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Historical Responses Of Marine Turtles To Global Climate Change And Juvenile Loggerhead Recruitment In Florida

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HISTORICAL RESPONSES OF MARINE TURTLES TO GLOBAL CLIMATE CHANGE AND JUVENILE LOGGERHEAD RECRUITMENT IN FLORIDA

by

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ABSTRACT

Marine turtle conservation is most successful when it is based on sound data incorporating life history, historical population stability, and gene flow among populations. This research attempts to provide that information through two studies. In chapter I, I identify historical patterns of gene flow, population sizes, and contraction/expansion during major climatic shifts. In chapter II, I reveal a life history characteristic of loggerhead turtles previously undocumented. I identify a pattern of juvenile recruitment to foraging grounds proximal to their natal nesting beach. This pattern results in a predictable recruitment pattern from juvenile foraging ground aggregations to local rookeries.

This research will provide crucial information to conservation managers by demonstrating how sensitive marine turtles are to global climate change. In the second component of my research, I demonstrate how threats posed to juvenile foraging grounds will have measurable effects on rookeries proximal to those foraging grounds. The addition of this basic life history information will have dramatic effects on marine turtle conservation in the future, and will serve as the basis for more thorough, forward-looking recovery plans.

This thesis is dedicated to my loving wife, who has been my sole source of inspiration and support during my studies.

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LIST OF ACRONYMS/ABBREVIATIONS

CITES	Convention on International Trade of Endangered Species
DNA	Deoxyribonucleic Acid
GIS	Geographic Information System
IRL	Indian River Lagoon
IUCN	World Conservation Union
MEX	Mexico
MCMC	Markov-chain Monte Carlo
mtDNA	Mitochondrial Deoxyribonucleic Acid
NCA	Nested Clade Analysis
N_{ef}	Effective Population Size
NEFL	Northeast Florida
NH	Natal Homing
N_m	Number of Migrants per Generation
NSF	Nest Site Fidelity
NWFL	Northwest Florida
SEFL	Southeast Florida
SWFL	Southwest Florida
USFWS	United States Fish and Wildlife Service

CHAPTER ONE: INTRODUCTION

Marine turtles as a group are globally threatened with decline and extirpation. Loggerhead turtles (*Caretta caretta*) were listed as threatened by the United States Fish and Wildlife Service (USFWS) in 1978. The green turtle (*Chelonia mydas*) is listed as endangered by the USFWS and globally threatened by the IUCN Redlist. Hawksbills (*Eretmochelys imbricata*) are listed as endangered by the USFWS and as globally critically endangered by the IUCN. Global conservation efforts to recover these species have been impeded by their complicated migratory pathways and enigmatic dispersal patterns. Molecular genetic studies have greatly improved these conservation efforts by revealing intricate population structure, gene flow among rookeries, and the ties between juvenile aggregations and their contributing rookeries.

Despite tremendous advances in molecular phylogeographic studies of historical population genetic structure, the marine turtle conservation movement has in many cases failed to incorporate these tools. My research directly targets two life history characteristics that are integral in understanding and conserving marine turtles in the future.

My research first targets discerning patterns of historical population structure in three species of marine turtles in the face of global climate change. The dramatic changes in climate and sea levels during the Pleistocene affected marine turtle populations in discernable patterns of modified gene flow and source-sink rookery populations. Understanding the historical responses in a mosaic of rookeries will have predictive value on future conservation efforts aimed at maximizing survival capabilities during future climate changes. I examined previously published data on the population structure of a combined total of 36 loggerhead, hawksbill, and

green turtle rookeries. I used coalescence and nested clade analyses to discern historical patterns of population expansion and contraction, sensitivity to climatic shifts, gene flow between major rookeries, and historical population sizes.

In addition to discerning historical patterns of gene flow and population growth/decline, it is important from a conservation standpoint that researchers also understand general life history characteristics which dictate population dynamics. Current conservation efforts are based on limited data describing the relationship between nesting beach rookeries and coastal foraging grounds. My research directly addresses determining the factors which dictate the recruitment of juvenile loggerheads from a nesting beach to a foraging ground. My research strongly indicates that threats posed to juvenile foraging ground aggregations primarily affect rookeries proximal to those foraging grounds. My findings suggest that recruitment to foraging grounds is predictable and based on the distance from the rookery and the rookery size. Inherent in this pattern is the fact that juvenile loggerheads make a second major migration before the well-established reproductive migration. Although this hypothesis has been supported by recently published data (Bowen et al. 2004), it was entirely unknown prior to my research.

CHAPTER TWO: HISTORICAL PERSPECTIVES ON POPULATION GENETICS AND CONSERVATION OF THREE MARINE TURTLE SPECIES

Introduction

Population genetics and phylogeography of natural populations are intimately tied to life-history, which for marine turtles is marked by long maturation time, enigmatic dispersal characteristics, and isolated nesting habitats. The unique biology of marine turtles includes a complex life history characterized by multiple distinct phases, each with unique habitat and geographic range associations. Although marine turtle species share many life-history characteristics, patterns of genetic diversity vary among species based on life-history strategies and historical population processes (Bowen *et al*. 1994, Bass *et al*. 1996, Encalada *et al*. 1996). Here I reexamine and compare published data on three species of marine turtles that use Western Atlantic and Mediterranean beaches as nesting grounds, the loggerhead (*Caretta caretta*), hawksbill (*Eretmochelys imbricata*), and green (*Chelonia mydas*) sea turtles. As threatened or endangered species within and outside of the United States, these species have been subjects of increased conservation efforts over the past 20 years.

The wide geographic distribution of marine turtles over their lifetime contrasts with the discrete subset of that total range over which reproductive events are localized. Marine turtle migratory behavior often consists of vast journeys spanning entire ocean basins (Carr 1978, Witzell & Banner 1980, Parmenter 1983, Mortimer & Carr 1987, Limpus *et al*. 1992, Bolten *et al*. 1998). Despite these tendencies for massive migratory events and the potential for mixing of populations, each of these three species generally displays high levels of mitochondrial genetic

separation between nesting aggregations. Two key life-history traits have led to this separation, natal homing and nest site fidelity. Natal homing refers to the propensity for mature females to return to the nesting beach of their natal origin for deposition of eggs. Nest site fidelity refers to the subsequent return, year after year, to the same beach for nesting. The combination of these traits results in the localization of the same female genetic stock on the same beach, generation after generation. Here, I refer to natal homing (NH) and nest site fidelity (NSF) as a complex of forces (NH/NSF) that may affect the dispersal patterns of marine turtle matrilines.

Natal homing and nest site fidelity (NH/NSF) have been demonstrated to some degree for each of the species in question. Florida loggerheads have been observed to exhibit NH/NSF on a scale of approximately a hundred kilometers (B. Bowen, pers. comm.). Green turtles in Florida display a higher degree of NH/NSF, often within 10 kilometers of their previous nest deposition (Carr *et al*. 1978, Balaz 1980, Limpus *et al*. 1992). High levels of NH/NSF in hawksbills have been demonstrated in the Caribbean (Bass *et al*. 1996); however, movement between adjacent beaches or islands has been reported in the Indian and Pacific Oceans (Diamond 1976, Limpus *et al*. 1983).

In addition to differences in migratory and nesting behavior across species, marine turtles occupy unique ecological niches. Loggerheads nest primarily in warm temperate regions (Pritchard & Trebbau 1984), whereas green turtles and hawksbills nest primarily in the tropics (Bowen *et al*. 1992, Bass *et al*. 1996). Efforts to conserve marine turtle species require consideration of these complex life-history traits, along with an understanding of how the population biology of these species has shaped genetic population structure through time, and will continue to do so in the future.

I employ an array of statistical estimates of among-population geneflow and historical population processes, including nested clade analysis (NCA; Templeton 1998), to address large-scale patterns of population history and genetic structure in three marine turtle species. Traditional interpretations of NCA need to be altered slightly in the case of marine turtles because reproduction is confined to only a small subset of their otherwise wide overall ranges. I employ NCA to infer processes centered on the geography of the reproductive portion of marine turtle populations (i.e. nesting beaches), which drastically contrasts with inferences regarding their overall distribution. Inferences of population fragmentation must be modified from the traditional interpretation derived from terrestrial systems, because in the case of marine turtles, fragmentation of nesting colonies occurs when NSF/NH sufficiently constrains geneflow between nesting populations. In addition to NCA, I employ neutrality test statistics, analyses of relationships between geographic distance and population genetic differentiation, and observed distributions of pairwise nucleotide differences to infer historical patterns of population dynamics and differentiation. I compare estimates of historical patterns of population contraction and effective population size to integrate inferences of historical demography with phylogeography. Overall, my goal was to exploit the overlap in inference capability across statistical methods to formulate broad cross-validated hypotheses for historical patterns and processes, decreasing the biases or shortcomings of any single analytical method (e.g., Knowles & Maddison 2002, Masta *et al*. 2003). I believe that such robust historical perspectives represent a useful resource for constructing long term goals for marine turtle conservation, given the insight they provide on historical and ongoing patterns of geneflow, and their predictive potential under scenarios of habitat loss, population extirpation, and global climatic change.

Methods

In this study, I analyzed previously published data on the same portion of the mitochondrial control region (~400 bp) in three marine turtle species. Loggerhead turtles (*Caretta caretta*) were evaluated using data from Pearce (2001) and Laruent *et al*. (1998). This combined dataset includes 417 individuals from 20 populations in Florida, Mexico, Brazil, and the Mediterranean. Hawksbill turtles (*Eretmochelys imbricata*) were evaluated using data from Bass *et al*. (1996) including 93 individuals from seven populations in the Caribbean and Brazil (excluding hawksbill/loggerhead hybrids). Green turtle (*Chelonia mydas*) populations were evaluated using data from Encalada *et al*. (1996) including 147 individuals from nine populations on the east and west coasts of the Atlantic Ocean and the Mediterranean.

