STATUS AND TRENDS OF THE LOGGERHEAD TURTLE POPULATION IN SOUTH CAROLINA

Final Report to the National Marine Fisheries Service

under

Section 6 Endangered Species Cooperative Agreement

Contractor:

South Carolina Wildlife and Marine Resources Department
P.O. Box 12559, Charleston, South Carolina 29422-2559
W. Brock Conrad, Jr. Director,
Wildlife & Freshwater Fisheries Division

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Compiled by Sally R. Hopkins-Murphy, Principal Investigator

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INTRODUCTION

Management and protection of sea turtles is a difficult task. The foremost problem in management and eventual recovery of sea turtle populations is the lack of basic biological information, especially when they are at sea. Most of the information gathered to date has been on nesting females, nests and to a lesser extent, hatchlings. Longterm tagging studies provide data on: remigration intervals, mean number of nests laid per gravid female per season, and the average number of eggs laid per season. Nesting beach protection projects provide information on the number of successful nests, and the number of hatchlings produced. (For a recent review, see Dodd 1988). Data on other population attributes are badly needed in order to manage the population and determine the status of stocks. These include: age to sexual maturity, adult to immature ratio, sex ratio, survivorship (mortality) and most importantly of all, the identification of the stock that is being affected, both the nesting females and the sub-adults.

To effectively manage sea turtle stocks and to determine the efficacy of nest protection activities, it is necessary to determine the origin of immature turtles. Such knowledge could be of major importance if progeny from specific nesting beaches exhibit different behavior, movements or foraging ranges than turtles from other beaches. Such differences could result in higher mortalities in some nesting populations and lower mortality in other populations (NMFS, 1990).

Knowledge of sub-adult turtles is scanty and comes mostly from stranded animals. Knowing the sex ratio of the sub-adult segment of a population, coupled with stock identification of that population, could be used to assess past management practices such as artificial incubation. There also may be differential mortality relative to sex for different age classes of turtles. Turtles hatching from different geographic regions may experience higher mortality than those from another part of the range. Data from other areas need to be compared with areas such as South Carolina and Georgia, in which certain age classes have received high mortality from shrimp trawling.

Monitoring of strandings is needed to document the temporal and spatial relationship of sea turtle mortality. The continued use of TEDs (turtle excluder devices) should decrease the number of carcasses each season.

Loggerhead turtles have deferred maturity, requiring perhaps several decades to reach adulthood. The lag time between when population declines are first monitored and when recovery may be accomplished is related to the age class being impinged upon and the age class where management is applied. Declines in nesting could result from reduced recruitment from the nesting beaches, immature mortality or adult mortality. Depending upon which of these causes was involved, it could be many years before population declines were realized and even longer before management could mitigate them. Thus, there is a need to quantify various population attributes, especially for the sub-adult segments of the population. Monitoring changes in population attributes is needed to evaluate the status of sea turtle stocks relative to

reclassification or delisting.

This research was divided into two jobs. Job 1, Sea turtle stranding network, documented the spatial and temporal occurrence of stranded sea turtle carcasses in South Carolina. Job 2, immature sex ratio and stock identification, determined the sex ratio of sub-adult loggerheads and the breeding population origins for nesting females. The breeding population origins for the sub-adults is undetermined at this time, pending further investigations.

This work was a collaborative effort among several investigators. The stranding data was collected by the Principal Investigator. Sex ratios were determined by Dr. David Owens at Texas A&M University. Genetic origins of nesting females were determined by Dr. Brian Bowen, University of Georgia. Genetic origins of sub-adults are currently being researched by Ms Connie Sears, graduate student at the University of Charleston. This research is being conducted at the National Marine Fisheries Service Charleston Laboratory under the direction of Dr. Sylvia Galloway. Each aspect of this research will be presented in separate sections of this report.

title is "Turtles, TEDS and trawl fisheries: Implications from a population projection model for loggerhead sea turtles (Caretta caretta)."

In addition, a more detailed analysis of the stranding data is being prepared for a manuscript which will include a time series analysis to show the effectiveness of TEDs.

Table 1.

MARINE TURTLE CARCASSES DOCUMENTED FROM SOUTH CAROLINA BEACHES, 1991

for two week periods beginning:

	4/1	4/16	5/1	5/16	6/1	6/16	7/1	7/16	8/1	8/16	9/1	9/16	10/1-31	11/1-30	TOTAL
Myrtle Beach	1	2.1*	0	1*	1	0	0	0	1	0	0	0	0	0	7
Litchfield Beach	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Pawley's Island	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Debidue Beach	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
North Island	0	0	0	1	0	0	0	0	0	1	0	0	1	4	7
Sand Island	0	0	0	1	0	0	0	0	0	0	0	0	0	1	2
South Island	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cedar Island	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Murphy Island	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cape Island	0	0	0	0	0	1.1*	0	1	0	0	0	0	0	0	3
Lighthouse Island	0	0	0	0	0	1	2	0	0	0	0	0	0	0	3
Raccoon Key	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Bulls Island	0	0	1	2.2*	0	0	0	1	0	0	1	0	0	0	7
Capers Island	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dewees Island	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
Isle of Palms	2	0	0	1*	1	1	0	0	0	2	0	0	0	0	7
Sullivans Island	0	0	0	1	1	1	0	0	0	0	0	1	0	0	4
Morris Island	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Folly Beach	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Kiawah Island	0	(1)	1	1.3*	0	0	0	(1)	0	0	0	1	1	0	5
Seabrook Island	0	0	0	0	0	1	0	0	0	0	0	1	0	0	2
Botany Bay	0	0	0	1	1	0	0	0	0	0	0	0	0	0	2
Edingsville Beach	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Edisto Beach	0	0	0	1*	0	0	0	(1)	0	0	0	0	0	0	2
Pine & Otter Islands	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Harbor Island	0	0	0	0	0	0	0	0	1§	0	1	0	0	0	2
Hunting Island	1	0	0	0	0	0	0	0	0	(1)	0	0	0	0	2
Fripp Island	0	0	0	0	0	0	1	0	0	0	0	0	(1)	0	2
Pritchards Island	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Little Capers	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
St. Phillips	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bay Point	0	1	1	0	1	0	0	0	0	0	0	0	0	0	3
Hilton Head	0	0	1.1*	1	0	1	1	0	0	0	0	0	0	0	5
Daufuskie Island	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Turtle Island	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Misc.	0	0	0	1#	0	0	0	0	0	2¶	0	1(1)**	0	0	5
1991 TOTALS	5	6	6	17	5	9	5	5	2	6	2	5	3	5	81

Table 2.

