2020

Genetic Structure of Green Sea Turtle (Chelonia mydas) Foraging Aggregations on the East Coast of Florida

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GENETIC STRUCTURE OF GREEN SEA TURTLE (*CHELONIA MYDAS*) FORAGING AGGREGATIONS ON THE EAST COAST OF FLORIDA

by
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B.S. University of Central Florida, 2020

A thesis submitted in partial fulfillment of the requirements
for the degree of a Bachelor of Biology
in the Department of Sciences
in the College of Biology
at the University of Central Florida
Orlando, Florida

Spring Term
2020

ABSTRACT

The genetic structure of juvenile green turtles (*Chelonia mydas*) foraging on the east coast of central Florida is not well understood, nor has it been examined over time. In the last three decades, the dramatic increase in the number of green sea turtle nests in Florida, in association with other population parameters, has led to this species being down-listed under the Endangered Species Act from "endangered" to "threatened" in the northwest Atlantic. However, it was unclear if the exponential growth in Florida nest numbers had any influence on the genetic structure of juveniles in nearby foraging aggregations. To understand this potential impact mixed-stock analysis was conducted using mitochondrial DNA fragments that were over 800 base pairs long on samples taken from juveniles captured from 2002-2005 and 2016-2018 in the central Indian River Lagoon and Trident Submarine Basin in Port Canaveral. Results indicate the sampled foraging sites are genetically distinct habitats. In both sites, recruitment from Florida nesting beaches remained low despite increases in nesting while contributions from rookeries in Costa Rica and Mexico dominated both foraging aggregations across time. Haplotype diversity and nucleotide diversity decreased at both foraging sites over time. The foraging sites shared the two most frequently occurring haplotypes, but also had haplotypes that were unique to the site or sample period. Our results highlight the need for broader sampling of rookeries and foraging aggregations to understand the impacts of nesting increases in one rookery on juvenile diversity. Future studies should include all life stages of green turtles to enhance understanding of both the census population and effective population to better inform conservation policies necessary for a continued recovery.

ACKNOWLEDGEMENTS

Thank you to the members of the UCFMTRG over the years for their diligent sample collection and preservation without which this study would not have been possible. A special thank you to the Hoffman Lab and UCF Honors College for their helpful insights throughout the project. This research was conducted under Florida MTP-231, MTP-186, NMFS Permit 19508, and predecessors. The project was partially funded by the Sea Turtle Grants Program.

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INTRODUCTION

Maintenance of genetic diversity in wild populations is vital for a species' ability to thrive and adapt to a changing environment. Understanding the genetic diversity of threatened species is fundamental for proper conservation planning. A recent review on the conservation status of the green sea turtle (*Chelonia mydas*) down-listed this species from “endangered” to “threatened” in Florida under the Endangered Species Act (Seminoff, J.A 2004). Among several factors analyzed, researchers considered that the significant increase in number of nests laid in Florida every year is contributing to the overall improvement of the conservation status (Dublin 2019). The Archie Carr National Wildlife Refuge (ACNWR) in the East coast of central Florida is an important nesting beach for the green turtle in the United States (Weishampel 2003; National Marine Fisheries Service and U.S. Fish and Wildlife Service. 1991). Coastal waters in Florida are also recognized as important developmental areas for juveniles of this species (Llewellyn et al. 2007; Redfoot 1997).

When considering conservation strategies for endangered species it is vital to look at their historical context and monitor changes in structure to inform decisions (Avise 1992). Green turtles are distributed throughout the oceans but exhibit defined populations due to isolation. Populations are defined by ecological and genetic data which are used to inform conservation decisions (Marandel 2017; Davy and Murphy 2014). Genetic information can be used to determine gene flow between rookeries and populations to inform population models and thus conservation strategies.

Given the importance of reliable and up-to-date information on genetic population parameters to any species, we sought to understand how the increase in number of nests in Florida may have impacted the genetic structure of the population of juveniles in foraging sites. My goals are to 1) assess the genetic structure of juveniles in coastal waters over time; from 2003 to 2018 and 2) determine the stocks of origin of juveniles; and 3) estimate variation in genetic diversity over time.