Haplotype diversity (h; Nei 1987), nucleotide diversity (π; Nei 1987), and the average number of nucleotide differences (k; Tajima 1983) for each species and per population were estimated in DnaSP v4.0 (Rozas *et al*. 2003). The number of migrants (N_m) was estimated for each species (according to Nei 1973) and F_{st} values between populations were estimated (based on Hudson *et al*. 1992) in DnaSP. Geographic distance and F_{st} values between populations were plotted to investigate correspondence between geographic and genetic distance across populations. Linear regressions were fit to F_{st} vs. geographic distance plots for groups of populations for each species to estimate correlation statistics. The overall significance of correlations between F_{st} and geographic distance matrices (per species) was tested using one-tailed mantel tests with 10,000 permutation replicates implemented using the Poptools v2.5.9 (Hood 2003) software supplement in MS Excel. To test the hypothesis that control region variation per species does not differ from neutral expectations, Fu's *Fs* (Fu 1997) and Tajima's *D* (Tajima 1989) tests of neutrality were conducted in DnaSP.

I calculated the effective female population sizes (N_{ef}) for each species from the formula theta (θ) = $2N_{ef}\nu$ (Tajima 1993). Theta (per DNA sequence) was estimated from the infinite-site equilibrium relationship between the number of segregating sites and the sample size (Watterson 1975), implemented in DnaSP. To solve this equation I estimated ν (= sequence length [m] x the mutation rate per generation [μ]). I used an approximation of the generation time at 30 years for each species (L. Ehrhart, P. Pritchard, pers. comm.), and the mutation rate estimated by Encalada *et al*. (1996) for the control region of green turtles at ~2% per million years. Pairwise haplotype mismatch distributions (Rogers & Harpending 1992, Rogers 1995) under a model of constant population size and a model of population growth/decline were performed in DnaSP to identify patterns of historical population expansion or contraction. Based on models of population growth/decline, estimates of the time since the last major population contraction were calculated from tao (τ). The number of years since population contraction was estimated from the equation $\tau = 2\mu t$, where t is the number of generations since population contraction. The N_{ef} prior to population contraction was estimated from θ initial (θi), following the calculations for N_{ef} above.

Evolutionary relationships among haplotypes within species were inferred by constructing 95% plausible parsimony networks in TCS v1.13 (Clement *et al*. 2000) based on statistical parsimony (Templeton 1998). For these analyses, gaps in alignment were treated as a 5th character, multiple base gaps were coded as a single character/event, and cladograms were estimated for each species independently. Statistical parsimony networks estimated in TCS were used to construct the nesting design for NCA analyses (Templeton 1998). Nested cladograms were constructed by hand based on a haplotype network following the guidelines of Templeton *et al*. (1992) and Templeton (1998). Since all species analyzed are marine, I used GIS software (ArcView v3.2, ESRI) to estimate the shortest over-water (oceanic) distance between all sampled

sites and used these distances to construct a distance matrix between sites for each species (e.g., Fetzner *et al*. 2003). The nested clade design was used along with geographic location, designated by the constructed distance matrix, to analyze geographic associations among hierarchically nested clades using the program GeoDis v2.0 (Posada *et al*. 2000). Statistical significance was calculated by comparison with a null distribution generated from 10,000 random permutations of clades against sampling localities. The results of GeoDis analyses were interpreted based on the revised inference key provided by Templeton (2004).

Results

Loggerheads

Ten mitochondrial control region polymorphisms were documented on Florida, Mexico, Brazil, and Mediterranean beaches. Overall haplotype diversity, although moderately high (h = 0.5867), was the lowest observed across the three species (Table 1). Overall nucleotide diversity (π = 0.027) and the average number of nucleotide differences (k = 7.891) were the highest among the three species. These relative trends in h, π, and k were observed across a majority of eastern Atlantic populations, yet contrasted with trends of low h and π in Mediterranean populations. The estimated N_m across populations of loggerheads was the highest among species (N_m = 0.76; Table 1). Neutrality statistics rejected neutral evolution (D = 4.015, P < 0.01; Fs = 22.548, P < 0.01; Table 1), suggesting that loggerhead control region haplotypes defy neutral patterns.

The estimate of θ from the number of segregating sites was θ = 2.9386 (Table 1). This provided an estimate of the current N_{ef} for loggerheads at 64,106 (Table 1). The distribution of the pairwise nucleotide differences across loggerhead haplotypes was bimodal and independent

	Parameter	Loggerhead *Caretta caretta*	Hawksbill *Eretmochelys imbricata*	Green *Chelonia mydas*
	Number of Sequences	417	93	147
	Number of Sites	391	382	496
	Segregating Sites (s)	20	16	18
	Unique Haplotypes	7	17	17
	Haplotype Diversity (h)	0.587	0.823	0.830
	Nucleotide Diversity [per site] (π)	0.021	0.010	0.010
	Average Number of Nucleotide Differences (k)	7.891	3.745	4.700
	N_m	0.76	0.53	0.45
Neutrality Tests	Tajima's D	4.01499*	0.74663	1.03341
	Fu's Fs	22.548*	-2.248	0.106
Current N_{ef} Estimates	Theta [per seq.] (θ)	3.026	2.939	3.235
	Current N_{ef}	66,185	64,106	54,351
Historical Inferences	Theta initial [per seq.] (θi)	7.848	2.297	2.908
	Tao (τ)	4.341	1.427	1.793
	Time since population contraction (years before present)	2,848,425	933,901	903,730
	N_{ef} prior to population contraction	171,654	50,109	48,858

Table 1. Summary of population genetic statistics for all three species sampled . (* = significant at P<0.001).

peaks were well-differentiated (Figure 1a). This observed distribution differed substantially from null models assuming either constant population size or population growth/decline primarily because these models are unimodal, whereas my data were bimodal (Figure 1a). Based on a model of population growth/decline, a population contraction was inferred to have taken place ca. 2.8 million years ago, prior to which, the N_{ef} was inferred at 171,654 (Table 1).

The haplotype network construction in TCS identified haplotypes B and D as ancestral haplotypes (Figure 2) based on root probability density criterion (Templeton 1998). The two ancestral loggerhead haplotypes differed by 16 mutational steps (excluding multiple base gaps). Restricted geneflow and dispersal, possibly with limited long-distance dispersal, was inferred (clade 1-1) among all Florida and Mediterranean populations sampled, except Amelia Island (Table 2). Continuous range expansion was inferred for clade 2-1 between Brazil and all Florida sites (Table 2).

Pairwise geographic distances between populations plotted against pairwise F_{st} distances between populations showed a moderate overall trend suggesting isolation by distance across populations evidenced by positive correlation between F_{st} and geographic distances (overall r = 0.443). The mantel test comparing the geographic distance and F_{st} matrices indicated that F_{st} and geographic distances between populations were correlated ($P < 0.05$).

Hawksbills

A majority of hawksbill control region polymorphisms (15 of 17) were unique to individual nesting colonies (as reported by Bass *et al*. 1996). Haplotype diversity was high in hawksbills (h = 0.823) although overall nucleotide diversity (π = 0.010) and the average number of nucleotide differences (k = 3.745) were comparatively low (Table 1). These trends were consistent across populations of hawksbills. The estimated N_m across populations of hawksbills

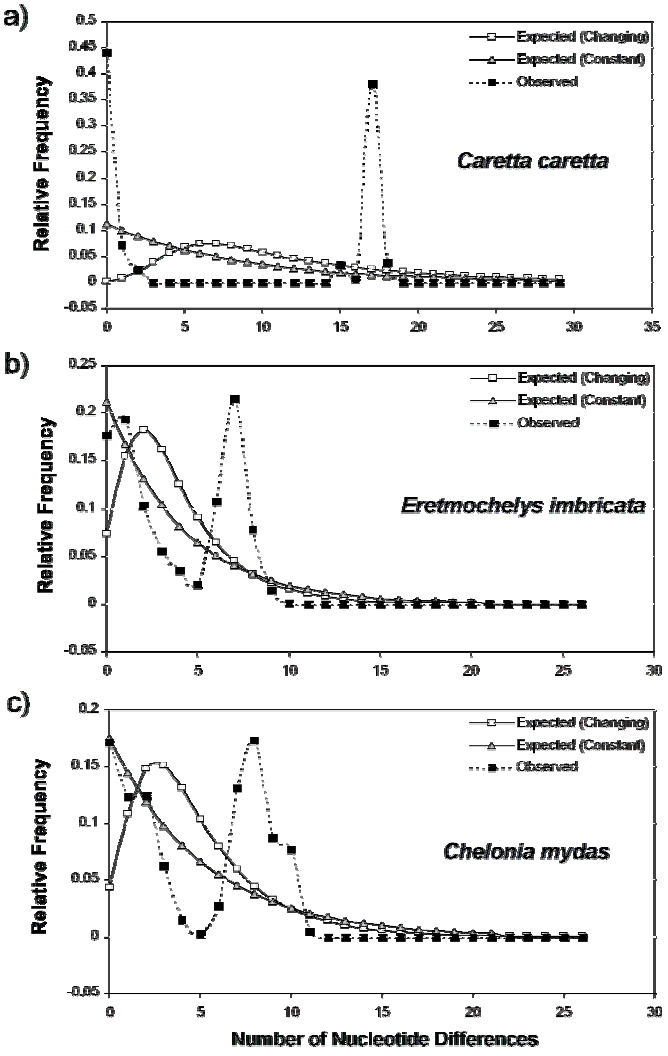

Figure 1. Pairwise nucleotide differences. Distribution of observed frequencies of pairwise nucleotide differences, with frequencies expected under models of constant population size and models of population growth/decline for each species of marine turtle sampled. (a) Distribution of pairwise differences for loggerheads (*Caretta caretta*). Expected frequencies generated with θi = 2.297, θ final = infinite for constant population size model, and θ final = 1000 for growth/decline model. (b) Distribution of pairwise differences for hawksbills (*Eretmochelys imbricata*). Expected frequencies generated with θi = 2.908, θ final = infinite for constant population size model, and θ final = 1000 for growth/decline model. (c) Distribution of pairwise differences for green turtles (*Chelonia mydas*). Expected frequencies generated with θi = 7.848, θ final = infinite for constant population size model, and θ final = 1000 for growth/decline model.

