*ΦST*) between the BP samples and Western Gulf samples is 0.0369, with a *p*-value of 0.17686±0.0067 (Table 6), demonstrating the two groups could be one population as they are genetically very similar and the existing differences are not significant (Craig, 2007).

To fully determine where the turtles sampled after the BP oil spill and on the east coast originate from, a larger quantity of high quality genetic samples would be needed for the spill affected area. Additionally, it would be necessary to utilize haplotype information from the primary nesting beach in Rancho Nuevo. To the author's knowledge, a sampling program is not currently in effect in that area, so it is difficult to distinguish between breeding stocks (if any) of Texas and Mexico. The ridleys originating from Mexico should display a wider range of haplotypes than turtles sampled on the Texas coast, as the original population has had more time to diversify.

3) Origin of East Coast Samples

The total frequency distributions for Haplotypes 1 and 2 were almost identical between the Western Gulf and East coast samples. East coast samples had the second highest variety of haplotypes of the four geographic regions, with the Western Gulf being the most variable. The similar haplotype frequency indicates that there is a chance that these individuals came from the

Texas area. Also, these Haplotype 1 and 2 frequencies are indicative of those found from the Western Gulf, BP samples, and (Frey, 2014).

East coast samples were taken from juveniles, and therefore cannot be considered a separate population as these individuals predominantly return to the coast of Mexico and Texas to nest at sexual maturity (Putman, 2010). Additionally, when comparing the East Coast samples to the Western Gulf samples, the resulting fixation index (*ΦST*) was -0.00621 with a *p*-value of 0.52562±0.0083 (Table 6). This demonstrates that these two groups were also too genetically similar to be considered separate populations. However, there has been infrequent nesting along the South Carolina and Florida east coast since 1989 (Meylan, 1990), so the potential for haplotype divergence is increasingly possible.

The two captive samples were two separate haplotypes. The captive ridley in Texas matched Haplotype 1 (Lk 4.1) and the captive ridley from the east coast matched Haplotype 2 (Lk 6.1). Both these haplotypes were found on either coast, however the east coast samples in this study typically matched Haplotype 1 (Lk 4.1).

Part II: Mitochondrial Genomes

1) Description of the First Complete Kemp's Ridley Mitochondrial Genome

After complete assembly of ten mitochondrial genomes, it was determined that most of the variation in the Kemp's ridley *mt*DNA occurs in the hypervariable control region. When looking at the complete *mt* genome, it is also evident that some genes are more variable or more conserved than others. 12s, ATP6, ATP8, ND2, ND3, ND4L, ND6, and COX2 were all perfectly conserved across individuals. For COX1 and ND4, only one out of ten individuals expressed var-

iation in the gene. 16s varied across multiple individuals, as did ND1 and ND5, and CYTB. COX3 was the least conserved.

2) Comparison of the Genetic Resolution of Full *mt* Genomes to the Control Region.

Targeting solely the control region limited the detection of variation between individuals. This was apparent early in the study when the depth of the phylogenetic tree reconstructions expanded and collapsed in resolution depending on using control region sequences or entire genomes. Though patterns can be seen within the control region, this study indicates that full genomes convey a more robust analysis. Targeting solely the control region is adequate for assigning individuals into haplotype groups, however specimens still contain differences from each other within the *mt* genome. Analysis of full *mt* genomes result in more haplotypes, which produces greater maximum likelihood tree resolution. Using full *mt* genomes make it more likely to detect population differences if any exist. The twelve full mitochondrial genomes showed twelve unique haplotypes. If only the control region is analyzed for these twelve specimens, the number of haplotypes is reduced to only 3, showing a dramatic loss of resolution. Furthermore, haplotypes based on the control region may lead to erroneous conclusions. For example, Genbank JX454982, which belongs to control region Haplotype 2, is clearly different from Nests 16, 14 and 23, which also belong to control region Haplotype 2, but according to the full mitochondrial genome these actually belong to a clade containing members of control region haplotypes 6 and 1 (Fig 6). Past studies have relied on the mitochondrial control region to determine levels of variation between individuals. From my findings, this indicates that they may have underestimated diversity within the Kemp's ridley population, but more importantly, they may have missed genetic structuring due to lack of resolution.