Life Cycle

Green sea turtles are long living organisms with a complex migratory life cycle involving migrations and ontogenetic shifts as the turtles move through size classes (Lutz et al. 2003). Their journey begins as hatchlings emerge from their nesting beach and enter the so-called "lost years". The hatchlings make their way into the ocean where they swim out until they reach floating *Sargassum* mats (Putman et al. 2016; Putman et al. 2015). *Sargassum* is a brown alga which floats on the surface of the ocean providing camouflage to the hatchlings as well as their prey items (Carr 1987; Putman et al. 2016). As they grow in the *Sargassum* habitat they feed on hydroids, anthozoan, gastropods, swimming crabs, bryozoan and fragments of sargassum that was encrusted with colonies of hydroids (Witherington et al. 2012). Once they are large enough, they make their way to coastal areas where it is believed that green turtles experience a diet shift from omnivory to herbivory (Cardona et al. 2010). The turtles continue to feed and grow in coastal areas until they are large enough and make their way back into the ocean to new feeding grounds (Lutz et al. 2003). They continue to feed on sea grass beds and mature until they are ready to make the migration back to their natal nesting beach. Each turtle will remain in the

nesting beach area during the nesting season laying one to seven of clutches per season with an average being two to three clutches per turtle per season (National Marine Fisheries Service and U.S. Fish and Wildlife Service 1991). They will make their way back to the nesting beach every two or more years to nest and subsequently return to their foraging grounds during interesting periods (Carr et al. 1978).

The foraging grounds shared by adult sea turtles are not the exclusive to each nesting beach population. Satellite tags of nesting females has revealed that adults nesting on the same beach do not all go to the same areas to feed during the off season (Baudouin et al. 2015; Godley 2008). Likewise, juvenile foraging aggregations do not originate from the same nesting beach populations and will vary seasonally (Mendonça 1983; Avens & Lohmann 2004). Despite their long ranging distributions, green turtles exhibit strong natal philopatry and will nest on the beach from which they originated (Avens & Lohmann 2004).

Florida Population History and Conservation Status

The population of green sea turtles in the Archie Carr National Wildlife Refuge from 1979-1992 ranged from 62 to 2,509 nest per season (Meylan et al. 1995). This nesting accounted for 1.9% of the total sea turtle nesting in Florida. The IUCN red list reports that the Florida subpopulation of green turtles has seen a 113% increase in nesting from 1980 to 2001 (IUCN Supplemental Information Seminoff, J.A 2004). As of 2017, green sea turtle nesting in the Archie Carr NWR accounted for a third of green turtle nesting in the US (U.S. Fish and Wildlife Service 2019). While the nesting numbers are increasing worldwide; it is important to consider that green turtles exhibit an alternating nesting pattern of high and low years which is a poorly

understood pattern (Bjorndal 1999; Ghazali & Jamil 2019; Seminoff et al. 2015). For example, in 2017 Brevard County had 25,891 nests, but the following year had 1,598 (FWC Statewide Nesting Beach Survey Program Database 2019). This increase in nesting abundance is cause for optimism; however, the cause of this increase is poorly understood, and the long-term impacts are unclear due to the long complex life history of sea turtles.

In 1978 the green sea turtle was globally listed as endangered under the United States Endangered Species Act of 1973 (National Marine Fisheries Service and U.S. Fish and Wildlife Service. 1991). However, in 2016 the Florida and Mexico breeding populations were down-listed as Threatened partly due to the increase in annual nesting. Threatened species listed as such because they are likely to become endangered or in danger of extinction within the foreseeable future (Seminoff, 2004).

Genetic Markers

Mitochondrial DNA, mtDNA, is passed to offspring from the mother via the mitochondria of the egg cell that is fertilized (Clayton 1982). Within the mtDNA there are variations of genes that are inherited together and represent a specific version of the genetic information, a haplotype, which can be used to distinguish populations (Costa Jordao et al. 2017). Previous mtDNA studies of green turtles have used a 490-base pair (bp) control region to delineate populations, but through the development of new primers LCM15382 and CM16437 an 800-bp region can be identified allowing for greater resolution (Abreu-Grobois et al., 2006; Shamblin et al., 2012). The frequencies of mtDNA haplotypes in a foraging aggregation can be

compared to known nesting populations to identify likely source populations for the juvenile aggregations (Jones et al. 2018).

METHODS

Study Sites

Samples were collected from two studies sites. The Indian River Lagoon (IRL) and Trident Submarine Basin (Trident) at Port Canaveral (Figure 1) are in-water sites for foraging juvenile green turtles of unknown sex.