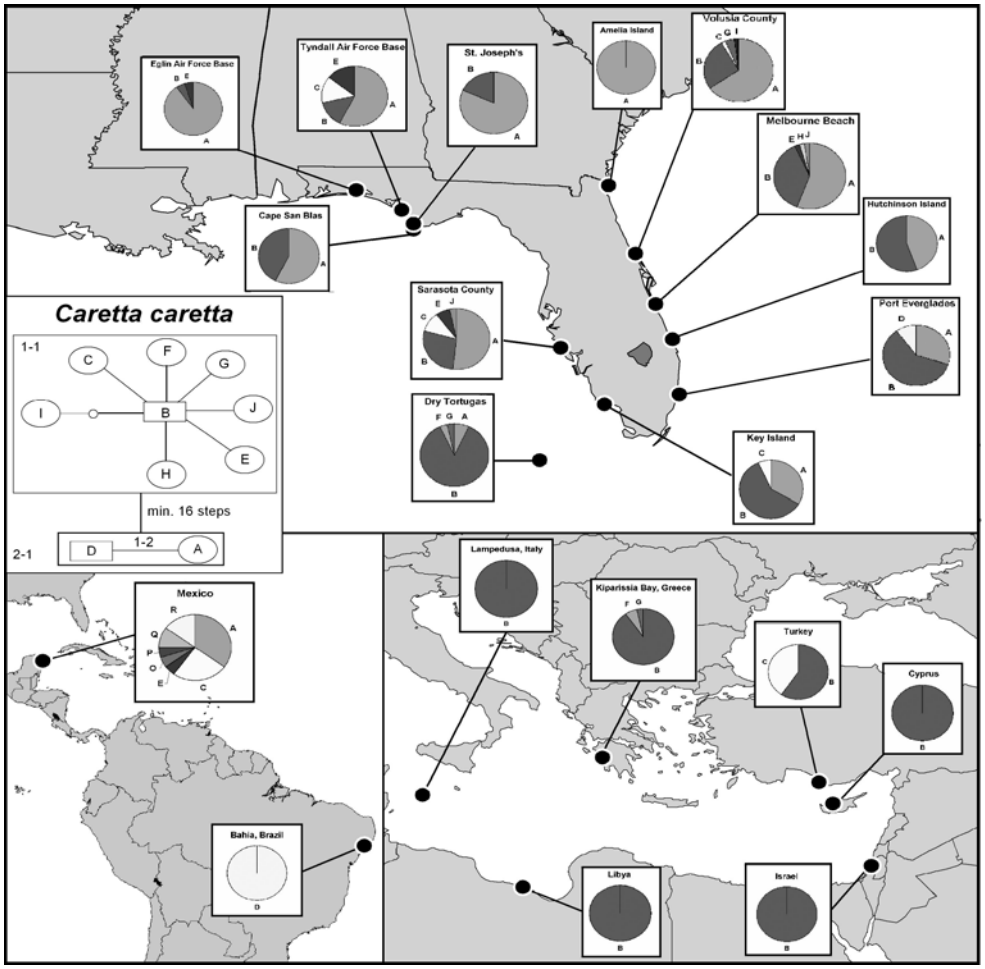

Figure 2. Sampled loggerhead nesting populations. Haplotype frequencies per nesting population are shown in pie charts. The nested cladogram for loggerheads is also given (ovals represent sampled haplotypes, small circles represent inferred haplotypes not sampled, squares represent haplotypes inferred as ancestral by TCS).

Nested clade	Chain of inference	Final inference
Loggerheads		
1-1	1-2-3-5-6-7	Restricted geneflow/dispersal but with some long-distance dispersal
1-2	1-2-11-17	Inconclusive outcome
2-1	1-2-11-12	Continuous range expansion
Hawksbills with unresolved loops		
1-3	1-2-11-17-4	Restricted geneflow with isolation by distance
1-4	1-2-11-17-4	Restricted geneflow with isolation by distance
2-1	1-2-11-17-4-9	Allopatric fragmentation
2-2	1-2-11-17-4	Restricted geneflow with isolation by distance
2-3	1-19	Allopatric fragmentation
3-1	1-2-11-12	Continuous range expansion
Hawksbills with estimated resolved loops		
1-1	1-2-3-4	Restricted geneflow with isolation by distance
1-4	1-2-3-5-6-7-8	Restricted geneflow/dispersal, but with some long-distance dispersal; or past geneflow followed by extinction of intermediate populations
2-1	1-2-11-17-4-9	Allopatric fragmentation
2-2	1-2-11-17-4	Restricted geneflow with isolation by distance
2-3	1-19	Allopatric fragmentation
3-1	1-2-11-12	Continuous range expansion
Green Turtles		
1-3	1-2-3-5-6-13	Long-distance colonization with subsequent fragmentation
1-7	1-2-11-12	Continuous range expansion
2-1	1-2-3-5-15-21	Long-distance colonization, possibly associated with fragmentation event
2-2	1-2-11-12	Continuous range expansion
3-1	1-2-11-17-4-9	Allopatric fragmentation

Table 2. Results from nested clade analysis for loggerhead, hawksbill, and green turtle haplotype data. Inferences based on Templeton 2004.

was intermediate among the three species (N_m = 0.53, Table 1). Neutrality statistics did not rejected neutral patterns of evolution for hawksbill haplotypes (Table 1).

The estimate of theta (θ = 2.9386; Table 1) was the lowest among species, yielding an estimate of N_{ef} for hawksbills at 54,351. The distribution of the pairwise number of nucleotide differences was bimodal and independent peaks were moderately differentiated. This observed distribution differed substantially from null models assuming either constant population size or population growth/decline (Figure 1b). Based on a model of population growth/decline, a population contraction was inferred ca. 900,000 years ago, prior to which, the N_{ef} was inferred at 50,109 (Table 1).

The nested cladogram for hawksbills included one 3-step clade, three 2-step clades, and seven 1-step clades (Figure 3). The central haplotype (Q) was found only in Mexico. Two homoplacious loops were inferred between clades 1-1 and 1-3, and between 1-3 and 1-4. Alternative resolutions of these loops were analyzed via NCA to determine their potential effects on historical inferences. In all cases, the inferences of the two step clades were the same; with the exception that clade 2-2 (in one alternative) resulted in long-distance colonization rather than restricted geneflow with isolation by distance (Table 2). The majority of loop resolutions resulted in restricted geneflow with isolation by distance for clade 2-2 (Table 2). In the resolved nesting scheme, I find evidence for restricted gene flow with isolation by distance in the majority of one and two step clades (Table 2). I also find evidence for restricted geneflow/dispersal or past geneflow followed by the extinction of intermediate populations for haplotypes in Mexico, Puerto Rico, and Belize (clade 1-4). Overall, past fragmentation was inferred for Caribbean populations, with evidence for subsequent long distance colonization and range expansion in the total cladogram including all clades.

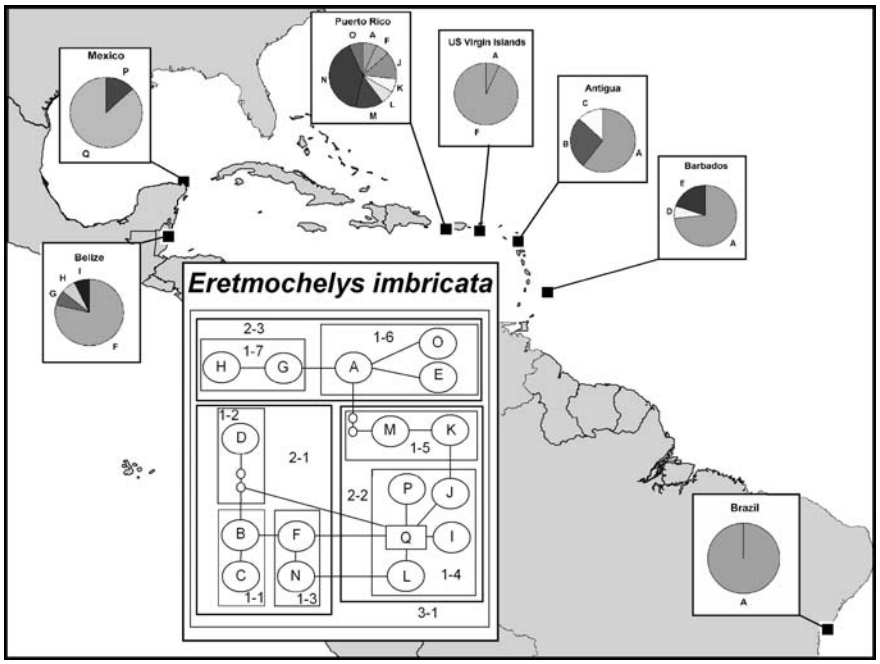

Figure 3. Sampled hawskbill nestintg populations. Haplotype frequencies per nesting population are shown in pie charts. The nested cladogram for hawksbills is also given (ovals represent sampled haplotypes, small circles represent inferred haplotypes not sampled, squares represent haplotypes inferred as ancestral by TCS).