MARINE TURTLE CARCASSES DOCUMENTED FROM SOUTH CAROLINA BEACHES, 1980 - 1991
for two week periods beginning:

	4/1	4/16	5/1	5/16	6/1	6/16	7/1	7/16	8/1	8/16	9/1	9/16	10/1-31	11/1-30	Total
1980	0	20	28	6	22	64	145	141	67	37	22	18	17	0	595
1981	10	22	4	7	4	12	62	90	55	10	25	13	7	0	121
1982	3	35	7	23	26	30	58	27	20	14	2	9	9	0	263
1983	0	6	9	17	22	18	43	23	14	8	7	2	1	1	171
1984	4	13	12	2	0	31	43	13	7	4	0	3	0	0	132
1985	2	14	2	1	2	17	29	13	9	5	4	1	2	0	101
1986	0	2	5	0	11	40	34	41	15	11	4	5	9	8	185
1987	0	0	7	22	115	28	33	19	8	7	12	12	5	0	268
1988	0	2	10	5	0	2	22	21	17	9	5	5	4	3	105
1989	1	0	4	8	23	8	5	14	11	4	7	4	2	4	95
1990	4	5	5	2	6	13	14	3	7	1	8	16	21	11	116
1991	5	6	6	17	5	9	5	5	2	6	2	5	3	5	81

legend:

¶ harbor dredge kill
* indicates leatherback
() indicates Kemp's ridley ** found floating in Ft. Johnson boatslip
stranded Dewees Inlet
§ indicates green turtle

Table 3.

MARINE TURTLE CARCASSES DOCUMENTED FROM SOUTH CAROLINA BEACHES, 1992

for two week periods beginning:

	4/1	4/16	5/1	5/16	6/1	6/16	7/1	7/16	8/1	8/16	9/1	9/16	10/1-31	11/1-30	Totals
Myrtle Beach	0	0	0	0	3	0	1	2	0	0	0	0	0	0	6
Litchfield Beach	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pawley's Island	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Debidue Beach	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1
North Island	0	0	0	0	1	0	0	1	0	0	0	0	0	1	13
Sand Island	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
South Island	0	0	0	0	0	1	1	0	0	0	0	2	0	0	4
Cedar Island	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Murphy Island	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cape Island	0	1	1	1	0	1	1	1	0	0	0	0	0	0	6
Lighthouse Island	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Raccoon Key	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
Bulls Island	0	0	2	1	0	0	0	0	0	0	0	1	0	0	4
Capers Island	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dokers Island	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Isle of Palms	0	0	0	2	2	1	0	0	0	0	1	0	0	0	6
Sullivans Island	0	0	3	0	2	0	1	0	0	0	0	0	0	0	6
Morris Island	0	0	0	2	0	0	0	0	0	0	0	0	1	0	3
Folly Beach	0	0	0	0	1	2	0	0	0	0	1	0	0	0	4
Kiawah Island	0	0	0	1	0	3	1	1	1	2	0	0	0	0	9
Seabrook Island	0	1	1	3	0	0	1	0	1	0	0	0	0	0	7
Botany Bay	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Edingsville Beach	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
Edisto Beach	0	0	0	4	0	0	0	1	0	1	0	0	0	0	6
Pine & Otter Islands	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Harbor Island	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hunting Island	1	0	2	1	0	0	0	1	0	0	0	0	0	0	5
Fripp Island	0	0	0	3	0	0	1	1	0	0	0	0	0	0	5
Pritchards Island	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Little Capers	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
St. Phillips	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bay Point	0	1	0	1	0	0	0	0	0	0	0	0	0	0	2
Hilton Head (Jan. 1)	0	2	2	3	0	0	1	1	0	0	1	0	0	0	11
Daufuskie Island	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Turtle Island	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Misc.	0	0	1	1	0	0	0	1	1	0	0	0	0	0	4
1992 TOTALS	2	6	13	26	9	7	9	11	4	3	4	3	0	1	107

Table 4.

MARINE TURTLE CARCASSES DOCUMENTED FROM SOUTH CAROLINA BEACHES, 1980 - 1992
for two week periods beginning:

	4/1	4/16	5/1	5/16	6/1	6/16	7/1	7/16	8/1	8/16	9/1	9/16	10/1-31	11/1-30	TOTAL
1980	0	20	28	6	22	64	145	141	67	37	22	18	17	0	595
1981	10	22	4	7	4	12	62	90	55	10	25	13	7	0	321
1982	3	35	7	23	26	30	58	27	20	14	2	9	9	0	263
1983	0	6	9	17	22	18	43	23	14	8	7	2	1	1	171
1984	4	13	12	2	0	31	43	13	7	4	0	3	0	0	132
1985	2	14	2	1	2	17	29	13	9	5	4	1	2	0	101
1986	0	2	5	0	11	40	34	41	15	11	4	5	9	8	185
1987	0	0	7	22	115	28	33	19	8	7	12	12	5	0	268
1988	0	2	10	5	0	2	22	21	17	9	5	5	4	3	105
1989	1	0	4	8	23	8	5	14	11	4	7	4	2	4	95
1990	4	5	5	2	6	13	14	3	7	1	8	36	21	11	116
1991	5	4	6	17	5	9	5	5	2	6	2	5	3	5	81
1992	2	6	13	26	9	7	9	11	4	3	1	3	9	1	107

Species Summaries: 1992

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
Loggerhead	1	0	0	4	10	12	19	6	7	6	1	
Green	0	0	0	0	1	0	0	0	0	1	0	
Kemp's ridley	0	0	0	0	2	0	1	0	0	2	0	
Leatherback	0	0	0	3	26	4	0	0	0	0	0	
Unidentified	0	0	0	0	0	0	0	1	0	0	0	

Location of Misc. Strandings: Buzzards Roost Tarpon, Stono River, Johns Island

Job 2 Immature Sex Ratio

Dr. David Owens

Objective: To determine the sex ratio of sub-adult loggerheads in Charleston Harbor entrance channel.

Rationale

A 16 month survey of the turtle population of the Charleston Harbor entrance channel was carried out by Marine Resources Division personnel. The objectives were to (1) document the seasonal and diurnal variability in turtle densities and (2) evaluate the distribution of turtles in the survey area (Van Dolah et al. 1992). These surveys hoped to gain information that would prevent sea turtle mortality from hopper dredges that were deepening and widening the channel. Job 2 was initiated to gain more information on this segment of the population than the objectives stated above.

