

Final Report for Project Titled:

Genetic identities of loggerhead turtles stranded in the Southeast United States

Prepared by

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## **Introduction**

The loggerhead nesting aggregate in the southeastern United States is the largest in the world, and additional important nesting populations (as defined by genetic distinctiveness) occur in the western Atlantic. The likelihood that turtles from genetically distinct stocks share coastal and pelagic developmental habitats may raise doubts regarding the effectiveness of conservation strategies based on geographical or political boundaries (Carr and Stancyk, 1975; Bowen and Witzell, 1996). Tagging studies indicate that a cohort of loggerhead turtles may hatch in one country, grow through adolescence in a second territory or territories, feed as adults in a third jurisdiction, and swim through a dozen more territorial waters enroute to and from these destinations. These life history traits provide an unprecedented challenge to wildlife management agencies. In particular, the origin of loggerhead turtles residing in U.S. coastal waters needs to be determined as a prerequisite for effective management strategies.

Recent research has demonstrated that most sea turtle nesting colonies are genetically distinct in terms of mitochondrial (mt) DNA haplotype frequency shifts. This finding allows the possibility of using mtDNA data to identify rookery cohorts on feeding grounds (Broderick et al., 1994; Bass et al., 1998). Utilizing existing databases (Encalada et al., 1998 and unpublished data) and molecular techniques, tissue samples from stranded marine turtles can be used to estimate the origin of animals inhabiting U.S. coastal waters. These data are generally

collected in a quicker time frame than results from tagging studies and can provide information on cryptic migratory behavior (Bolten et al., 1998).

The main objectives of this project were two-fold: 1) to determine the genetic relationships of Atlantic rookeries that have not been previously sampled, and 2) to determine the genetic identity of loggerhead turtles stranded in the southeast United States. Analyses of stranded loggerheads along the Atlantic coast of the United States will indicate which nesting populations are foraging in US waters. Consequently, populations that may be affected by coastal or maritime activities can be identified and the potential impacts can be assessed.

## **Materials and Methods**

Tissue samples from previously unsurveyed Atlantic rookeries were obtained through collaboration with researchers working at selected sites. Tissue samples from stranded loggerheads were collected by NMFS personnel and colleagues in collaboration with the Sea Turtle Stranding and Salvage Network state coordinators and placed in 15 ml of saturated salt preservation buffer or 95% EtOH. Samples were then transferred to the University of Florida for analysis. Standard phenol/chloroform DNA isolation protocols were conducted on the tissue samples and a 380 bp fragment of the mitochondrial DNA control region was amplified using primers designed for sea turtles (Allard et al., 1994; Norman et al., 1994). Individual fragments were sequenced and compared to known *Caretta caretta* nesting beach haplotypes. Individuals were

then assigned a haplotype based on designations from Encalada et al. (1998), Bolten et al. (1998) and our unpublished data.

Maximum likelihood (ML) analysis for mixed stock identification (Grant et al., 1980) was used to estimate the contributions of nesting populations to stranded loggerhead turtles. This method estimates the most likely contributions of source populations based on the haplotype frequencies in the source populations and in the mixed population (stranded individuals in this case). The maximum likelihood programs GIRLSEM and UCON were used (Masuda et al., 1991). As a starting point in ML iterations using GIRLSEM, it was assumed that all source populations had an equal probability of contributing (i.e. population size, distance from the foraging location, etc. did not have an impact on the percentage of animals recruiting to a particular area). The conditional maximum likelihood estimates from GIRLSEM were then used as the initial point to generate estimates in the unconditional maximum likelihood program, UCON. Standard errors and 95% confidence intervals of the point estimates were generated from 100 bootstraps of the stock and mixture data sets using GIRLSEM (see Pella et al., 1998).

## **Results**

### *DNA Analysis*

Of the 18 individuals from the Dry Tortugas, 17 possessed haplotype B and one individual had haplotype J. Three haplotypes were observed among the 8 individuals sampled from the Florida panhandle (A = 8, B = 1, and M = 1).

Thus far, haplotype M had not been observed in the previously sampled nesting locations.

Table 1. Location of rookeries sampled or resampled.

<b>Location</b>	<b>Sample Size</b>	<b>Haplotypes</b>
<b>Dry Tortugas</b>	24	17 B; 1 J
<b>Florida Panhandle</b>	8	8 A; 1 B; 1 M

Table 2 provides a synopsis of the number of individuals received (excluding the first batch from NC) and the number of individuals for which sequences have been generated. Appendix 1 contains a listing of all samples analyzed.

Table 2. Locations from which samples from stranded animals have been taken.

(NW = Not Working)

<b>Location</b>	<b># Received</b>	<b># Analyzed</b>
<b>Texas</b>	129	121 (8 NW)
<b>South Carolina</b>	101	95 (6 NW)
<b>Florida</b>	94	93 (1 NW)
<b>North Carolina</b>	66	60 (6 NW)
<b>Virginia</b>	36	35 (1 NW)
<b>Delaware</b>	9	8 (1 NW)

<b>New Jersey</b>		8	6 (2 NW)
<b>Totals</b>		444	419 (25 NW)

Haplotypes A and B were ubiquitous at all sampling localities (Table 3). In general, similar haplotype compositions were observed in all seven states. The low sample size from Delaware and New Jersey precludes the discovery of the rarer haplotypes observed in other localities.