Pairwise geographic distances between populations plotted against pairwise F_{st} distances between populations showed a moderate trend indicating isolation by distance ($r = 0.419$). The mantel test comparing geographic distance and F_{st} matrices did not indicate a significant difference between matched and randomized values ($P > 0.05$). Subdividing populations by major region resulted in a weak correlation between F_{st} and geographic distance across Caribbean populations ($r = 0.150$) and a moderately strong correlation between Brazil vs. Caribbean populations ($r = 0.708$).

Green Turtles

Similar to hawksbills, green turtle haplotype diversity was high ($h = 0.830$; Table 1) and nucleotide diversity and the average number of nucleotide differences was comparatively low ($\pi = 0.010$, $k = 4.700$; Table 1). Haplotype and nucleotide diversity varied considerably across populations of green turtles. Mexican and Brazilian populations had the highest h while populations in Guinea Bissau, Cyprus, and Costa Rica had the lowest h. The estimated N_m across populations of green turtles was the lowest among species ($N_m = 0.45$; Table 1). Neutrality statistics did not rejected neutral evolution for green turtle haplotypes (Table 1).

The estimate of theta was $\theta = 3.235$, yielding an estimate of the N_{ef} for green turtles at 54,351 (Table 1). The distribution of the pairwise number of nucleotide differences was bimodal and independent peaks were not completely differentiated. This observed distribution did not correspond well with distributions from null models assuming either constant population size or population growth/decline (Figure 1c). Based on a model of population growth/decline, a population contraction was inferred to have taken place ca. 900,000 years ago, prior to which, the N_{ef} was inferred at 48,858 (Table 1).

The nested cladogram included 18 unique haplotypes and 5 inferred unsampled haplotypes, nested as one 3-step clade, two 2-step clades, and seven 1-step clades (Figure 4). Allopatric fragmentation was inferred for the total cladogram (Table 2). Long distance colonization, possibly associated with a fragmentation event, was inferred for clade 2-1 including haplotypes from Surinam, Aves Island, Mexico, Brazil, Ascension Island, and Guinea Bissau. Long-distance colonization and subsequent fragmentation of populations in Brazil, Ascension Island, and Guinea Bissau was inferred for clade 1-3 (Table 2). Continuous range expansion was inferred for both nested clades that included Caribbean and Mediterranean haplotypes (1-7 and 2-2; Table 2).

Pairwise geographic distances between populations plotted against pairwise F_{st} distances between all populations showed a moderately correlated positive trend suggesting isolation by distance ($r = 0.546$). The mantel test comparing geographic distance and F_{st} matrices did not show significant correlation between matched values and randomized values ($P > 0.05$).

Discussion

Loggerheads (*Caretta caretta*)

Among the three species in this study, loggerheads were characterized by relatively low endemism, depressed genetic structure, and elevated levels of haplotype panmixia and geneflow between populations (Table 1). These data are consistent with the hypothesis that loggerheads have the lowest levels of NH/NSF among the three species studied. The relationship between F_{st} and geographic distance between populations was significantly non-random (based on a mantel test), although these were only moderately correlated ($r = 0.443$), implicating distance as a

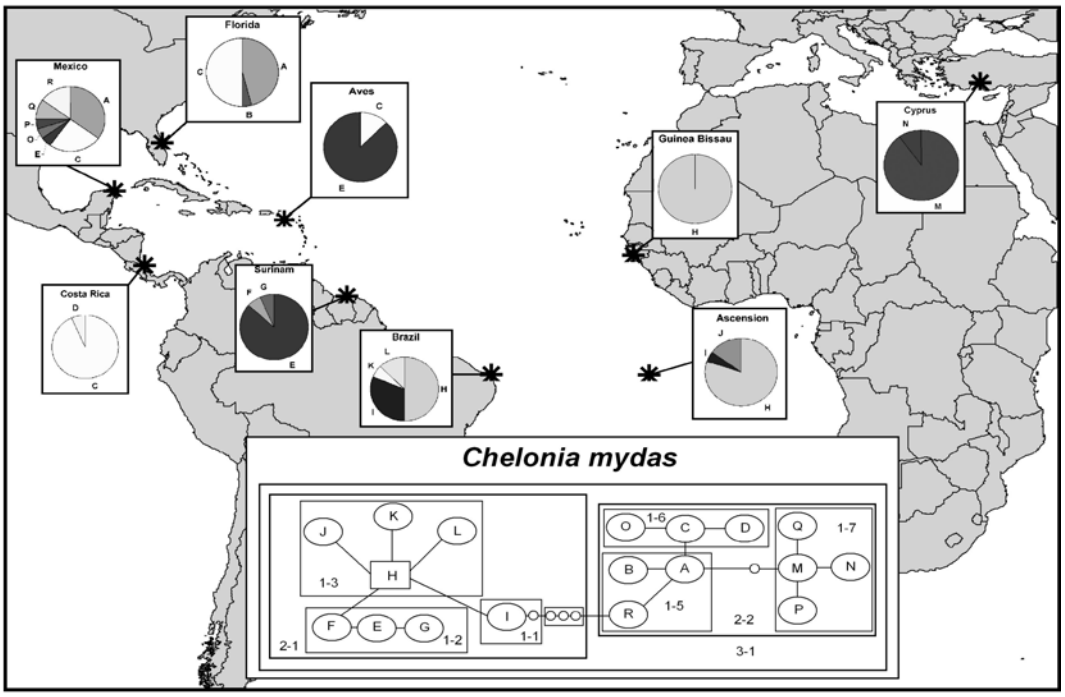

Figure 4. Sampled green turtle nesting populations. Haplotype frequencies per nesting population are shown in pie charts. The nested cladogram for green turtles is also given (ovals represent sampled haplotypes, small circles represent inferred haplotypes not sampled, squares represent haplotypes inferred as ancestral by TCS).

contributing factor determining relationships and geneflow among populations. Evidence for similar patterns was observed in NCA analyses (clade 1-1).

Results of my analyses on loggerheads in the Mediterranean and Atlantic suggest a metapopulation scenario with strong source-sink relationships among distant populations. Historical periods of climatic depression are thought to have shifted the range of suitable nesting habitat towards the equator, while simultaneously affecting dispersal patterns via changes in the geography of coastlines resulting from sea-level fluctuations (Nairn & Stehli 1986). Loggerheads require sand temperatures of at least 25 degrees (C) to successfully nest (Dodd 1988). Climatic depression at the Pliocene-Pleistocene border would likely have confined loggerhead nesting to southern Florida, the Caribbean, and near-equatorial regions. Thus, northern portions of Florida may not have been continuously suitable for loggerhead nesting until after the last glacial period (Hedgpeth 1954, Pearce 2001). Moreover, Pleistocene fluctuations in sea level have resulted in a tremendously dynamic Florida coastline over the last two million years (Nairn & Stehli 1986, Webb 1990). Therefore, over recent evolutionary time, Florida loggerhead populations have been continually displaced as the coastline has cyclically changed. Additionally, Mediterranean nesting grounds would have been unsuitably cold during much of the Pleistocene to harbor nesting populations of loggerheads (Bowen *et al*. 1994, Encalada *et al*. 1996).

Restricted geneflow with some long-distance dispersal, inferred for clade 1-1 (Figure 2), suggests populations in Florida and Mexico may have been sources for colonization of Mediterranean populations after that area again became suitable for nesting (as suggested by Bowen *et al*. 1993 and Encalada *et al*. 1998). The relationship between F_{st} and geographic distance between populations yielded moderately strong correlations only in comparisons of Mexican vs. other Caribbean and Florida populations (r = 0.744). Collectively these data support

the hypothesis that more tropical nesting grounds, including Mexico/southern Florida may have acted as Pleistocene refugia for Western Atlantic loggerhead populations.

The haplotype diversity of loggerheads shows a unique pattern relative to the other species in this study, including a deep divergence between two haplotype clades (clades 2-1 and 2-2; Figure 2), yet otherwise shallow population genetic structure. These patterns are also evident in the distribution of pairwise differences (Figure 1a) in the form of well-differentiated bimodal peaks also suggesting a historical population subdivision. The significantly positive value of Tajima's *D* statistic (4.015) further supports the hypothesis of a substantial historical bottleneck (Tajima, 1993). The strongly positive, significant value of Fu's *Fs* (22.548) suggests loggerhead population expansion (Fu 1997, Aris-Brasou & Excoffier 1996) following this bottleneck. Evidence for expansion is corroborated by NCA inferences of continuous range expansion throughout Florida (clade 2-1). Collectively, loggerhead data suggest a panmictic ancient population may have become subdivided and experienced a major bottleneck, probably prior to the Pleistocene (given deep divergence between 2-step clades; Figure 2), and subsequently experienced substantial population expansion.