Methods

Loggerhead turtles captured by the trawler RV "Lady Lisa" in Charleston Harbor entrance channel were sampled for blood using the technique reported by Owens and Ruiz (1980).

Two 15ml blood samples were drawn from each captured turtle. These samples were immediately centrifuged and the serum and blood cells placed in separate vials. All vials were frozen and stored at -30 degrees at the NMFS Charleston Laboratory.

Serum samples were shipped frozen to Texas A&M University, Department of Biology and testosterone assays were run as described in Owens et al. (1978).

Results and Discussion

Twenty-five samples were analyzed. Nineteen were females and 6 were males or 76% females and 24% males for the Charleston Harbor. These percentages are slightly higher than Wibbles et al. (1987) calculations. They found that the pooled sex ratio of sub-adult loggerheads along the Atlantic coast of the United States was 66.3% females. The results from Charleston show a more than 2:1 female sex ratio.

A manuscript on observations and implications of sex ratio in sub-adult sea turtle populations is being prepared.

Job 2 Genetic structure of immature loggerhead turtles

Ms Connie Sears

Objective: To determine the breeding population origins for immature loggerhead turtles in South Carolina.

Rationale

Mitochondrial DNA can be used as an internal "tag" to identify the maternal ancestry. This is especially useful to identify stocks of sea turtles since only the females come ashore to nest. Stock identification is necessary for managers to make informed decisions for the protection and conservation of these threatened species. It is now finally possible to look at the genetics of an individual loggerhead turtle within a local population and determine the identity of that individual's natal rookery. The conservation and management potential of this knowledge is broad.

Methods

Whole blood cells will be used to extract mitochondrial DNA to determine the stock identification of sub-adults. A specific genotype will be determined for each turtle sampled by analyzing mtDNA. Restriction fragment patterns will be generated from isolated mtDNA using several restriction endonuclease enzymes. These data will be compared with Georgia, South Carolina and Florida stocks. The mitochondrial DNA analysis will be conducted at the NMFS Charleston Laboratory under the direction of Drs. Cheryl Woodley-Miller and Sylvia Galloway.

Results

It is expected that the results of this study will demonstrate the natal origins for the population of sub-adult loggerhead turtles found in the Charleston Harbor entrance channel. However, techniques using mtDNA on blood samples are still being developed and refined. This portion of the research is in partial fulfillment of requirements for a MS degree for Ms Sears and will be reported on by 31 January as requested in the last quarterly report.

Job 2 Genetic structure of nesting females

Objective: To determine the breeding population origins for nesting females in South Carolina.

Dr. Brian Bowen

Rationale

To assess population genetic structure and evolutionary relationships among loggerhead turtle rookeries, samples were analyzed from four nesting beaches in the West Atlantic Ocean and one in the Mediterranean Sea for mitochondrial (mt)DNA variation.

Nineteen hatchlings from Cape Island, Cape Romain National Wildlife Refuge were acquired during this study and transported to the University of Georgia Genetics Department for analysis.

The results of these samples along with others are found in the draft manuscript that has been submitted to Conservation Biology. The title is "Population structure of loggerhead turtles (Caretta caretta in the West Atlantic Ocean and Mediterranean Sea" by B.W. Bowen, J.I. Richardson, A.B. Meylan, D. Margaritoulis, S.R. Hopkins-Murphy and J.C. Avise. A draft is included as part of this report for information only. Please do not cite this manuscript as it is still under review.

DRAFT

POPULATION STRUCTURE OF LOGGERHEAD TURTLES (*CARETTA CARETTA*) IN THE WEST ATLANTIC OCEAN AND MEDITERRANEAN SEA

Brian W. Bowen[1,2,7], James I. Richardson[3], Anne B. Meylan[4], Dimitris Margaritoulis[5], Sally R. Hopkins-Murphy[6] and John C. Avise[1]

[1]Department of Genetics, University of Georgia, Athens, GA., 30602; [2]present address: DNA Sequencing Core, P.O. Box 110699, University of Florida, Gainesville, FL., 32611 and Archie Carr Center for Sea Turtle Research, 223 Bartram Hall, University of Florida, Gainesville FL., 32611; [3]Institute of Ecology, University of Georgia, Athens, Georgia 30602; [4] Florida Marine Research Institute, 100 Eighth Ave., S.E., St. Petersburg, FL., 33701-5095; [5] Sea Turtle Protection Society of Greece, Solomou 35, GR-106 82, Athens, Greece; [6] South Carolina Wildlife and Marine Resources Dept., P.O. Box 12559, Charleston, S.C. 29422-2559; [7] To whom correspondence and reprint requests should be addressed.

Running head: loggerhead turtle population structure

ABSTRACT

To assess population genetic structure and evolutionary relationships among loggerhead turtle (*Caretta caretta*) rookeries, we analyzed 113 samples from four nesting beaches in the West Atlantic Ocean and one nesting beach in the Mediterranean Sea for mitochondrial (mt) DNA variation. Significant differences in haplotype frequency between nesting beaches in Florida versus Georgia/South Carolina, and between both of these assemblages and the rookery in the Mediterranean, indicate substantial restrictions on contemporary gene flow between regional populations, and therefore a strong tendency for natal homing by females. Nonetheless, this regional genetic structure appears shallow, indicating recent evolutionary connections among rookeries. Data from tag returns and mtDNA, as well as geological considerations, suggest that over short evolutionary timescales (perhaps a few thousand years), dispersal by female loggerheads is sufficient to allow colonization of appropriate habitat in proximity to established rookeries but is too low to significantly impact the population dynamics of rookeries on a contemporary timescale. These data indicate that nesting populations of the loggerhead turtle must be managed as demographically independent units. The West Atlantic population subdivision based on mtDNA analysis is concordant with previously-reported distinctions between Florida and Georgia/South Carolina nesting populations based on environmental markers, tag recaptures, and morphology.