### *Maximum Likelihood Analysis*

Data analyses were conducting using state designations as groupings (i.e. due to a lack of location information all samples from Texas were included in a single maximum likelihood (ML) analysis). No stratification into size classes or time period was included.

For all state stranding data, a ML analysis was conducting including all potential source populations (analyses not shown). If the estimate generated had a standard deviation greater than the percent contribution, then the associated source population was removed from the analysis. In addition, NWFL was removed from all analyses due to the small size of the population. We are assuming that we cannot detect contributions from this relatively small population [100-200 turtles annually (Meylan et al. 1995)]. Inclusion of this population in the analysis(or other populations that contribute less than 1% of overall nesting effort), generates overestimates of contribution and can compromise the accuracy of the estimates for the other source populations.

Table 4. Maximum likelihood estimates of contribution by source populations to stranded loggerhead cohorts from Texas (n = 115). Three potential source populations, NWFL , Brazil, and Greece, were removed from the analysis. Estimates were generated using UCON. Standard errors and 95% confidence intervals were generated from 100 bootstraps of both the stock and mixture using GIRLSEM.

<i>Source Population</i>	<i>Contribution (PE)</i>	<i>Standard Error</i>	<i>95% C. I.</i>
SFL	0.8336	0.2124	0.7915-0.8757
NEFL-NC	0.1033	0.1219	0.0792-0.1274
MEXICO	0.0630	0.0875	0.0457-0.0803

Table 5. Maximum likelihood estimates of contribution by source populations to stranded loggerhead cohorts from Florida (n = 89). The potential source populations, NWFL , Brazil, and Greece, were removed from the analysis. Estimates were generated using UCON. Standard errors and 95% confidence intervals were generated from 100 bootstraps of both the stock and mixture using GIRLSEM.

<i>Source Population</i>	<i>Contribution (PE)</i>	<i>Standard Error</i>	<i>95% C. I.</i>
SFL	0.7286	0.3178	0.6656-0.7916
NEFL-NC	0.1982	0.1715	0.1642-0.2322
MEXICO	0.0728	0.0598	0.0610-0.0846

Table 6. Maximum likelihood estimates of contribution by source populations to stranded loggerhead cohorts from South Carolina (n = 92). The potential source populations, NWFL , Brazil, and Greece, were removed from the analysis. Estimates were generated using UCON. Standard errors and 95% confidence

intervals were generated from 100 bootstraps of both the stock and mixture using GIRLSEM.

<i>Source Population</i>	<i>Contribution (PE)</i>	<i>Standard Error</i>	<i>95% C. I.</i>
SFL	0.6566	0.1901	0.6189-0.6943
NEFL-NC	0.2455	0.1325	0.2193-0.2717
MEXICO	0.0977	0.0683	0.0842-0.1112

Table 7. Maximum likelihood estimates of contribution by source populations to stranded loggerhead cohorts from North Carolina (n = 56). The potential source populations, NWFL , Brazil, and Greece, were removed from the analysis.

Estimates were generated using UCON. Standard errors and 95% confidence intervals were generated from 100 bootstraps of both the stock and mixture using GIRLSEM.

<i>Source Population</i>	<i>Contribution (PE)</i>	<i>Standard Error</i>	<i>95% C. I.</i>
SFL	0.6546	0.2574	0.6036-0.7056
NEFL-NC	0.2857	0.1647	0.2531-0.3183
MEXICO	0.0595	0.0702	0.0456-0.0734

Table 8. Maximum likelihood estimates of contribution by source populations to stranded loggerhead cohorts from Virginia (n = 33). The potential source populations, NWFL , Brazil, and Greece, were removed from the analysis. Estimates were generated using UCON. Standard errors and 95% confidence intervals were generated from 100 bootstraps of both the stock and mixture using GIRLSEM.

<i>Source Population</i>	<i>Contribution (PE)</i>	<i>Standard Error</i>	<i>95% C. I.</i>
SFL	0.4628	0.2777	0.4077-0.5179
NEFL-NC	0.4673	0.1843	0.4308-0.5038
MEXICO	0.0698	0.0894	0.0521-0.0875

## **Discussion**

Estimates of contributions from 5 of the 7 states sampled indicate that the highest contributions to stranded loggerheads in the south are from the large SFL nesting population. Clinal variation becomes apparent as one moves north with a visible decline in the contribution from the southern population (Figure 1). In these cases, recruitment to local foraging populations is dominated by local nesting populations. Contributions from the Mexican nesting population appear to be evenly distributed along the surveyed areas and may be controlled by current patterns. The contribution from the NWFL population is most likely evenly distributed among the estimates of contribution from the SFL and NEFL-

NC nesting populations. Contributions from the nesting populations in Brazil and Greece were not detected in any of these analyses. There may be occasional strays from these nesting populations into US waters, but their numbers are too small to detect.

Overall, coastal marine activities in US waters would affect the SFL, NEFL-NC, and Mexican rookeries.

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