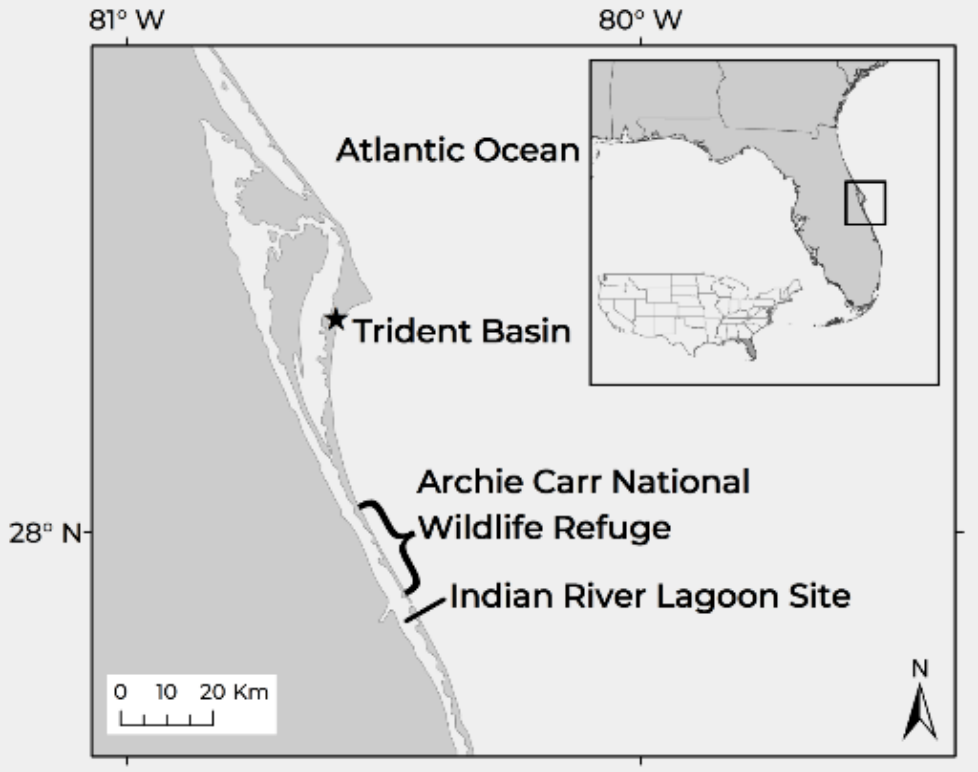

Figure 1: Sampled juvenile foraging sites around the Archie Carr Wildlife Refuge

The Indian River Lagoon is a slow-moving estuary with a sea grass-based ecosystem extending 156 miles along the eastern coast of Florida from Ponce de Leon Inlet to Jupiter Inlet (Dybas 2002). The average depth throughout the lagoon is 1.8 m with little variation but the

width varies from half a mile to five miles (Steward and Green 2007; Dybas 2002). Water is exchanged from the IRL to the Atlantic Ocean through Sebastian Inlet, Fort Pierce Inlet, Haulover Canal, and a canal-lock facility in Port Canaveral (Steward and Green 2007). The IRL is primarily home to juvenile stage turtles but will occasionally host adults during the nesting season (Ehrhart et al. 2007). The most common species encountered are green turtles, but loggerheads (*Caretta caretta*) are not uncommon; there have been sightings of hawksbills (*Eretmochelys imbricata*), leatherbacks (*Dermochelys coriacea*), and hybrids turtles in the lagoon as well (UCF MTRG unpub data; Ehrhart et al. 2007). Green turtles are recruited to the IRL during the juvenile stage where they become primarily herbivorous and can feed on the sea grass beds and algae in the lagoon. The age of these turtles remains unknown, but the straight carapace length (SCL) varies from 24.3 cm to 78.6 cm with a mean SCL 43.7 cm ± 10.0 cm (SD) (Ehrhart et al. 2007).

Our second study site is the Trident Submarine Basin at Port Canaveral located near the mouth of the Port Canaveral Ship Channel within the Cape Canaveral Air Station (Kubis et al. 2009). The manmade basin ranges from 15 m in the center to 10 m along the edges. The depth throughout most of the basin does not allow light to penetrate deep enough to support a sea grass ecosystem, but the basin is surrounded by large submerged boulders which provide a substrate for algae to grow on (Holloway-Adkins and Hanisak 2017) . These algae include *Gelidium americanum, Hypnea cervicornis, Solieria filiformis, Polysiphonia subtilissima,* and *Ulva lactuva* which serve as a food source for the green turtles foraging in the habitat (Redfoot 1997). The species of turtles encountered at Trident Basin are typically green turtles but there is the occasional loggerhead which tend to be injured or lethargic. The straight carapace length of

juvenile green turtles caught in the basin ranges from 22.8 cm to 48.1 cm with a mean SCL of 29.8 ± 3.8 cm (SD) (Kubis et al. 2009).