INTRODUCTION

The loggerhead turtle, *Caretta caretta*, is distributed widely in warm temperate and subtropical oceans. At intervals averaging two to three years, adult loggerheads depart from the foraging grounds on reproductive migrations that range in distance from a few kms to thousands of kms (Meylan 1982; Limpus et al. 1992). Tagging data indicate that most females return faithfully to the same nesting beach, and both sexes return to the same foraging areas between reproductive migrations (Limpus et al. 1992). In the Atlantic, loggerhead hatchlings leave the nesting beach to occupy oceanic current systems such as the north Atlantic gyre, where they drift passively for five or more years before recruiting to coastal neritic zones (Carr 1986). Subadults may occupy coastal feeding grounds for a decade or more before their first reproductive migration (Carr 1987). Estimates of the age at maturity range from 12 to 30 years in the West Atlantic (Frazer & Ehrhart 1985; Zug et al. 1986; Klinger & Musick, 1992), and 30+ years in eastern Australia (Limpus 1985).

In recent reports, mtDNA analyses have proven useful for defining demographic and evolutionary units within marine turtles *Chelonia mydas* (green turtle) and *Lepidochelys* spp. (ridley turtles) (Bowen et al 1991, 1992). The maternal inheritance of mtDNA lends this approach special significance in evaluating aspects of marine turtle life history, including the possibility of natal homing by females (Meylan et al. 1990). Tag recapture experiments indicate that female loggerheads typically return to the same nesting area, but it is unclear whether this site fidelity is a product of natal homing behavior. In principle, a variety of mechanisms could explain female

nest site fidelity, including imprinting of hatchlings, genetic programming, or social interaction (see Owens et al. 1982). Notably the latter mechanism can account for nest site fidelity without invoking natal homing: first-time nesters may follow experienced females from feeding grounds to suitable nesting locations and then fix on that location for all subsequent nesting efforts. Under this "social facilitation" scenario, turtles may recruit to non-natal rookeries, providing an avenue of female-mediated gene flow between rookery populations that overlap on feeding grounds. Alternatively, under a "natal homing" scenario, female-mediated gene flow between rookeries would be limited or absent. Thus these contrasting models, social facilitation versus natal homing, generate distinct predictions about the population genetic structure of female nesting assemblages that can be tested with mtDNA analysis.

Significant differences in the concentrations of heavy metals in eggs (Stoneburner et al. 1980), and in the composition of epibiota on the carapaces of adult females (Caine 1986), indicate that loggerheads in the southeastern U.S. are divided into at least two demographic units corresponding to Florida and Georgia/South Carolina nesting assemblages. However, metal concentrations and epibiota are presumably acquired through prolonged contact with a particular environment, and therefore are a product of the habitats utilized by turtles during non-nesting intervals. Thus these "acquired" markers demonstrate differences in feeding areas or migration pathways but leave open the question of whether Georgia/South Carolina and Florida rookeries are genetically distinct. Another type of acquired marker, physical tags applied to adult

determine whether Mediterranean rookeries are genetically isolated from North Atlantic nesting populations. To address these questions we obtained samples from the western Peloponnesus coast of Greece, one of the largest nesting aggregates in the Mediterranean (Margaritoulis 1988*a*).

Finally, these population genetic issues are relevant to management concerns for the loggerhead turtle. Nesting habitat in many areas has been diminished by human activity, and post-juveniles may be severely impacted on feeding grounds as well. Prior to the recent application of turtle excluder devices, an estimated 10,000 to 55,000 marine turtles (mostly loggerheads) were drowned annually in the shrimp fishery of the southeastern U.S. (Henwood & Stuntz 1987, National Research Council 1990). To assess the impact of these factors on loggerhead survival, accurate population and demographic data are required. Thus this study was designed to assess the genetic integrity of nesting beaches both within a region (the southeastern U.S.) and among rookeries on a geographic scale consistent with known loggerhead migrations (the North Atlantic and Mediterranean basin). Given the magnitude of loggerhead movements, both micro- and macro-geographic scales are relevant to management concerns. As noted by Limpus et al. (1992), "no one country controls the fate of a given turtle population".

MATERIALS AND METHODS

Between 1987 and 1991, 113 nests were sampled from: 1) Kiparissia Bay, western Peloponnesus, Greece (n = 21); 2) Cape Romain, South Carolina, USA (n = 19); 3) Cumberland Island, Georgia USA (n = 44); 4) Broward County and St. Lucie County, east Florida,

USA (n = 15); and 5) Key Island (Collier County), west Florida, USA (n = 14). Cumberland Island samples represented either alpha (n = 12) or beta (n = 32) turtles, corresponding to (untagged) new arrivals or (previously tagged) remigrants respectively, to test for evidence that new arrivals may include strays from other nesting populations.

The sampling strategy was designed to minimize impact on natural populations. The high natural mortality of eggs and hatchlings (estimated at 99.8% prior to maturity-- Frazer 1986) made this life history stage the most appropriate period for collection. Each nest was sampled for two eggs or one hatchling, and precautions were taken not to resample subsequent nests from the same female. Two eggs were taken to offset mortality during transportation, as loggerhead eggs are very sensitive to motion during the first few weeks of development (Limpus et al. 1979). Eggs were incubated for six to eight weeks prior to processing. Hatchlings were euthanized at appropriate lab facilities. Since siblings are expected to be identical with respect to mtDNA genotype, sample sizes refer to the number of nests sampled.

Closed-circular mtDNA was isolated from soft tissues (hatchlings) or whole embryos (eggs) by CsCl-ethidium bromide density gradient centrifugation (Lansman et al. 1981). Purified mtDNAs were digested with the 17 informative four-, five- and six-base cutting restriction enzymes listed in Table 1. In addition, representative samples were digested with *Bam*HI, *Cla*I, *Eco*RI, *Kpn*I, *Nsi*I, *Sac*I, *Sal*I, and *Sma*I, but these enzymes proved to be phylogenetically uninformative, producing either one or no cuts in

loggerhead samples. Digestion fragments were end-labelled with ^{35}S nucleotides and separated on 1.0-1.7% agarose gels. Restriction fragments were visualized by autoradiography and assigned molecular weights on the basis of comparison to a 1-kb ladder standard.

Estimates of nucleotide sequence divergence (*p* values) were calculated by the "site" approach of Nei & Li (1979), and haplotype and nucleotide diversities were estimated by the methods described by Nei & Tajima (1981) and Nei & Li (1979) respectively. Restriction fragment profiles were described using composite letter codes, and were joined into a parsimony network that interrelates observed restriction fragment patterns.