Unimodal models of either constant population size or population growth/decline failed to closely fit the observed frequencies of nucleotide differences among loggerhead haplotypes (Figure 1a). Therefore, I discuss demographic estimates derived from these models tentatively, verifying inferences with independent lines of evidence. Both frequency peaks in the plot of nucleotide differences across haplotypes are well differentiated and steep-sloped (Figure 1a), potentially indicative of expansion (from two sources) following a bottleneck that resulted in two population subdivisions (Slatkin & Hudson 1991, Rogers & Harpending 1992). Several authors (e.g. Aris-Brasou & Excoffier 1996, Schneider & Excoffier 1999) have shown that estimates of

tao and N_{ef} prior to bottlenecks can be made without much bias (even in poorly fitting models), whereas estimates of the present-day θ (and hence N_{ef}), are commonly biased upwards. Under a population growth/decline model, a population contraction is inferred to have taken place ca. 2.8 million years ago. This estimate falls within the Late Pliocene and is generally coincident with a number of significant historical events including: 1) the final closing of the Central American Isthmus leading to major changes in nutrient load, upwelling, and current patterns in the western Atlantic and Caribbean (Allmon *et al*. 1996, Coates & Obando 1996, Kameo 2002), 2) the initiation of glacial cycles and sea-level fluctuations that would continue through the Pleistocene (e.g., Cronin 1990, Jansen *et al*. 1988), and 3) patterns of widespread extinction of western Atlantic and Caribbean invertebrates and vertebrates (reviewed by Allmon 2001). A major historical bottleneck in loggerhead populations is consistent with historical events associated with the late Pliocene and the effects I would expect these events to have on a sub-tropical nesting marine turtle. These conclusions also support those of Bowen *et al*. (1994) that processes of rookery extinction and recolonization over time have homogenized populations and prevented accumulation of extensive mutational separation among nesting populations and extant haplotypes in loggerheads.

The loggerhead population is currently estimated at 21,831 nesting females in the Atlantic and Mediterranean (Bolten & Witherington 2003). These estimates are conservative due to inconsistent population surveys and limited data on remote nesting beaches. My estimates of the current effective population size of females (N_{ef} = 66,185; based on θ) overestimate current census population sizes.

The parameters used to estimate N_{ef} (mutation rate and generation time) are admittedly rough estimates of poorly known life history and evolutionary processes for marine turtles.

Additionally, the estimate of θ used to derive N_{ef} is biased by its dependence on nucleotide diversity. In the case of loggerheads this value of θ is inflated due to the large divergence between haplotype clades (yet depressed within-clade diversity). Despite potential sources of error, my estimates of the current N_{ef} are not unreasonably high considering the dramatic population decline estimated to have occurred over the last few centuries. The long female generation time and lifespan characteristic of marine turtles would be expected to result in a substantial lag period between the timing of a recent bottleneck and the time required for a population decline to be manifest in the genetic composition of populations (see also Zhang *et al*. 2003). Thus, I expect estimates of present-day N_{ef} to be largely insensitive to population decline occurring in recent generations, probably since the beginning of major human impacts over the last few centuries. Estimates of N_{ef} preceding an inferred Late Pliocene bottleneck and genetic estimates of the current N_{ef} (66,185), together with recent estimates of decline that I assume has not had sufficient time to affect genetic estimates of N_{ef}, suggest that loggerheads have suffered an ongoing long-term trend of population decline since the Late Pliocene.

Hawksbills (*Eretmochelys imbricata*)

A high proportion of beach-specific (endemic) haplotypes were observed among populations of hawksbills. Relative to the more temperate-nesting loggerheads, hawksbills show elevated numbers of unique haplotypes, elevated haplotype diversity, and reduced overall N_m (Table 1). These trends correspond well with elevated levels of NH/NSF reported for hawksbills relative to loggerheads. In contrast to loggerheads, hawksbill populations were highly structured, and genetic distance among haplotypes did not show a comparably deep bifurcation, leading to overall lower nucleotide diversity in hawksbills.

Inferences from NCA analyses reinforce the potential for hawksbill rookeries to be genetically isolated by distance, consistent with elevated levels of hawksbill NH/NSF. Collectively, these inferences also suggest all populations except Mexico may have experienced moderate geneflow historically, and have since become allopatrically isolated, largely due to isolation by distance.

Relative to loggerheads, nesting density of the more tropical hawksbill is more focused in the Caribbean. Pleistocene effects on climate, together with changes in sea level, represent major potential forces dictating historical patterns of geneflow among hawksbill populations. Most notably, several large, shallow shelves or platforms exist in the Caribbean, which would have drastically changed the surface geography of the basin, altering over-water connectivity and the distribution of nesting and foraging habitats. Enormous expanses of area associated with these shelves or platforms are submerged between 10 and 100m and would have formed extensive landmasses during even slight decreases in sea level during the Pleistocene (Stuart 1966, Westphal 1998). The emergence of the Nicaraguan Rise (Stuart 1966), drastically extending the NW coastline of Honduras, probably forged a broad barrier separating rookeries in northern and southern Middle America and those of the northern and southern Caribbean. The emergence of the Campeche Bank (doubling the area of the Mexican Yucatan Peninsula) and the Florida Shelf (expanding the Florida Peninsula), would have decreased the distance between Mexican and Florida rookeries, while reducing marine connectivity across the Caribbean (Stuart 1966, Nairn & Stehli 1986, Westphal 1998). As a result of increasing fragmentation of the Caribbean during the Pleistocene, hawksbill populations may have become increasingly isolated, decreasing geneflow and substantially isolating matrilines. I suggest that the formation of these land barriers

may explain the separation of Mexico from eastern Caribbean populations and the overall trend of high haplotype endemicity.

I expect that cyclic patterns of climatic and sea-level change during the Pleistocene likely resulted in corresponding population contraction and expansion, affecting population sizes and extirpation/recolonization of populations, as suggested by Encalada *et al*. (1996) for tropically nesting green turtles. These historical population dynamics are distinct from Pliocene-Pleistocene effects on loggerheads because I expect a significant portion of the nesting range of hawksbills (more equatorial populations) to have remained constant through the Late Pliocene and Pleistocene. The non-significant neutrality statistics estimated for hawksbills (Table 1) implies that they have not experienced major contractions or expansions since, perhaps, the Late Pliocene. This is consistent with the complex genetic structure observed across hawksbill populations (c.f. Bertorelle & Slatkin 1995), refuting the hypothesis of a major historical bottleneck in hawksbill populations, which would have resulted in a substantial reduction in haplotype diversity (contrary to loggerheads).

Pleistocene effects on tropical climates are incompletely resolved (e.g., Stanley & Ruddiman 1995, Hostetler & Mix 1999, Allmon 2001), although available evidence suggests that Pleistocene climatic change drastically altered climates at middle and high latitudes, while having reduced effects on low latitudes (e.g. Hostetler & Mix 1999, Liu & Herbert 2004). Also, Excoffier & Schneider (1999) have shown multiple bottlenecks and/or multiple population expansion events may obscure resolution of historical demographics from DNA sequence data (especially neutrality statistics). Therefore, despite non-significant neutrality statistics, I expect that hawksbills have undergone minor population contractions and expansions with climatic cycling during the Pleistocene. These climatic changes likely concentrated impacts on peripheral

populations/rookeries at higher latitudes, with decreased impacts on lower latitude populations where effects on sea level and population connectivity (rather than temperature) predominated.

Unimodal models of either constant population size or growth/decline did not fit the distribution of nucleotide differences for hawksbills (Figure 1b), thus inferences based on these models are tentative. The observed distribution is bimodal, implicating past population subdivision. The distinction between modes is much less dramatic than that for loggerheads, suggesting a more recent subdivision, and potentially a shorter duration of subdivision and/or a less dramatic population contraction associated with subdivision (Slatkin & Hudson 1991, Rogers & Harpending 1992, Excoffier & Schneider 1999). In contrast to loggerheads, peaks in frequency of nucleotide differences for hawksbills are left-skewed, suggesting historical population expansion following relatively minor population contractions and subdivision (Slatkin & Hudson 1991, Rogers & Harpending 1992). This interpretation is supported by the expectation that the geographic range of suitable nesting habitat for hawksbills has expanded since the last glacial period (and during historical glacial periods in general).

I estimated a historical population contraction in hawksbills, based on estimates of tao, to have taken place ca. 900,000 years ago, which is broadly consistent with expected contractions of nesting habitat during Pleistocene glacial cycles. This time estimate corresponds well with the Mid-Pleistocene transition that is associated with increased amplitude of climatic cycles, an increased impact of cycles on tropical climates, and a change in climatic cycle periocity (e.g., Raymo *et al*. 1997, Diekmann & Kuhn 2002, Liu & Herbert 2004). Alternatively, this historical estimate may represent the center of a time period over which multiple contractions and expansions took place.

The number of nesting adult hawksbills in the Caribbean and Brazil was estimated by Meylan (1999) at no more than 5,000 and 600, respectively. Meylan (1999) estimated that hawksbills have experienced an 80% reduction in population size over the last three generations and that globally there were <15,000 nesting females. My estimates of the current N_{ef} (64,106) are reconciled by those of Meylan (1999) that suggest a population of approximately 75,000 nesting females in the early 1900s. Interestingly, estimates of current N_{ef} (64,106) and N_{ef} before historical population contractions (ca. 50,000) are similar and suggest minimal net (long-term) depletion of genetic diversity resulting from Pleistocene climatic cycling.

Green Turtles (*Chelonia mydas*)

An intermediate number of green turtle haplotypes were endemic to single populations. A majority of population genetic parameters and patterns for green turtles are strikingly similar (or identical) to those observed for hawksbills (Table 1, Figure 1c). These similarities are justifiable since both species are tropical nesters and are estimated to demonstrate similar levels of NH/NSF (Bass et al. 1996, Encalada et al. 1996, Laurent et al. 1998, Pearce 2001). Estimated migration among populations of green turtles was the lowest ($N_m = 0.45$), and evidence of genetic isolation of populations by distance (based on F_{st} vs. geographic distance) was the strongest observed across species ($r = 0.546$).

