Report on the Analysis of green turtles from the Pamlico-Albemarle Estuarine Complex, North Carolina

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Introduction

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., 1996) and molecular techniques, tissue samples from 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 objective of this project were to determine the genetic identity of green turtles foraging in the Pamlico-Albemarle Estuarine complex, North Carolina. Analyses of foraging green turtles 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

Blood samples and carapace length measurements were collected from juvenile loggerheads that were caught by pound net fisherman in the study area (the Core Sound, eastern Pamlico and Abermarle Sounds, North Carolina) throughout the months of September – December from 1995 to 1997. Blood samples were also taken from green turtles foraging in the study area (n=106). Approximately 1 ml of blood was placed in 9 ml of lysis buffer (100mM Tris-HCL, 100mM EDTA, 10mM NaCl, 0.5% SDS; pH 8.0)

and stored at room temperature. Whole DNA isolations from blood samples were conducted with the phenol/chloroform method described by Hillis et al. (1996).

A 510 base-pair fragment located in the control region of the mtDNA genome was amplified using the primers LTCM1 and HDCM1 of Allard et al. (1994) and standard reaction conditions. Cycle sequencing was conducted with an ABI Prism kit and fluorescently labeled dideoxynucleotides at the University of Florida DNA Sequencing Core and the labeled extension products were analyzed with an automated DNA sequencer (Applied Biosystems model 373A). Sequences were compared to known green turtle haplotypes (Encalada et al., 1996) and assigned a haplotype designation.

Maximum likelihood (ML) estimates of percent contribution of regional rookeries were obtained using the program SPAM (ADGF, 1999). The regional rookeries correspond to those listed in Encalada et al. (1996): Florida and Mexico, Costa Rica, Aves Island and Surinam, Brazil, Ascension Island and Guinea Bissau and Cyprus. To measure variation due to sampling errors in both the foraging population and source populations, 1000 bootstrap resamplings were conducted.

Results and Discussion

Of the 108 samples obtained, 106 samples yielded readable sequences. 97 of the sequences matched haplotypes observed at previously surveyed nesting locations (Table 1; see Appendix 1 for complete listing). The majority of animals were classified as haplotypes CM I (n = 34) and CM III (n = 43). Seven individuals were characterized as

CM VIII, 5 as CM V, 3 as CM XVIII, 2 each as CM II, XVI and XVII, and one as CM XV. The haplotypes of 3 animals matched a haplotype previously identified in Florida, here designated as CM28*. The remaining 4 animals represent newly identified haplotypes, CM26* and CM27* (Appendix 2).

Table 1. Haplotype frequency and origin for green turtles sampled from the Pamlico-Albemarle Estuarine Complex, North Carolina.

Haplotype	***Frequency***	***Location***
CM I	34	Florida, Mexico
CM II	2	Florida
CM III	43	FL, Yucatan, Costa Rica, Aves Island
CM V	5	Yucatan, Aves Island, Surinam
CM VIII	7	Brazil, Ascension, Guinea Bissau (West Africa)
CM XV	1	Mexico
CM XVI	2	Mexico
CM XVIII	3	Mexico
		Foraging Ground or Stranded
CM XXII	2	St. Lucie Power Plant
CM 26*	2	North Carolina
CM 27*	2	North Carolina
CM 28*	3	Florida Stranded

Maximum likelihood analyses using SPAM indicated that 4 of the potential 6 source populations contributed at detectable levels (Table 2). The highest contribution was from the combined Florida and Mexico "population" with ~75%. The second largest contribution was from Costa Rica with 15%. The nesting populations at Ascension Island and Guinea Bissau contributed 7% and the remaining fraction was attributed to the nesting populations at Aves Island and Surinam.

Table 2. Maximum likelihood estimates from the program SPAM for the green turtle foraging aggregation in the Pamlico-Albemarle Estuarine complex.

Source Population	***Estimate***	***Standard Deviation***	***95% Confidence Intervals***
FL/MEX	0.7464	0.1113	0.4915-0.9071
Costa Rica	0.1452	0.1022	0.0001-0.3543
Aves/Surinam	0.0362	0.0284	0.0000-0.1019
Brazil	0.0000	0.0001	0.0000-0.0001
Ascension/Guinea Bissau	0.0721	0.0266	0.0309-0.1339
Cyprus	0.0000	0.0000	---------

The majority of individuals foraging in North Carolina originated from the combined nesting populations located in Florida and Mexico. The relatively high estimate for turtles originating from the distant Ascension Island and Guinea Bissua population is surprising and may actually represent Brazilian turtles.

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Appendix 1. Haplotypes of individual turtles analyzed from the Pamlico-Albemarle Estuarine Complex, North Carolina.