Because we are interested in genetic relationships among particular pairs of nesting beaches (e.g., those that may occupy the same feeding grounds), some of the analyses described below include pairwise rookery comparisons (although results of multiple pairwise comparisons are not statistically independent). Pairs of rookeries were tested for significant differences in haplotype frequency by the *G* test with Yates' correction for small sample size (Sokal & Rohlf, 1981). Pairwise estimates of inter-rookery gene flow (*Nm*) were also calculated from G_{ST} values ($Nm = 1/2a\ (1/G_{ST} - 1)$, where $a = (L/L-1)^2$ and *L* is the number of demes-- Takahata & Palumbi 1985, Nei 1987). Calculations were conducted under assumptions of large *L* (hence a @ 1) rather than small *L* (hence a = 4), providing a more conservative estimate of population structure in the sense that the resulting *Nm* values are four-fold higher. Finally, an estimate of mean migration rate (*Nm*) among rookeries was

calculated by the private-allele method (Slatkin 1985), using the equation in Slatkin & Barton (1989).

RESULTS

Five genotypes were observed among the 113 loggerhead nests sampled (Table 1), with a mean of 85 restriction sites scored per individual. Digestion profiles are available from the senior author upon request. All restriction site changes could be explained by specifiable gains or losses of particular restriction sites.

Turtle species tend to exhibit relatively low levels of genetic variation and differentiation (Avise et al. 1992; Karl et al. 1992; but see Scribner et al. 1986), and previous studies indicate that loggerhead turtles have comparatively low genetic diversity at protein electrophoretic loci (Smith et al. 1978; Guyris & Limpus, 1988). The current study extends this qualitative conclusion to mtDNA: overall haplotypic and nucleotide diversities in surveyed loggerheads were 0.505 and 0.0018, respectively (Table 2). These estimates, and the levels of sequence divergence among all observed haplotypes (Table 3), are at the low end of the spectrum of such values reported for comparisons of conspecific vertebrates (Avise et al. 1987, 1989).

Two distinct groups of haplotypes were observed in the phylogeny of Atlantic loggerhead mtDNA (Fig. 1), and these differ at a mean level of sequence divergence $p = 0.008$. This magnitude of divergence is similar to the deepest fork observed in a global phylogeny for green turtle mtDNA ($p = 0.007$), which proved to partition Atlantic-Mediterranean from Indian-Pacific phylads (Bowen et al. 1992). In contrast, both major groupings in the

loggerhead mtDNA phylogeny occur within the Atlantic Ocean and overlap on Florida nesting beaches.

On the basis of a mtDNA clock calibration derived from other marine turtle species (0.2-0.4% divergence between lineages per million years-- Bowen et al. 1991, 1992; Avise et al. 1992), these loggerhead mtDNA genotypes may have been separated for approximately 2-4 MY. The presence of such divergent haplotypes in one ocean basin may reflect maintenance of distinct mtDNA lineages within the Atlantic basin over evolutionary timescales. Alternatively, this co-occurence of divergent genotypes may result from recent contact between geographically isolated lineages, perhaps the product of gene flow between Indian and Atlantic Ocean Basins.

To some extent, the low level of genetic variation observed in this study impairs our ability to resolve regional population issues. Rookery samples are dominated by two mtDNA genotypes (B and D-- see Table 1). Nevertheless, the distribution of these genotypes has a strong geographic component. Genotype D, observed in 67% of eastern Florida samples (n = 15) and 64% of western Florida samples (n = 14), is completely absent from the Georgia (n = 44) and South Carolina (n = 19) collections. Genotype frequencies differed significantly in eight of ten pair-wise rookery comparisons (Table 4). Notably, the cases where genotype frequencies are not significantly different involve proximal nesting beaches: 1) east Florida and west Florida; and 2) Georgia and South Carolina. We conclude that loggerhead rookeries of the southeastern U.S. comprise at least two genetic populations, between which contemporary gene

flow is low (Table 4). Further genetic analyses, possibly including direct sequencing of the mtDNA control region, may reveal additional population subdivision.

The Mediterranean sample of loggerheads was fixed for a mtDNA genotype (clone D, Table 1) that was observed at 64-67% frequency in Florida samples and absent in Georgia and South Carolina. Pairwise comparisons of the Mediterranean versus West Atlantic rookeries produced significant *G* values, and estimates of inter-rookery migration are low, ranging from $Nm = 0.0$ to $Nm = 1.3$ migrants per generation (Table 4). Thus the Mediterranean rookery differs significantly in genetic composition from West Atlantic nesting beaches, including those in Florida.

In general, values of *Nm* greater than about 1-4 indicate that gene flow is sufficient to maintain a relatively homogeneous gene pool, whereas lower values indicate that gene flow is not sufficient to prevent dramatic divergence of isolated gene pools by genetic drift (Slatkin 1987; Birky et al. 1983). However, several caveats concerning *Nm* estimates merit consideration. First, the estimates generated here are from a single gene genealogy. More accurate estimates of gene flow would be expected from an analysis of many independent loci (although they would not then apply strictly to female lineages that are of special interest here). Second, some of the rookeries surveyed may be the product of recent colonization events (see below), such that assumptions of population equilibrium are not met (although Slatkin & Barton [1989] suggest that their estimates of *Nm* are relatively insensitive to this type of bias). Finally, the theoretical basis for these estimates is still under

development, and empirical calibrations are currently unavailable. For these reasons, *Nm* estimates should be interpreted as *general* indicators of the magnitude of genetic exchange.

DISCUSSION

The nesting beaches of the southeast United States, taken together, comprise the second largest nesting aggregate in the world, with approximately 35,000 nesting females (Murphy & Hopkins 1984). However, this area is subdivided into several discontinuous nesting habitats. In particular, the nesting beaches in Florida and Georgia-South Carolina are separated by several hundred kms of beach in which loggerhead nesting is effectively absent (Murphy & Hopkins-Murphy 1989). In the Mediterranean, prominent nesting aggregates are reported from Pelopponesus and Zakynthos in Greece and along the adjacent coastline of Turkey (Groombridge 1988). Notably, the loggerheads which nest at these Mediterranean locations are significantly smaller than those that nest in the west Atlantic (Margaritoulis 1982, 1988b).