The distribution of pairwise nucleotide differences across green turtles shows a bimodal pattern in which the peak size, shape, and mean frequencies are similar to hawksbills (Figures 1c). This suggests that, like hawksbills, Atlantic green turtle populations may have been historically subdivided, probably around the same time as were hawksbills. Estimates of tao from a model of population growth/decline suggested a population contraction in green turtles, as in

hawksbills, during the Mid-Pleistocene (ca. 900,000 years ago). The nested cladogram for green turtles was nested into two 2-step clades; 2-1 comprises haplotypes possibly derived from the ancestral near-equatorial haplotype H, and clade 2-2 comprises haplotypes exclusively found in the Caribbean and Mediterranean (Figure 4). The bimodality of nucleotide differences and geographic distributions of haplotypes in these 2-step clades suggest an ancient subdivision of Atlantic green turtle populations into near-equatorial and Caribbean subpopulations.

Encalada *et al*. (1996) suggested climatic depressions of the Pleistocene, accompanied by decreases in sea-level (ca. 100m), likely confined green turtles to a more narrow band of habitat straddling the equator with one refuge in the Caribbean and one in Brazil, similar to my hypothesis for hawksbills (and, in part, loggerheads). The ancestral haplotype (H) inferred by TCS for green turtles is endemic to Brazil, Ascension Island, and Guinea Bissau. Across these populations, long-distance colonization with subsequent fragmentation was inferred by NCA to explain haplotype distributions for clade 1-3 (Figure 4). Bowen *et al*. (1992) suggested that the geology of Ascension Island probably would have resulted in extirpation of this rookery during sea-level changes in the Pleistocene. Taken together, these lines of evidence suggest that Brazil may have historically acted as a significant source for populations along West Africa and adjacent islands. At a larger scale, these data also suggest that this near-equatorial metapopulation (especially Brazil and Guinea Bissau) may have acted as a Pleistocene refuge for Atlantic green turtles. Results from NCA further reinforce these conclusions through broad scale inferences of long distance colonization with subsequent fragmentation (clade 2-1) followed by continuous range expansion in the Caribbean (clade 2-2).

This bifocal (near-equatorial and Caribbean) refugia hypothesis is similar to that for hawksbills, and suggests that the distribution of these two tropically nesting species may have responded similarly to historical climatic cycles. Also, like hawksbills, the observed distribution of nucleotide differences among green turtle haplotypes was bimodal with left-skewed peaks (Figure 1c), suggesting population growth/expansion from multiple subpopulations. Considering the evidence available, I conclude that both tropical nesting species of marine turtles, hawksbills and green turtles, appear to have experienced very similar population patterns and processes over the last several million years.

Among the three species studied, historical and current population sizes of green turtles has been the most intensively studied (Seminoff *et al*. 2002). The current population in the Atlantic and Mediterranean is estimated at 79,054-83,873 nesting females, compared to 43,593-94,000 nesting females approximately 150 years ago. My estimate of N_{ef} for green turtles (based on θ) is 54,351, well within the range of population sizes estimated to have been characteristic of the last few centuries. Corresponding with a number of close similarities observed between the population genetics of hawksbills and green turtles, estimates of the current N_{ef} (ca. 54,000) and the N_{ef} before historical population contraction (ca. 48,000) are similar. These data for both tropically nesting species suggest a minimal net (long-term) depletion of genetic diversity and effective population sizes resulting from Pleistocene climatic cycling.

Conclusions

My findings provide strong evidence for long term, broad scale metapopulation dynamics within marine turtles, including corroborative evidence for complex source – sink relationships among populations. Also, these relationships appear to be plastic and even reversible over time,

with long-term dynamics probably driven by cycles of global climatic change. Relative levels of NH/NSF and nesting habitat preferences (i.e. temperate vs. tropical) appear highly correlated with patterns of genetic population structure and inferred historical responses to climatic cycling.

These data suggest differential effects of the Pleistocene glacial cycles across species, although the most drastic differences are observed in the effects on temperate vs. tropical nesting species. Tropical species show no net long-term trend of population decline or depressed genetic diversity as a result of Pleistocene climatic change. Apparently, tropical species experienced population subdivision and possibly population contraction, yet not at a level substantial enough (in duration or severity) to result in a major genetic bottleneck. Tropical species appear to have undergone this subdivision and possible contraction at some time around the Mid-Pleistocene, which indirectly implies they were not significantly impacted by environmental changes associated with the global onset of climatic cycling beginning in the Late Pliocene.

The temperate nesting loggerhead is inferred to have undergone substantially different population dynamics through the last several million years. This period, associated with dramatic climatic cycling, appears to have resulted in a net long-term trend of population decline and loss of genetic diversity, probably associated with an earlier and more dramatic bottleneck (in terms of duration and/or severity).

Conclusions regarding the differential patterns of response to global climatic change across species offer important insights for forecasting the impact of contemporary patterns of climatic change (i.e. global warming). In general, my findings suggest that tropical species are robust (in terms of population size and genetic diversity) to climatic change, particularly depression of global temperatures. In contrast, my data suggest that loggerheads may be negatively impacted (in population size and genetic diversity) by climatic change, although

details of how elevated temperatures (rather than depressed glacial temperatures) may affect this species are unclear from my data other than that they may induce rookery decline (or extirpation) as the distribution of optimal nesting habitat shifts. Already there is evidence of temporal shifts in the median nesting day of loggerheads on the east coast of Florida (Weishampel *et al*. 2004) consistent with similar shifts in migration and breeding patterns thought to be associated with global warming (Hughes 2000, Gitay *et al.* 2002, Root & Schneider 2002, Walther *et al.* 2002, Archaux 2003).

Present levels of genetic diversity, along with my estimates of N_{ef}, provide an optimistic perspective for conservation of marine turtles. Despite global decline in marine turtle populations resulting from several centuries of negative human impacts, the long generation time of these species has buffered rates of decline of genetic diversity. This suggests that the preservation of current levels of genetic diversity across species will rely heavily on the ability of conservation efforts to facilitate population recovery before the genetic reservoir maintained through long generation times is exhausted.

Future Research

Mitochondrial haplotype analysis has been the predominant method for analyzing population genetic patterns in marine turtles. Due to the nature of inheritance of mitochondrial haplotypes, my conclusions are limited to a matrilineal perspective of population structure and historical processes. Male-mediated gene flow has been detected in green turtles through comparisons of mitochondrial and nuclear polymorphisms (Karl et al. 1992; FitzSimmons et al. 1997; Roberts et al. 2004) although the important question remains: How reliable are mitochondrial polymorphisms at representing overall population genetics and gene flow across marine turtle populations?

Bi-parentally inherited molecular markers (e.g., microsatellite loci) have been employed in marine turtles in multiple paternity studies (Moore & Ball 2002), and polyandry and polygamy have been demonstrated (FitzSimmons 1998, Hoekert 2000, Crim 2002). Although the potential for sex-biased dispersal has been suggested by early studies and preliminary studies (Karl *et al.* 1992; Casale *et al*. 2002), FitzSimmons *et al*. (1997) observed strong tendency for male philopatry in Australian green turtles, supporting a broader population-wide interpretation and application of inferences based on matrilineal patterns of population dynamics. Pearce (2001), however, found that populations of Florida loggerheads with low mitochondrial diversity displayed normal levels of nuclear diversity.

Recently, Roberts *et al.* (2004) provided evidence, based on microsatellite data, that male-mediated gene flow might be more widespread than previously thought in green turtles. Rogers *et al*. (2004) employed four microsatellite loci, each characterized by excessive numbers of alleles and, thus, subject to high amounts of homoplasy (as discussed in Rogers *et al.* 2004). Based on this feature of the molecular marker employed, it is difficult to assess the accuracy of estimates of male-mediated gene flow across green turtle populations suggested by Rogers *et al.* (2004). To date, no study has examined an extensive number of populations using an effective array of bi-parentally inherited molecular markers sufficient to address the relationship between nuclear and mitochondrial patterns of genetic diversity. Future studies incorporating a larger number of microsatellite loci not subject to excessive numbers of alleles per locus are required before a clear understanding of the impacts of sex-biased gene flow on marine turtle population genetics is resolved. It is possible that inferences based on mitochondrial gene polymorphisms

will require revision if nuclear diversity does not correlate well with the patterns observed in mtDNA based studies.

CHAPTER THREE: MIXED STOCK ANALYSIS OF JUVENILE LOGGERHEADS IN THE INDIAN RIVER LAGOON, FLORIDA: IMPLICATIONS FOR CONSERVATION PLANNING

Introduction

Conservation of marine animals often is limited by the ability of researchers to identify biological trends and potential threats to organisms that make long distance migrations. For example, salmon production in California may be affected by logging hundreds of miles inland (Cafferata et al. 1998) and marine turtle bycatch during Mediterranean shrimping operations decreases breeding populations on nesting beaches in Florida and Mexico (Laurent et al. 1998). Attempts to solve this problem by censusing breeding aggregations, while logistically feasible, fail to incorporate predictive modeling and primarily record the results of trends displayed by juvenile aggregations in the prior decade. In the case of Florida marine turtles, the primary measure of population change is the number of nests deposited on a beach, which ignores the effects of juvenile mortality caused by disease (Work 2001), commercial fisheries (Crowder 1995), and pollution on the future breeding population. Understanding the factors dictating juvenile recruitment permits a more forward looking, predictive approach that incorporates the effects of pollution, disease, natural disasters, and commercial fisheries by-catch on juvenile populations to predict future trends.