Sample Collection

The University of Central Florida Marine Turtle Research Group, UCFMTRG, began year-round bimonthly netting trips in the Indian River Lagoon, or IRL, in 1985 (Llewellyn et al. 2007). To capture the turtles, two large mesh tangle nets of 40 cm knot to knot stretch are suspended in the IRL and constantly monitored by two boats by pulling along the length of the net. Turtles swim into the net where they become tangled and are removed by the patrol boats. Upon retrieval turtles were brought to a third boat where morphometric data as well as skin and blood samples are collected. The turtles were tagged with flipper tags on the upper trailing edge of their front flippers and a passive integrated transponder, or PIT tag, was inserted into the right front flipper. After data were collected and the turtles were tagged, they were released back into the IRL away from the tangle nets to avoid recapture.

Trident Submarine Basin in Port Canaveral is visited biannually in the spring and late summer by the UCFMTRG for data collection. At this site two mesh tangle nets, one 40 cm knot to knot stretch and another smaller net of 30.5 cm knot to knot stretch are used to accommodate the smaller average size of juvenile turtles caught in this sight compared to the IRL. In addition to the tangle nets, turtles are opportunistically caught using dip nets along the rocks of the basin where they forage on algae. Sample and morphometric data collection follow the same protocol used in the IRL.

For all study sites, skin samples were preserved in EtOH and blood was collected in heparinized vacutainers until the plasma is separated from the sample. All blood was preserved in freezers prior to DNA extractions and skin was preserved in EtOH. Samples were chosen by the year in which the turtle was first tagged to represent to represent their respective aggregation (table 1). For the juvenile in water samples, turtles first tagged between 2003-2005 in the IRL and Trident represent to old aggregation. Those first tagged between 2016-2018 represent the current new aggregation.

Table 1: Number of foraging aggregation samples

	IRL	**Trident**
"Old" 2003-2005	34	41
"New" 2016-2018	38	39

Genetic Sequencing

Mitochondrial DNA was extracted from blood and skin samples using the a Serapure bead-based protocol (Rohland & Reich 2012) with adaptations (Faircloth & Glenn 2016). After extraction, 152 samples were amplified for analysis of a 817 bp fragment of the mitochondrial control region (CR) using primers LCM15382 (Abreu-Grobois et al., 2006) and CM16437 (Shamblin et al., 2012). We used 20 µL polymerase chain reactions (PCR) with 20 mM Tris HCl pH 8.4, 50 mM KCl, 0.25 mM dNTPs, 1.5 mM $McCl_2$, 0.5 µM of each primer, 1 unit of *Taq* DNA polymerase, approximately 10 ng of genomic DNA, and H_2O. The reaction was placed in thermal cyclers as follows: 95°C for 5 min; 40 cycles of 95°C for 30 s, 57°C for 30 s, 72°C for

80 s; and a final extension at 72°C for 10 min; holding at 10°C. The reactions were purified using Exonuclease I (EN0581) and FastAP (EF0651) following manufacturer's protocol (Thermo Fisher Scientific).

Analysis

Pairwise F_{st} values were calculated using the software Arlequin v3.5 and associated p-values were estimated with 1000 permutations. A Hierarchical Bayesian model was used for mixed-stock analysis (Bolker et al. 2007) using package mixstock in R. The haplotype diversity (*h*) and nucleotide diversity (π) were calculated in Arlequin (Excoffier and Lischer 2010). The haplotype network was made with Median Joining Network in PopART. The SCL for each sample site and time period were compared using boxplot analysis.

RESULTS

The straight carapace lengths, SCL, of samples used for the analysis were grouped and compared by sample year category. Analysis indicated that the SCL did not significantly vary in individual study areas across sample periods suggesting that size class recruitment to the foraging sites remained consistent (Figure 2). The SCL between the foraging sites are different and recapture data between the foraging sites report insignificant overlap in resident turtles (UCFMTRG unpublished), indicating they are different distinct habitats.