In terms of mtDNA lineages, Atlantic and Mediterranean nesting populations are structured genetically, a conclusion consistent with results of a protein electrophoretic analysis of loggerhead rookeries in Queensland, Australia (Gyuris & Limpus, 1988). However, we observed more sharing of mtDNA genotypes among loggerhead rookeries than observed in green turtles, a fact reflected in higher estimates of mean migration rate based on Slatkin's (1985) private allele method: $Nm \approx 2.0$ for Atlantic loggerheads, as compared to $Nm \approx 0.3$ for Atlantic green turtles (Bowen et al. 1992). It is possible that these migration estimates reflect a higher level of dispersal in

female loggerhead turtles relative to green turtles: movement between spatially distinct green turtle rookeries is extremely rare (Meylan 1982). On the other hand, the higher migration estimate for loggerheads may be an artifact of sampling design: four out of five sampled nesting beaches are in one geographic province (the southeast United States). Furthermore, higher migration estimates may merely reflect differences in nesting habitat: loggerhead nesting beaches are typically located on continental coastlines, while many green turtle rookeries are located on oceanic islands where regional dispersal is a physical impossibility. Thus, it remains to be determined whether nesting loggerhead turtles are less site-specific than nesting green turtles.

Evolutionary History of the North Atlantic and Mediterranean rookeries

Climatic processes have undoubtedly influenced the contemporary distribution and population structure of loggerhead nesting assemblages. Loggerhead eggs require a minimum of 60 days above 25° C to successfully incubate, such that cold temperate conditions in the Mediterranean (Buckley et al. 1982; McCoy 1980) may have precluded nesting here during the most recent glacial period (18,000 to 12,000 years BP). In the West Atlantic, loggerheads possibly nested in southern Florida during glacial intervals, but almost certainly not at present-day rookery locations in Georgia, South Carolina, and North Carolina (see Hedgepeth 1954). Thus the contemporary distribution of nesting beaches in the southeast U.S. may be the product of colonization events over the last 12,000 years, as loggerhead females extended the northern

boundary of the nesting range. One consequence of this colonization process could be a progressive loss of mtDNA diversity in more northerly rookeries, as maternal lineages are filtered through a series of colonization bottlenecks. Estimates of haplotypic and genotypic diversity (Table 2) are consistent with this scenario.

In the southeast U.S., this colonization process has apparently been sufficient to extend the northern limits of nesting by 1,000 kms within the last 12,000 years (roughly 600 loggerhead generations). In the Mediterranean, habitats that were too cold to support nesting and feeding 12,000 years ago are now utilized extensively by loggerhead turtles. The presence of the "D" genotype at 100% frequency in Greek samples indicates that this colony shared a recent common ancestor with North Atlantic populations. Thus conclusions drawn from climatic history, mtDNA data, and tagging studies are concordant in indicating that loggerheads are active colonizers that can occupy newly opened habitat over relatively short evolutionary timescales. In other words, movement between loggerhead rookeries in the West Atlantic and Mediterranean has been sufficient to prevent pronounced evolutionary divergence, despite a restriction of contemporary gene flow between nesting assemblages (Table 4).

Regional population structure and population dynamics--southeast U.S.

mtDNA data indicate that nesting turtles in the southeast United States are divided into Georgia/South Carolina and Florida cohorts. Notably, many coastal marine organisms of the southeast United States show a phylogeographic discontinuity in this area (Bowen &

Avise 1990, Avise 1992), including other turtles (Lamb & Avise 1992). This conclusion also is consistent with loggerhead population subdivisions defined with environmental markers (Stoneburner et al. 1980, Caine 1986), morphology (Stoneburner 1980), and geography (nesting is effectively absent along the Atlantic coast of Florida from Jacksonville to Cape Canaveral National Seashore [Murphy & Hopkins-Murphy 1989]). However, this conclusion is difficult to reconcile with some aspects of tag-recapture data: while most nesting females return to the same beach in successive nesting seasons, a small component of tagged turtles have been observed nesting at alternate sites (Dodd 1988). Bjorndal et al. (1983) reviewed 25 cases of nesting beach relocations in the southeast region, and Lebuff (1974) reported that a female tagged on a west Florida nesting beach was observed nesting on an east coast of Florida (550 km distant) four years later. More directly relevant to this discussion, 11 tagged loggerheads were observed to nest at both Georgia and East Florida locations during the period 1978-1985 (J.I.R., unpublished data).

The significant difference in observed mtDNA haplotype frequencies between Florida and Georgia/South Carolina rookeries appears to be inconsistent with this propensity for nesting relocations, because the exchange of even one migrant per generation is sufficient in theory to maintain alleles in similar frequencies in populations at equilibrium (Slatkin 1987, but see Allendorf 1983). One possible explanation is that Georgia/South Carolina and Florida nesting populations have not reached an equilibrium condition, such that the genetic consequences of nesting

relocations may not be detectable with current sample sizes. Support for this scenario stems from: 1) climate data: the Georgia/South Carolina nesting assemblage may have a relatively recent origin, as this area was probably unsuitable for nesting 12,000 years (or about 600 loggerhead generations) BP, and 2) contemporary demographics: nesting populations have been reduced in the last several decades by mortality associated with incidental capture in the shrimp fishery (National Research Council 1990). Either of these factors could be sufficient to abrogate assumptions of population equilibrium. A related explanation is that these nesting relocations are a relatively recent phenomenon, perhaps induced by human encroachment on nesting grounds and adjacent internesting habitat. Inferential support for this scenario is provided by field observations: turtles disturbed while nesting are more likely (than undisturbed turtles) to relocate to an adjacent beach, and may use the new beach for subsequent nesting efforts (T.M. Murphy, pers. comm.).

The nesting beaches at Cumberland Island and Little Cumberland Island, Georgia have been the subject of a saturation tagging program for most of the last two decades (Richardson 1982 and unpublished data). Results of this program indicate a strong bimodal distribution of nesting frequencies: adult turtles typically make either one nesting visit (alpha turtles) or 4-6 nesting visits (beta turtles) during the reproductive season. Alpha turtles, which constitute approximately one third of the nesting females each year, are usually untagged "neophytes" that are rarely observed in subsequent nesting seasons. Beta turtles typically carry tags or tag

scars and usually return to nest at the Cumberland/Little Cumberland many times in subsequent nesting seasons.

One obvious explanation for this pattern is high mortality rate in neophyte nesters. However, this explanation is inconsistent with a typical survivorship curves for marine turtles (Iverson 1991), in which very high mortality at younger stages is offset by low mortality at reproductive stages. An alternate explanation, forwarded by Bell & Richardson (1978), is that alpha turtles may be strays from the adjacent nesting beaches in Florida. To address this issue we collected 12 Cumberland Island nest samples from female turtles classified (by J.I.R.) as alpha turtles. Eleven of these alpha samples contained genotype B (the common Georgia/South Carolina genotype), and the twelfth was the only individual in the study with genotype E (Table 1). We did not observe any putative strays with genotype D (the most common Florida genotype), as would have been expected if Florida rookeries were contributing strays to this Georgia nesting beach. Notably, the distribution of genotypes within the group of putative strays did not differ significantly from the remigrant beta sample ($G = 0.2$, N.S.). If the turtles that nest only once on Cumberland and Little Cumberland Islands are strays from other regions, they apparently are not predominantly from Florida. The phenomenon of single-nesting alpha turtles remains unexplained.