Here, I use mitochondrial DNA haplotypes to investigate whether the juvenile loggerhead (*Caretta caretta*) aggregation in Indian River Lagoon, Florida represented an investment of several nesting beaches outside the United States, or strictly Florida and Mexico nesting beaches. I also test the hypothesis that large rookeries in close geographic proximity to juvenile feeding

aggregations contributed more individuals to those populations than expected, given their relative size and geographic proximity. I tested both hypotheses with the most extensive quantitative and temporal sampling of juvenile marine turtles to date. My study directly addresses how management units are linked through juvenile foraging grounds in the North Atlantic.

Loggerheads nest on sandy beaches throughout temperate latitudes. The species was federally listed as threatened in the North Atlantic in 1978 and is a CITES Appendix I listed species. The Marine Turtle Specialist Group and the IUCN Red List consider the loggerhead to be endangered throughout much of its range (Marine Turtle Specialist Group 1996). Atlantic loggerheads leave their nesting beaches and enter oceanic current systems such as the Gulf Stream that later become part of the North Atlantic Gyre (Carr 1987). After circumnavigating the Atlantic for 3-10 years they recruit to a juvenile foraging ground for the next 10-12 years (Carr, 1986; Musick and Limpus, 1997). Much of the loggerhead population in the North Atlantic recruits to juvenile foraging grounds in the Azores and Madeira (Bolton 1998), but many others recruit to foraging grounds in Indian River Lagoon (IRL) on the east coast of central Florida.

Aggregations of juvenile marine turtles may include individuals from nesting beaches around the globe, but until very recently, biologists had not devised a method for modeling contributions of local rookeries to juvenile aggregations (Norrgard and Graves 1995, Lahanas et al. 1998, Bass and Witzell 2000, Witzel et al. 2002, Luke et al. 2004). Bowen et al. (2004) suggested that on the scale of the North Atlantic, juvenile loggerhead recruitment to a foraging ground was heavily influenced by distance from the natal rookery and natal rookery size. Loggerheads make two separate migrations, one reproductive migration and one migration to a

juvenile foraging ground proximal to their natal beach (Bowen et al. 2004). However, whether juvenile loggerheads from nesting beaches around the world use IRL as a foraging ground remained unknown.

Loggerheads nesting in Florida comprise 86% of all loggerhead nesting in the Atlantic (Bolten and Witherington 2003). The large juvenile aggregations in IRL offer an excellent opportunity to examine how trends in juvenile recruitment (e.g., Bowen et al. 2004) can influence marine turtle conservation. By understanding the mechanisms that influence rookery contributions to a mixed-source juvenile foraging ground, and the threats posed to those juvenile aggregations, marine turtles can be managed with a pro-active strategy. This approach incorporates predictive demographic data without the lag time required for trends to manifest in nesting adult populations.

Methods

Study Site

Indian River Lagoon comprises more than one third of Florida's eastern coastline and extends 250 km, from Ponce de Leon Inlet to Jupiter Inlet. The lagoon system spans 5900 km^2 and is the nation's most diverse estuarine system (Dybas 2002). Indian River Lagoon waters are shallow (3-4 m depth) and the prevailing current is caused by wind rather than tides (Trocine and Trefry 1993). Canals that drain heavily-irrigated farm and livestock lands further inland contribute massive quantities of freshwater to the brackish water system, artificially decreasing its salinity (Trocine and Trefry 1993). This freshwater influx brings pesticides, heavy metals, dissolved nitrogen, and fertilizer residues into IRL (Trocine and Trefry 1993, MacDonald et al. 1996), with certain areas having heavy metal concentrations up to ten times greater than natural levels (Trocine and Trefry 1993). Pollutants contributed to eutrophication of IRL from a clear,

oyster bed-supporting system in the early 1900's to its current low visibility, red/green algae-dominated community (Trocine and Trefry 1993). Polluted conditions are thought to have contributed to a high prevalence (up to 70%) of fibropapillomatosis in resident juvenile green turtles (Ehrhart 1991).

DNA Extraction and Haplotype Identification

I conducted mitochondrial DNA d-loop sequence analysis on nine years of samples of 168 juvenile loggerheads from Indian River Lagoon, approximately 2 km south of Sebastian Inlet, Indian River County, Florida. I used large-mesh tangle nets to capture juvenile turtles monthly from 1993-2004. I stored blood samples obtained from the dorsal cervical sinus (Owens and Ruiz 1980) in lysis buffer at room temperature and extracted DNA using standard phenol/chloroform extraction techniques (Hillis et al. 1996). Because Florida nesting populations were previously defined by Pearce (2001) based on a 400 bp fragment of the mitochondrial d-loop (Table 1), I analyzed this fragment using primers CR-1 and CR-2 (Norman et al. 1994). I subjected purified DNA to polymerase chain reaction (PCR) in 25 μL reactions by denaturing at 93°C for 3 min, followed by 39 cycles of (1) DNA denaturing at 93°C for 30 s, (2) primer annealing at 52°C for 30 s, and (3) primer extension at 72°C for 30 s, with a final primer extension cycle at 72°C for 10 min. I visualized PCR products on an agarose gel and removed and purified the 400 bp fragment using MinElute Gel Extraction Kits (Qiagen). I quantified purified PCR products post purification using an agrose gel with 1 kb size standard (Promega). I sequenced the purified products on a Beckman CEQ 8000 automated sequencer following the manufacturer's protocols. I manually edited sequence data in Sequencher (Gene Codes Corp. 1996) and aligned the sequence data in GeneDoc (Nicholas et al. 1997). I calculated haplotype frequencies in TCS (Clement et al. 2000).

I compared the haplotypes observed in IRL to those found on 12 nesting beaches that encompass all nesting regions of Florida, plus Quintana Roo, Mexico. Currently, 11 loggerhead mitochondrial haplotypes define the nesting beaches of Florida and the Yucatan Peninsula of Mexico (Pearce 2001). These haplotypes are widespread and common throughout the North Atlantic but occur in significantly different frequencies when grouped by management units recognized in the United States Fish and Wildlife Service (USFWS) recovery plan for the loggerhead (Figure 5). I also included Brazilian and Mediterranean nesting beach haplotypes (Bolten et al. 1998, Laurent et al. 1998) to determine their contribution of juveniles to IRL.

Estimating Contributions of Nesting Beaches to IRL Juvenile Aggregation

To estimate the contribution of various nesting beaches to the IRL juvenile aggregation, I performed a Bayesian Markov-chain Monte Carlo (MCMC) mixed stock analysis using the program BAYES (Pella and Masuda 2001). I used even prior expected distributions for all analyses to avoid biasing the results by incorporating rookery size or distance into the model. This method allows for contributions of rare haplotypes from rookeries when those haplotypes were not found in the juvenile aggregation sample (Pella and Masuda 2001). Bayesian MCMC methods also yield more accurate probabilistic estimates of contribution than maximum-likelihood point estimates (see Luke et al. 2004; Bolker et al. 2003). I calculated estimated rookery contributions to IRL juveniles based on 23,598 resamplings (as determined by BAYES) of one stock mixture expected to recruit juveniles from five nesting beach aggregations. Estimated contributions excluded the Brazil rookery because its sole haplotype was not found in

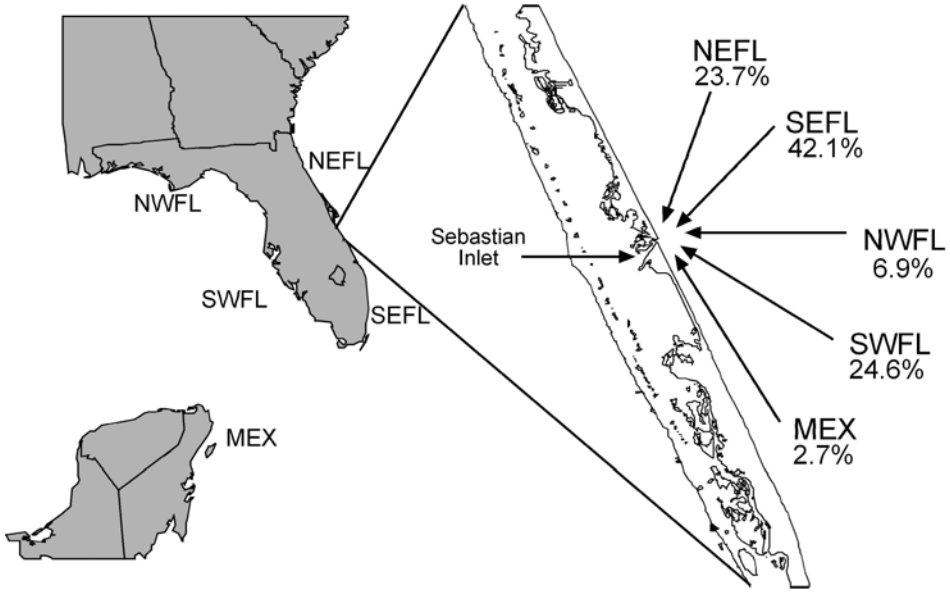

Figure 5. Map of Indian River Lagoon including Southeastern United States and Yucatan Peninsula, with estimated contributions from nesting beach regions. NEFL= Northeast Florida, SEFL=Southeast Florida, SWFL= Southwest Florida, NWFL=Northwest Florida, MEX=Mexico.

IRL. I also excluded Mediterranean rookeries for two reasons: 1) no endemic mitochondrial haplotypes are available that indicate their contributions, and 2) while juvenile loggerheads from Atlantic rookeries spend extended periods in the Mediterranean Sea, the opposite is not suggested in the literature; Mediterranean-born loggerheads may spend their entire juvenile period in that basin (Laurent et al. 1998). Similar MCMC analyses have been used to estimate stock composition and rookery contribution in other marine turtles and fishes (Fernandez et al. 2002, Fillatre et al. 2003, Herwerden et al. 2003, Luke et al. 2004, Ruzzante et al. 2004).