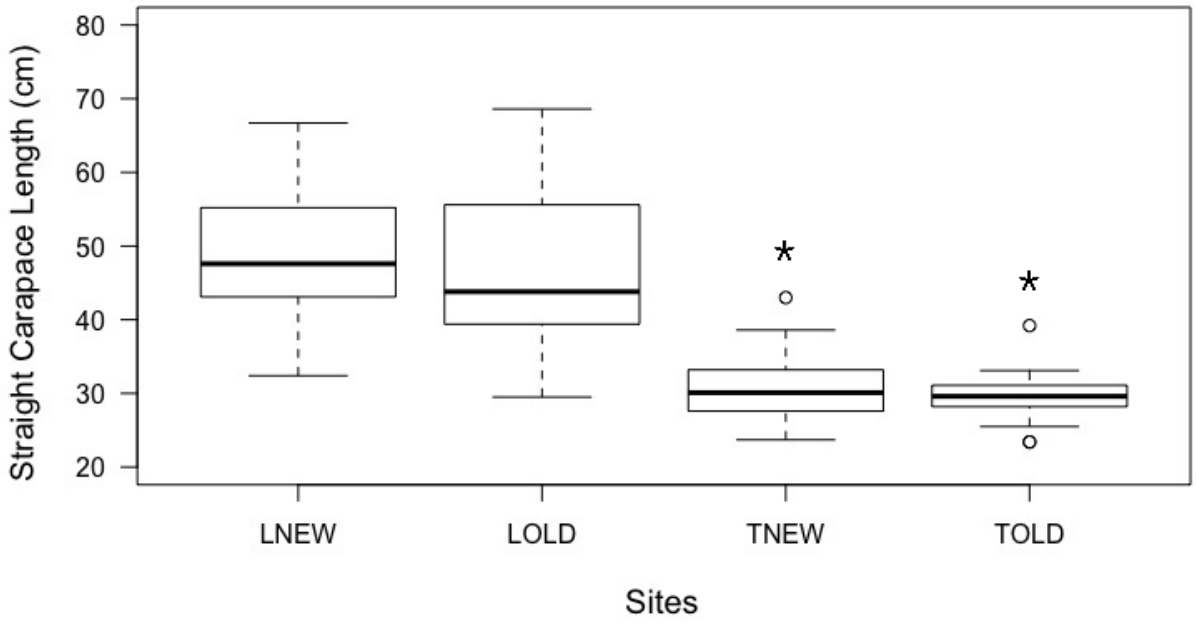

Figure 2: Boxplot showing the straight carapace length (SCL) of study sites per sample period.

SCL did not vary significantly over time in the sample sights suggesting consistent size class recruitment to the foraging areas. Additionally, the size class recruitment is distinct between the two foraging areas indicating they are different habitats.

F_{st} values calculated in Arlequin and their associated p-values were considered for all comparison combinations of old and new sample sets from both Trident and the IRL as well as for the foraging aggregations with all samples from both time periods (table 2). In addition to the SCL, the F_{st} and p-values calculated when considering all samples collected at each site indicate Trident and the IRL are genetically distinct foraging aggregations.

Table 2: F_{st} and P-values for sample set comparisons.

P- values were estimated with 1000 permutations. Comparisons with significant p-values are indicated in bold. When Trident and the IRL were compared by combining all samples from each site in a single group, they were found to be genetically different. Trident new and old were not significantly different from each other despite Trident new being distinct from both IRL old and new while Trident old was not.

Study Groups Compared		**F_{st} Value**	**P value**
Trident New	Trident Old	0.022	0.091
Trident New	**Lagoon New**	**0.060**	**0.008**
Trident New	**Lagoon Old**	**0.083**	**0.001**
Trident Old	Lagoon New	-0.003	0.436
Trident Old	Lagoon Old	0.027	0.088
Lagoon New	Lagoon Old	0.014	0.202
Trident All	**Lagoon All**	**0.030**	**0.016**

For the mixed stock analysis, nesting sites in the South Atlantic were grouped as a single contributing unit, SA, to account for isolation by distance from foraging site (figure 3). The MSA plot (Figure 4) error bars represent 95% confidence intervals.