Mediterranean recruitment and natal homing

The mtDNA data indicate that regional loggerhead nesting assemblages are genetically distinct populations, but a critical test of natal homing requires information on feeding ground composition as well. If nesting populations maintain separate feeding grounds,

then genetic expectations under natal homing and social facilitation models converge because turtles are not confronted with the option of following experienced breeders to a non-natal rookery. Therefore the best genetic tests of these competing hypotheses involve nesting populations that share feeding areas.

The supposition that North Atlantic loggerheads occur on Mediterranean feeding grounds is based on three lines of evidence. First, Carr (1987) noted that more juvenile loggerheads occupy Mediterranean feeding grounds than could be generated by the Mediterranean rookeries alone (see also Argano & Baldari 1983). Second, a juvenile loggerhead tagged in the Azores was subsequently recovered in the Mediterranean (A. Bolten, pers. comm.). Third, the north Atlantic current system (believed to passively transport juvenile loggerheads) branches into the Mediterranean (Estrada et al. 1985). Groombridge (1988) and Carr (1987) speculated that surface currents and oceanic topology may trap pelagic-stage (juvenile) loggerheads in the Mediterranean Basin, and that some of these strays may remain there to breed.

If one accepts the premise that North Atlantic juveniles occupy Mediterranean feeding grounds, then mtDNA data provide a critical test of natal homing for these turtles. Under a social facilitation model, some recruitment of North Atlantic juveniles onto Mediterranean nesting beaches is expected, resulting in a sharing of common mtDNA lineages. Contrary to these expectations, samples from one of the largest Mediterranean rookery (Kiparissia Bay, Greece) contain only one (D) of the two genotypes (B and D) which dominate North Atlantic samples. In terms of mtDNA haplotype

frequencies, *G*-tests and estimates of migration (Table 4) support the conclusion that gene flow between the North Atlantic and Mediterranean is limited. We conclude that these data are inconsistent with a major role for social facilitation. If juveniles are trapped in the Mediterranean as Groombridge (1988) suggests, they apparently are not recruiting to Mediterranean rookeries at levels sufficient to impact contemporary demographics. Although our data contradict expectations of a social facilitation model, a stronger test of natal homing would include mtDNA data from feeding grounds as well as rookeries. It remains to be seen whether mtDNA genotypes common in the North Atlantic occur on Mediterranean feeding grounds.

Acquired versus inherited population markers

Stoneburner et al. (1980) reported that loggerhead egg shells contain concentrations of heavy metals that differ significantly between Georgia/South Carolina and Florida rookeries. Presumably the metals are acquired from prey items ingested by females on North Atlantic feeding grounds, where loggerheads spend the majority of their adult lives. Caine (1986) reported distinct assemblages of epibiota on Florida versus Georgia/South Carolina nesting turtles, with the latter group carrying a more temperate subset of Atlantic invertebrates. Epibiota may accumulate on feeding grounds or migratory pathways, and have been used to reconstruct migratory behavior in other marine turtle species (Eckert & Eckert, 1988). Hence the significant differences in heavy metals and epibiota between Georgia/South Carolina and Florida nesting enclaves indicate segregation on feeding grounds or

migratory routes. However, these acquired markers provide no information on the population genetic structure of nesting assemblages. In contrast, the mtDNA tags employed in the current study indicate that loggerhead populations are genetically segregated by nesting beach, but these innate markers provide no information on feeding grounds demographics or migratory pathways.

Acquired and innate markers support a concordant geographic partitioning of nesting populations into Florida versus Georgia/South Carolina units. However, these two distinct classes of markers elucidate very different aspects of loggerhead natural history. The genetic data indicate that loggerhead females tend to nest in the vicinity of their natal rookery, whereas the environmental markers suggest that the Florida and Georgia/South Carolina nesting populations also tend to segregate on feeding grounds.

With regard to the heavy metal assays (Stoneburner et al. 1980), turtles cannot be assigned to particular feeding ground locations at present; this would require an extensive survey of the heavy metal signatures of candidate locations. In contrast, the epibiota assay (Caine 1986) allows turtles to be assigned to two distinct biogeographic regions. Coral species present on the carapaces of Florida nesting loggerheads are native to the tropical Caribbean, and anemones present on the South Carolina nesters occur in the warm-temperate Sargasso Sea. The non-overlapping geographic distribution of these indicator species even permit an estimate of the degree of loggerhead mixing on feeding grounds: 4.2% of Florida nesting turtles had the "northern" epibiota, while 13.4% of turtles in

the South Carolina-Georgia nesting population had the "southern" epibiota (Caine 1986).

Finally, we consider the information content of a third class of acquired markers-- human-applied tags. If loggerhead turtles from different nesting beaches overlap on feeding grounds, as is believed to be the case for populations of other marine turtle species (Meylan 1982), then one would expect little or no concordance between environmental and genetic markers. However, in reviews of loggerhead tag recoveries, Meylan (1982) and Meylan et al. (1983) noted that Florida nesting turtles were recovered on the east coast of the United States, in the Bahamas, and elsewhere in the Caribbean. In contrast, no loggerheads tagged in Georgia have been recovered in the Caribbean, but extensive tag recoveries are reported along the east coast of the U.S. (Bell & Richardson 1978 and unpublished data). Thus, both environmentally-acquired and human-applied tags point towards some level of segregation on feeding grounds.

Inherited and acquired markers both provide valuable but distinct kinds of information on the natural histories of marine turtles. Taken together, they allow a more complete picture of loggerhead migratory behavior to emerge.

Conservation concerns and prospects for future research

The mtDNA data reported here indicate a significant population structure for loggerhead turtle rookeries in the North Atlantic Ocean and Mediterranean Sea. Contemporary female-mediated gene flow between regions is negligible (Table 4), yet all loggerhead populations are related very closely in an evolutionary sense. What

do these results imply for the management of threatened and endangered populations? Over short evolutionary timescales (perhaps a few thousand years), dispersal apparently is sufficient to allow colonization of appropriate habitats in proximity to established rookeries. However, both tag returns and mtDNA data indicate that female gene flow is too low to have a significant impact on population dynamics on a contemporary scale. Therefore, if nesting females are depleted or extirpated at one rookery, regional dispersal will not be sufficient to replenish this resource over a timescale that is meaningful to wildlife management agencies. Accordingly, nesting populations must be considered demographically independent.