Lab#	Incident #	Haplotype
643		VIII
644		I
645		I
646		I
647		I
648		III
649		CM26*(NC-1)
650		CM28*(FL-1)
651		I
652		III
653		I
654		V
655		III
656		VIII
657		XVIII
658		III
659		V
660		XVIII
661		III
662		III
663		I
664		XVIII
665		III
666		I
667		I
668		I
669		III
670		V
671	QQS 241-242	CM28*(FL-1)
672	QQS 245-246	III
1108	382	I
1109	706	III
1110	641	I
1111	752	V
1112	710	III
1113	623	VIII
1114	609	III
1115	594	NW
1116	564	III
1117	379	III
1118	740	VIII
1119	741	I
1120	234	I
1121	593	NW

Appendix 1. Haplotypes of individual turtles analyzed from the Pamlico-Albemarle Estuarine Complex, North Carolina.

Lab#	Incident #	Haplotype
1122	608	I
1123	440	III
1124	275	I
1125	373	III
1126	380	III
1127	513	III
1128	516	I
1129	236	I
1130	172	III
1131	317	XXII
1132	95	I
1133	505	I
1134	509	I
1135	70	I
1136	60	III
1137	182	XVI
1138	325	V
1139	148	III
1140	83	CM27*(NC-2)
1141	207	II
1142	519	III
1143	103	CM28*(FL-1)
1144	310	CM26*(NC-1)
1145	269	I
1146	473	XVI
1147	524	I
1148	327	III
1149	460	I
1150	324	XXII
1151	326	I
1152	175	III
1153	322	III
1154	284	I
1155	86	III
1156	64	III
1157	254	VIII
1158	333	III
1159	328	II
1160	447	I
1161	47	CM27*(NC-2)
1162	526	I
1163	41	III
1164	33	III
1165	540	I

Appendix 1. Haplotypes of individual turtles analyzed from the Pamlico-Albemarle Estuarine Complex, North Carolina.

Lab#	Incident #	Haplotype
1166	296	III
1167	551	III
1168	116	III
1169	550	III
1170	456	III
1183	266	III
1184	770	III
1185	389	I
1186	359	III
1187	265	III
1188	588	III
1189	280	III
1190	241	III
1191	536	I
1192	400	III
1193	341	XV
1194	260	I
1195	781	I
1196	355	VIII
1197	444	VIII

Appendix 2. Control region sequence for haplotypes CM 26* and CM 27*. Lower case letters represent the transfer RNA on the 5' end of the control region. Solid lines under nucleotides indicate position of primers. Bold and underlined nucleotides are polymorphic sites in relation to each other and to haplotypes CMIII and CMV.

CM26*

cccaaaaccg gaatcctata attaaactat cctttg ACACAGGAAT AAAAGTGTCC
LTCM1

ACACAAACTA ACTACCTAAA TTCTCTGCCG TGCCCAACAG AACAATACCC GCAATACCTA

TCTATGTATT AT**C**GT**A**CATC TACTTATTTA CCAATAGCAT ATGACCAGTA AT**G**TTAACAG

TTGATTTGGC CCTAAACATA AAAAATCATT GAATTTACAT AAATATTTTA ACAACATGAA

TATTAAGCAG AGGATTAAAA GTGAAATGAC ATAGGACATA AAATTAAAC**T** ATTATACTCA

ACCATGAATA TCGTCACAGT AAT**T**GGTTAT TTC**T**TAAATA GCTATTCACG AGAAATAAGC

AACCCTTGTT AGTAAGATAC AACATTACCA GTTTCAAGCC CATTCA**G**TCT GTGGCGTACA

TAATTTGATC TATTCTGGCC TCTGGTTAG**T** TTTTCAGGCA CATACAAGTA **A**C**G**ACGTTCA

TTCGTTCCCC TTTAAAAGGC CTTTGGTTGA ATGAGTTCTA TACATT**A**AAT TTATAACCTG

GCATACGGTA GTTTTACTT
HDCM1

CM27*

cccaaaaccg gaatcctata attaaactat cctttg ACACAGGAAT AAAAGTGTCC
LTCM1

ACACAAACTA ACTACCTAAA TTCTCTGCCG TGCCCAACAG AACAATACCC GCAATACCTA

TCTATGTATT AT**C**GT**A**CATC TACTTATTTA CCAATAGCAT ATGACCAGTA AT**G**TTAACAG

TTGATTTGGC CCTAAACATA AAAAATCATT GAATTTACAT AAATATTTTA ACAACATGAA

TATTAAGCAG AGGATTAAAA GTGAAATGAC ATAGGACATA AAATTAAAC**C** ATTATACTCA

ACCATGAATA TCGTCACAGT AAT**T**GGTTAT TTC**C**TAAATA GCTATTCACG AGAAATAAGC

AACCCTTGTT AGTAAGATAC AACATTACCA GTTTCAAGCC CATTCA**G**TCT GTGGCGTACA

TAATTTGATC TATTCTGGCC TCTGGTTAG**T** TTTTCAGGCA CATACAAGTA **A**C**G**ACGTTCA

TTCGTTCCCC TTTAAAAGGC CTTTGGTTGA ATGAGTTCTA TACATT**A**AAT TTATAACCTG

GCATACGGTA GTTTTACTT
HDCM1