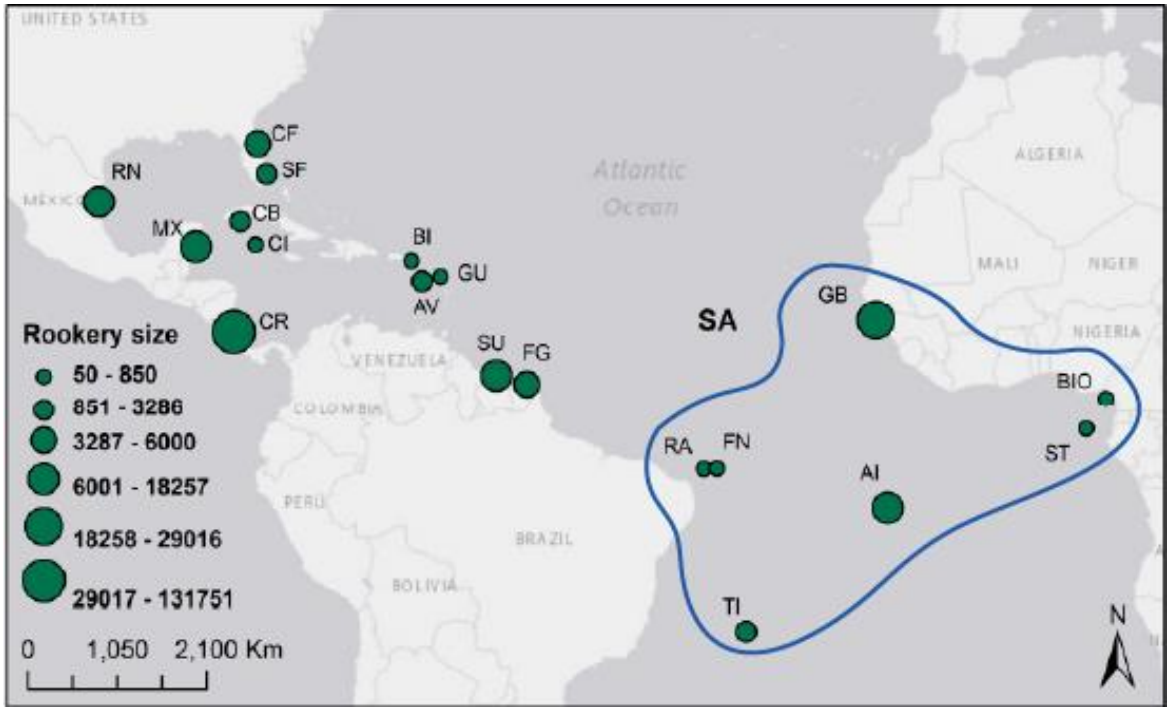

Figure 3: Location of rookeries which contributed juveniles to the foraging groups.

The South Atlantic was grouped together in the analysis to account for isolation by distance. CF, Central Florida, USA; SF, South Florida, USA; RN, Rancho Nuevo, Mexico; MX, Quintana Roo, Mexico; CB, Cuba; CI Cayman Islands; CR, Coast Rica; BI, Buck Island; AV, Aves Island; SU, Suriname; FG, French Guiana; SA, South Atlantic.

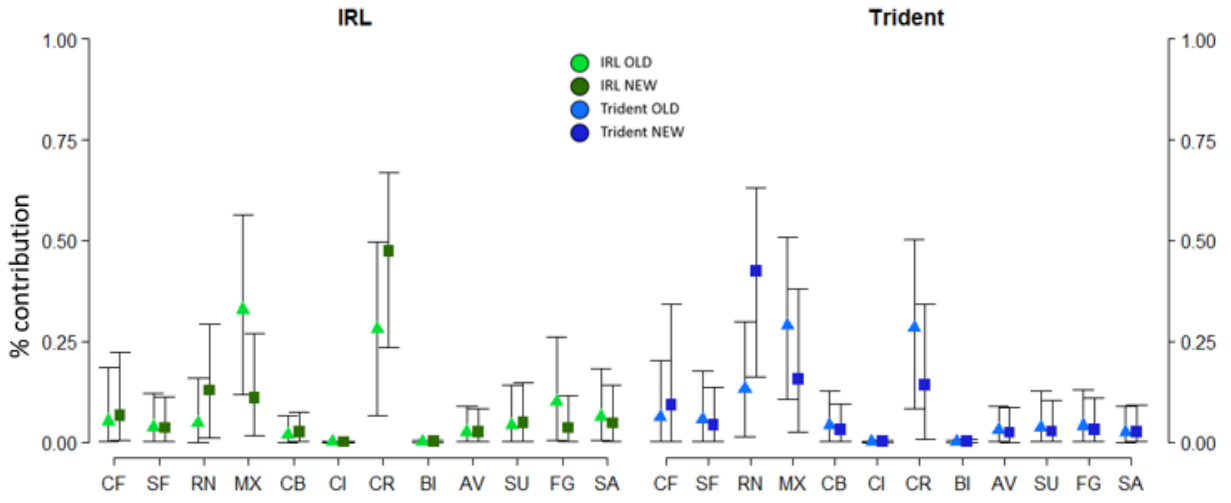

Figure 4: Mixed stock analysis of contributions to foraging groups.