Several lines of scientific investigation are suggested by the data presented here. Mitochondrial lineages are of special interest for addressing the migratory behavior and population structure of female loggerheads, but parallel questions about male dispersal remain unresolved. These issues could be addressed with nuclear DNA assays (see Karl et al. 1992). A second unresolved issue hinges on the distribution of loggerheads on North Atlantic and Mediterranean feeding grounds. We have concluded that the Mediterranean rookery at Peloponnesus is genetically distinct from West Atlantic rookeries. Are feeding areas in the Mediterranean and eastern Atlantic occupied by subadults from West Atlantic rookeries, as a few tag returns have suggested? Are West Atlantic nesting populations segregated on feeding grounds, as environmental and human-applied tags indicate? Both of these issues could be resolved with mtDNA surveys of feeding ground populations. Genetic

analyses still have much to offer in terms of life history and conservation information for marine turtles.

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TABLE 1. Description and distribution of mtDNA genotypes observed in loggerhead turtles. Italicized letters refer to mtDNA restriction fragment profiles produced by (from left to right): *Ava*II, *Bcl*I, *Bgl*I, *Bgl*II, *Bst*EII, *Bst*NI, *Dra*II, *Eco*RV, *Hind*II, *Hind*III, *Msp*I, *Nde*I, *Pvu*II, *Spe*I, *Sst*II, *Stu*I, and *Xba*I. For each enzyme adjacent letters in the alphabet indicate that fragment profiles differ by a single restriction site gain or loss; nonadjacent letters differ by at least two sites.

composite code	mtDNA genotype	rookery location	number of nests
A	*DCCCCCCCCACCCCCBC*	Georgia, U.S.A.	2 beta*
B	*DCCCCCCCCBCCCCCBC*	South Carolina, U.S.A.	19
		Georgia, U.S.A.	11 alpha*
		Georgia, U.S.A.	30 beta*
		East Florida, U.S.A.	4
		West Florida, U.S.A.	5
C	*DCCCCBCCCBCCCCCBC*	East Florida, U.S.A.	1
D	*ACCCCDCCCBBCCCCCC*	East Florida, U.S.A.	10
		West Florida, U.S.A.	9
		Zakynthos, Greece	21
E	*ACCCCDCBCBBCCCCCC*	Georgia, U.S.A.	1 alpha*

*alpha turtles indicate first-time arrivals, possibly including strays from other rookeries. Beta denotes turtles known to have nested in Georgia more than once-- see Bell and Richardson (1978) and text for explanation.

TABLE 2. Loggerhead turtle haplotype and nucleotide diversities (Nei and Li, 1979), overall, and within each nesting beach sample.

	Haplotype Diversity (h)	Nucleotide Diversity (π)
Greece	0.000	0.0000
South Carolina	0.000	0.0000
Georgia	0.132	0.0002
East Florida	0.514	0.0018
West Florida	0.495	0.0018
Overall	0.505	0.0018

Table 3. Estimates of sequence divergence between the mtDNA clones described in Table 1, based on the site approach of Nei and Li (1979).

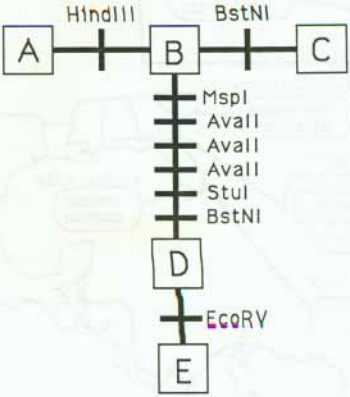
mtDNA clone	A	B	C	D	E
A	-	0.0010	0.0023	0.0089	0.0100
B		-	0.0012	0.0078	0.0089
C			-	0.0092	0.0103
D				-	0.0010
E					-

FIGURE LEGENDS

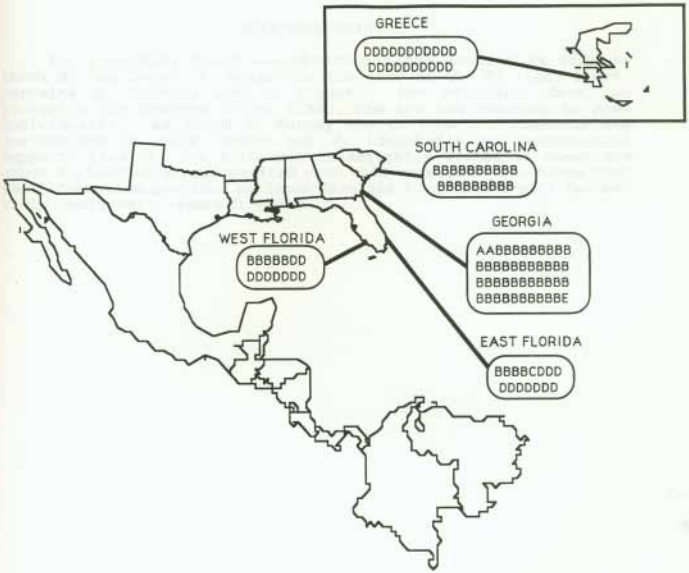
FIGURE 1. Parsimony network summarizing the interrelationships among mtDNA haplotypes observed in the survey of West Atlantic and Mediterranean loggerhead rookeries. Letters refer to the composite genotypes described in Table 1. Each dash corresponds to a single restriction site gain or loss, with the corresponding enzyme indicated above or to the left of the dash.

FIGURE 2. Collection sites and genotypes observed at each of five Atlantic and Mediterranean rookeries of the loggerhead turtle. Each letter refers to the composite mtDNA genotype of an individual turtle (Table 1).

DRAFT


HindIII
BstNI
A
B
C
MspI
AvaII
AvaII
AvaII
StuI
BstNI
D
EcoRV
E

DRAFT


GREECE
DDDDDDDDDDD
DDDDDDDDDD
SOUTH CAROLINA
BBBBBBBBBB
BBBBBBBBB
GEORGIA
AABBBBBBBBB
BBBBBBBBBBB
BBBBBBBBBBB
BBBBBBBBBBE
WEST FLORIDA
BBBBBDD
DDDDDDD
EAST FLORIDA
BBBBCDDD
DDDDDDD

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