I grouped haplotypes that characterize Florida and Mexico rookeries into northeast (NEFL), southeast (SEFL), northwest (NWFL), and southwest Florida regions (SWFL) and Mexico (MEX; Pearce 2001) and estimated distance from each rookery to Sebastian Inlet using GIS software (ArcView v3.2; ESRI). I estimated rookery size as the total number of nests recorded by the Florida Index Nesting Beach Program from 1988-2002. I used linear regression to test whether Bayesian MCMC estimated contributions depended on rookery size or distance. Independent variables were log-transformed to meet analysis assumptions. I computed Chi-squared tests between haplotype frequencies in the IRL and each rookery to support my model of multiple rookery contributions.

Results

I observed 8 haplotypes (Table 3) in IRL: CCA1 (48.2%), CCA2 (44.6%), CCA3 (1.8%), CCA7 (1.2%), CCA10 (1.8%), CCA13 (0.6%), CCA14 (0.6%), and CCA20 (0.6%). In a single individual, I observed one novel haplotype, which was one bp distant from CCA2. Haplotype frequencies in IRL indicate that its juvenile aggregation is a genetically diverse assemblage with contributions of juveniles from nesting beaches throughout the Atlantic (Table

	CCA1	CCA2	CCA3	CCA4	CCA5	CCA6	CCA7	CCA8	CCA9	CCA10	CCA20	Total
Northeast Florida	44	13	1				2		1			61
Southeast Florida	32	28		1	1			1		1	1	65
Southwest Florida	24	67	4		2	2	2			1		102
Northwest Florida	36	7	1		2							46
Mexico		11	2					1	1	5		20
Brazil				11								11
Mediterranean		78	13				1					92
Totals	136	204	21	12	5	2	5	2	2	7	1	397

Table 3. Frequency of loggerhead mtDNA control region haplotypes described by Bolten et al. (1998) and Pearce (2001).

4) and possibly the Mediterranean. However, due to the lack of endemic haplotypes, and the absence of evidence in the literature to support Mediterranean contributions in juvenile loggerhead aggregations, I will test my second hypothesis including only northern Atlantic rookeries.

The program BAYES estimated 23,598 resamplings as the minimum number of generations required for estimation of posterior probability densities. Results of Bayesian MCMC analyses using even prior expected distributions indicated contributions from rookeries in Mexico, southeast, southwest, northeast, and northwest Florida (Table 4). Chi-squared tests between haplotype frequencies in IRL and each rookery were all significantly different at α=0.05. The proportion of estimated contributions regressed significantly on both log distance ($F_{1,3}$=20.7, p<0.020, R^2=0.88) and log rookery size ($F_{1,3}$=21.6, p<0.019, R^2=0.88). Distance and rookery size were negatively correlated albeit not significantly (r = -0.86, p=0.063).

Discussion

Immature loggerheads spend 10-12 years in developmental habitats such as Indian River Lagoon, Florida. Previous studies of juvenile loggerhead aggregations suggest multiple contributions primarily from local rookeries (Norrgard and Graves 1995, Witzell et al. 2002). However, recruitment of juvenile loggerheads from Florida to the Azores and Madeira suggest that individuals are capable of migrating to a juvenile foraging ground upwards of 6400 kilometers from their natal nesting beach (Bolten et al. 1998). Contradicting the findings of Bolten et al. (1998), a study of multiple rookeries in the North Atlantic indicates that juvenile loggerheads primarily recruit to foraging grounds near their natal beaches (Bowen et al. 2004). My data support the findings of Bowen et al. (2004) and suggest that IRL juveniles migrate from

Nesting Stock	Mean	SD
Northwest Florida	0.069	0.112
Southwest Florida	0.246	0.175
Northeast Florida	0.237	0.196
Southeast Florida	0.421	0.250
MEXICO	0.027	0.044

Table 4. Estimated contributions of 5 rookeries to the juvenile aggregation in Indian River Lagoon, Florida, based on Bayesian Markov-chain Monte Carlo (MCMC) mixed stock analyses. Only haplotypes from IRL that previously were identified on a nesting beach were included.

local Florida and Mexico rookeries. However, the resolution offered by mitochondrial d-loop alleles is insufficient to rule out minimal contributions from Brazil or the Mediterranean, and mitochondrial DNA data supports limited contribution from these regions. A more detailed study of hypervariable nuclear loci (i.e., microsatellites) may further resolve the contributions of South American and the Mediterranean rookeries to juvenile aggregations in IRL. Juvenile green turtles in IRL have been shown through endemic mtDNA haplotypes to originate from Mediterranean nesting beaches (Bagley 2003). There are no endemic Mediterranean loggerhead haplotypes that would directly indicate such a contribution. The most common haplotypes found on Mediterranean nesting beaches also are found in the Northern Atlantic, so it is difficult to resolve relative contributions. Some juveniles hatched in Florida and Mexico recruit to juvenile aggregations in the Mediterranean (Laurent et al. 1993, 1998). It is possible (albeit somewhat unlikely) that the opposite also occurs. For the purposes of this study, I assumed that IRL strictly recruits juveniles from rookeries in Florida and Mexico.

I determined that distance was highly correlated with estimated contributions based on haplotype frequencies. Rookery size also was strongly correlated with estimated contributions of juveniles to the foraging ground. The SEFL aggregation, which comprises 86% of all Florida/Mexico loggerhead nesting, and is significantly closer to IRL than all other rookeries, only contributed an estimated 42.1% of the individuals in IRL. These data demonstrate that IRL receives individuals from nesting beaches throughout Florida and Mexico with proportional contributions from local, dense rookeries. My results generally confirm the patterns suggested by Bowen et al. (2004). Similar studies of green turtles suggested that distance either did not factor into recruitment (Luke et al. 2004) or was not as important as rookery size (Lahanas et al. 1998). Bass and Witzell (2000) did find a correlation between rookery size/distance and

contribution of juveniles, with distance having the greatest effect. My study had more than twice as many samples as the studies listed above. The larger sample size provides greater resolution of rare alleles, which may significantly affect estimated contributions. My study also incorporated 108 continuous months of sampling, whereas the previous studies cited (Lahanas et al. 1998, Bass and Witzell 2000, Luke et al. 2004) were 1-27 months duration. I suggest that longer periods of genetic sampling and greater sample size increase the accuracy of population assessment and support strong effects of rookery size and distance from the juvenile foraging ground.

The mixed stock composition of juvenile loggerheads in IRL suggests that nesting beach-based management units described by the USFWS recovery plan are linked though this important foraging ground. Overall, my results demonstrate the importance of IRL as a primary resource for juvenile loggerheads from the entire region. The large size of IRL, its abundant and high quality food sources (Holloway-Adkins 2001), and lack of most pelagic predators make it an ideal foraging ground for both loggerhead and green turtles. The strong correlation of estimated contributions with distance and rookery size indicates that juvenile turtle aggregations will recruit to contributing rookeries in a predictable manner. Commercial fishing impacts, pollution, and diseases affecting these juvenile aggregations will have measurable outcomes for the rookeries that depend on them. I suggest that these parameters be incorporated into predictive population-dynamic models based on monitoring of juvenile aggregations.

The fact that multiple rookeries contribute to a limited number of juvenile aggregations makes marine turtles as a group, and specifically the loggerhead, particularly vulnerable to the effects of pollutants in degraded juvenile foraging habitats such as IRL. Currently, researchers have little data on the effects degraded ecosystems, such as IRL, have on the long term

development and fitness of juvenile turtles. Certainly, in the case of green turtles, it is evident that high occurrence of fibropapillomatosis (upwards of 70%) in juveniles inhabiting IRL will have noticeably negative impacts on future populations through increased juvenile mortality and potential long term effects. While fibropapillomatosis is not as prevalent in juvenile loggerheads, it does occur in 4.5% of individuals captured from 1982 to 2004 (unpublished data). The nature of juvenile loggerheads recruiting from numerous nesting beaches, possibly from all over the world, to a select few foraging grounds, make the threats posed to these localized foraging grounds relevant at a global scale.

CHAPTER FOUR: CONCLUSION

My research attempts to provide information important to conservation efforts in marine turtles. I have added to our understanding of historical patterns of gene flow in three species of marine turtles during major climatic shifts. These findings are of significance to future conservation efforts by enhancing our understanding of where major population contractions will be centered during future climate shifts. This research also provides data on estimated population sizes during the Pleistocene. My findings on the coincidence of major climate changes and the severity of subsequent population contractions in the loggerhead, hawksbill, and green turtle demonstrate how sensitive marine turtles are to global climate change.

In addition to historical patterns of gene flow, I have demonstrated a previously unknown migratory pattern in the loggerhead turtle. My research supports a pattern by which juvenile loggerhead turtles make a major migration from circumnavigating the Atlantic to recruit to foraging grounds proximal to their natal nesting beach. This migration results in a predictable pattern whereby distance from the rookery and the relative size of the rookery are proportional to the contribution of juveniles. These findings demonstrate the link between threats posed to juvenile loggerhead aggregations and the direct implications on local nesting beaches. Incidental take by commercial fisheries and the effects of disease and other threats to a foraging ground will directly impact local nesting beaches. In addition, consistent monitoring of juvenile aggregations will serve as a predictive model for growth/decline of local rookeries. Because juveniles will recruit to the natal rookeries for reproduction much as they do for their subadult development, population dynamics in juvenile aggregations will be mirrored by mature rookery populations.

These findings are of great value to conservation managers and will better our understanding of loggerhead turtle life history.

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