Error bars indicate 95% confidence intervals. CF, Central Florida, USA; SF, South Florida, USA; RN, Rancho Nuevo, Mexico; MX, Quintana Roo, Mexico; CB, Cuba; CI Cayman Islands; CR, Coast Rica; BI, Buck Island; AV, Aves Island; SU, Suriname; FG, French Guiana; SA, South Atlantic. In the old populations, both Trident and the IRL were made up of juveniles predominantly from Quintana Roo, Mexico and Costa Rica not the nearby Florida beaches. The new aggregation of Trident saw a drop in recruitment from those areas and instead was dominated by Rancho Nuevo, Mexico turtles. The IRL did not experience as dramatic of a shift as Trident, but the new aggregation saw a drop in recruitment from Quintana Roo and an increase in recruitment from Costa Rica.

The top contributors to the old population of the IRL foraging aggregation consisted of Quintana Roo Mexico and Costa Rica but contributions from Quintana Roo decreased in the new population so that approximately 50% of turtles came from Costa Rica. For the old Trident foraging aggregation, Quintana Roo and Costa Rica were the main contributors. In the new Trident population contributions from both of those sites decreased and Rancho Nuevo, Mexico became the top contributor. Despite proximity to Florida nesting beaches, contributions from central and south Florida remained low in both sites across time.

Within each foraging aggregation the haplotype and genetic diversity decreased over time (table 3). With this decrease in diversity there was also a change in nesting beach contribution to

the aggregations (figure 4). Despite the less dramatic shift in contributions to the foraging site experienced in the IRL, it remained the most diverse throughout the study.

Table 3: Summary statistics of haplotype and nucleotide diversity.

Haplotype diversity and nucleotide diversity decreased over time in both foraging sites.

800 base pairs				
Site	n	No. of haplotypes	h (SD)	π (SD)
Lagoon Old	34	8	0.8 (0.049)	0.0036 (0.0021)
Lagoon New	38	8	0.65 (0.063)	0.0026 (0.0017)
Trident Old	41	9	0.734 (0.05)	0.002 (0.0013)
Trident New	39	6	0.553 (0.068)	0.0008 (0.0007)

The haplotype network (figure 5) indicated that the CMA1.1 and CMA3.1 haplotype were the most frequently occurring variety in both the IRL and Trident foraging aggregations across sample time periods. The old IRL foraging aggregation had three unique haplotypes; CMA16.1, CMA13.1, and CMA48.3, which were not present in the new IRL samples. The new IRL foraging aggregation had two unique haplotypes: CMA27.1 and CMA2.1, which had not been detected previously. Trident and the IRL both had one haplotype unique to the regions which were present across study periods, CMA1.4 and CMA8.5 respectively. These haplotype changes are indicative of changing contributions to the foraging aggregations over time.

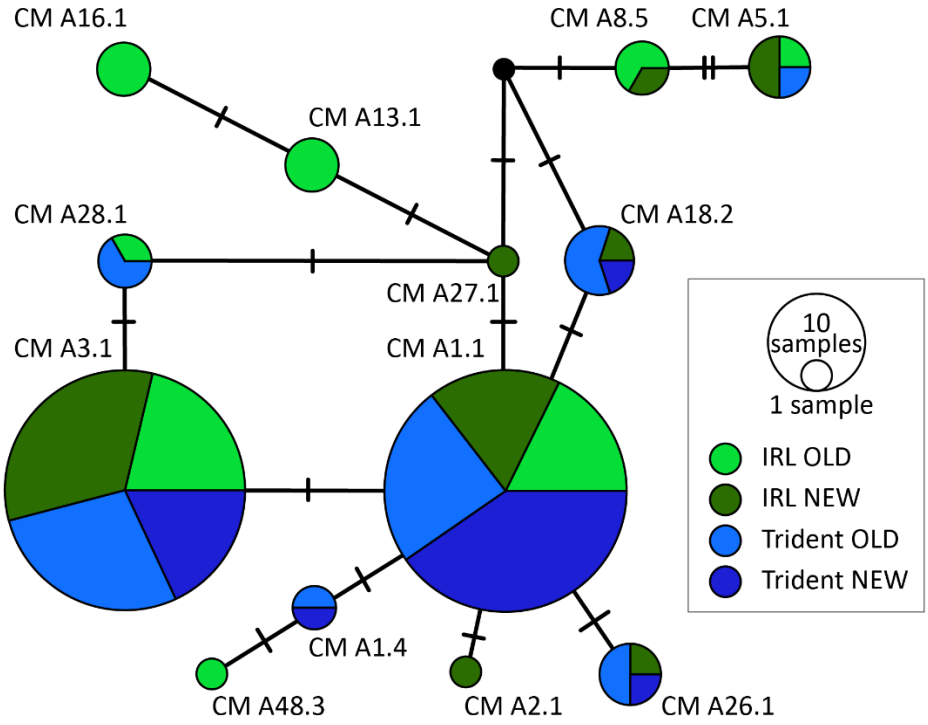

Figure 5: Haplotype network of foraging sites.