Conclusions

The findings of this study 1) contributed the first complete Kemp's ridley mitochondrial genome 2) demonstrated a sophisticated and streamlined process of obtaining complete mitochondrial genomes in sea turtles 3) illustrated the depth of genetic analysis capable by both full genome sequencing and targeted control region sequencing and 4) provided an initial analysis of the geographic distribution of haplotypes present within current and recent Kemp's ridleys located in the Gulf of Mexico and East coast of the United States.

The repeated mapping of the Kemp's ridley mitochondrial genome contributed ten more genomes to be referenced in conjunction with the partial *mt* genome data published in literature. Finding results similar to the two published genotypes supports the hypothesis that the nesting Kemp's ridleys on South Padre Island have low, but consistent genetic variability within the stock. The contribution of the complete mitochondrial genome of Kemp's ridley sea turtles benefits analysis of genetic divergence across sea turtle species, and provides a basis for comparison of genetic diversity among nesting individuals on North Padre Island, TX and Tamaulipas, Mexico; the two major nesting sites for Kemp's ridleys. Understanding the genetic diversity in each major nesting area will help target conservation focus.

Without past data to compare the results of this research against, the data presented in this paper can only provide a baseline for future research. To complete the investigation of whether or not there has been a bottleneck in Kemp's ridley population, it will be vital to collaborate with researchers to utilize past data from genetic samples collected before and after 2010 indicating the haplotype frequencies present in the mitochondrial DNA of nesting females in Texas and in

migrating juveniles located along the East Coast. Additionally, to investigate the plausibility of a bottleneck, researchers should strive to obtain genetic samples from nesting females utilizing the primary beaches in Mexico. Sampling from these areas will allow for robust genetic comparisons. Finally, the analysis of nuclear markers, not addressed in this study, would provide a fresh analysis of the present genetic variability in the population by including the influence of male-mediated gene flow. A comparison between the paternally defined population structure and the maternally defined population structure would expand our knowledge of the geetic diversity of this highly endangered and charismatic vertebrate: the Kemp's ridley sea turtle.

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BIOGRAPHICAL SKETCH

Hilary Renee Frandsen was born April 13, 1992 in Fort Wayne, Indiana. She received her Bachelor of Science in Biology from Principia College in 2014. During her education at Principia she travelled extensively, visiting Iceland, Peru, Chile, Argentina, Trinidad, and Costa Rica. Highlights from her travels included an in-water survey of green sea turtles, spending two months recording heart rates of nesting leatherbacks, and caring for rehabilitating sloths.

After graduation, Hilary interned at Mystic Aquarium in Connecticut, in their Research and Veterinary Services Department. Her project focused on contaminant concentrations in snapping turtles (*Chelydra serpentina*), and was presented to Connecticut's Department of Energy & Environmental Protection. Next, she interned with Sea Turtle Inc. in Texas, where she worked in the sea turtle hospital and patrolled for nesting sea turtles. Her project focused on the effect of off-road vehicle tire ruts on Kemp's ridley hatchling dispersal. She presented the results at the Second International Kemp's Ridley Symposium in 2014, and the Southeast Regional Sea Turtle Meeting in 2016. After her internship with Sea Turtle Inc. was finished, Hilary worked for The Nature Conservancy in St. Croix, USVI, as a Sea Turtle Specialist. There, she independently monitored three pocket beaches for nesting green and hawksbill sea turtles.

Afterwards, Hilary moved to Brownsville, TX to pursue her master's degree in Biology at the University of Texas Rio Grande Valley, and to intern at Sea Turtle Inc. Currently, she is a Biological Technician for the National Park Service on North Padre Island. She received her Master's in Biology in December 2017. Hilary can be reached at hilary.frandsen@gmail.com.