Transversal lines indicate 1 stepwise mutation. Two haplotypes dominated both study sites across sample periods. Three haplotypes were exclusive to the old IRL sample set and two were exclusive to the new IRL. Trident and the IRL both had one haplotype the was present only at their foraging sites but appeared in both sample periods.

DISCUSSION

Genetic Structure

The Indian River Lagoon and Trident Submarine Basin are two genetically distinct foraging aggregations (F_{st} = 0.030, p = 0.016) (table 2). This genetic differentiation is further supported by the different size class of juveniles which recruit to each habitat (figure 2). The comparisons of Trident Old to Lagoon Old (F_{st} = 0.083, p = 0.001) and Lagoon New (F_{st} = 0.060, p = 0.008) were significantly different; however, Trident Old and Trident new were not significantly different (F_{st} = 0.022, p = 0.091) (table 2). Samples from Lagoon Old and Lagoon New were also not significantly different (F_{st} = 0.014, p = 0.202).

Stocks of Origin

The stocks of origin determined through MSA in figure 4 show a low contribution to foraging sites from the nearby Florida nesting beaches; instead, high contributions come from two nesting sites in Mexico and Costa Rica. These rookeries are larger than those of the surrounding beaches of the Caribbean and Florida and thus have a greater output of hatchlings. In addition, the geographic position of these nesting beaches relative to the oceanic currents in the Gulf of Mexico and Atlantic Ocean may explain why hatchlings from these nesting sites come to Florida to forage (Putman et al. 2016). Rookeries with low contributions, below approximately 20%, in the old sample set remained low in the new samples as well. In the old populations of both Trident and the IRL Costa Rica and Quintana Roo Mexico contributed the most to the foraging aggregations. In the new Trident population, the previous top contributors

both decreased and Rancho Nuevo became the highest contributing rookery. The new IRL lagoon saw a decrease in contributions from Quintanna Roo and a corresponding increase from Coast Rica.

Genetic Diversity

Haplotype diversity and nucleotide diversity decreased over time in both foraging sites (table 3). The Indian River Lagoon remained more diverse than Trident in both time periods. Three haplotypes were found exclusively in the IRL Old period and two in the IRL New period. Trident and the IRL each had one haplotype that was unique to the foraging site but appeared across time (figure 5). Haplotypes which were unique to sites were found in low quantities and were genetically distant from the two haplotypes which were the most common in both sites over time. In this study sample size was limited and the presence of private haplotypes may be an artifact of low sampling. These private haplotypes could suggest a geographic substructuring within the larger populations which can be used to determine gene flow (Avise, 1992).

Summary

The pattern of haplotype diversity and nucleotide diversity loss in combination with the geographic substructuring demonstrated in the foraging aggregations on the east coast of Florida is indicative of recovery from a genetic bottleneck event (Fedorov and Stenset. 2001). Sea turtles are long lived and therefore take years to reach maturity where at which time they can contribute to the population. During the “lost years” at sea turtles are not effectively sampled so this life

stage has been neglected from analysis (Putman et al. 2015; Witherington et al. 2012). Consequently, there is a generational lag between nesting females and juveniles sampled which can dampen the effects of genetic drift events and make them harder to detect (Davey and Murphy 2014). As the effective population grows to reflect the previously increased census size the haplotype diversity and genetic diversity should increase until the reproducing females are representative of the population at which point there will be a steady level of variation.

CONCLUSION

While the number of green turtles nesting in the Archie Carr National Wildlife Refuge and Florida beaches has increased, the genetic and haplotype diversity of the surrounding juvenile foraging aggregations has decreased. Florida beaches which are experiencing increased nesting contribute approximately 15% of the Atlantic green turtle population while Tortuguero which is experiencing a decrease in nesting accounts for approximately 85%. With this consideration in mind, understanding the interplay of the increasing adult population in some areas and loss of diversity in surrounding juvenile populations will require long term study. The genetic and haplotype diversity changes demonstrated in the foraging aggregations of East Florida are characteristic of population recovery following a bottle neck event. The diversity of these aggregations should be monitored as the effective population size increases to match the new census size. To fully understand the status of green turtles in the Atlantic and inform conservation decisions, increased sampling efforts should include all rookeries and foraging aggregations to evaluate to population at different stages.

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