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ORIGINAL PAPER

Genetic structure of the southeastern United States loggerhead turtle nesting aggregation: evidence of additional structure within the peninsular Florida recovery unit

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Abstract The southeastern United States supports one of two large loggerhead turtle (*Caretta caretta*) nesting aggregations worldwide and is therefore critical to global conservation and recovery efforts for the species. Previous studies have established the presence of four demographically distinct nesting populations (management units) corresponding to beaches from (1) North Carolina through northeastern Florida, (2) peninsular Florida, (3) the Dry Tortugas, and (4) northwest Florida. Temporal and geographic genetic structure of the nesting aggregation was examined utilizing partial mitochondrial control region haplotype frequencies from 834 samples collected over the 2002 through 2008 nesting seasons from 19 beaches as well as previously published haplotype data. Most rookeries did not exhibit interannual genetic variation. However, the interannual variation detected did significantly impact the interpretation of spatial genetic structure in northeastern Florida. Based on pairwise F_{ST} comparisons, exact tests of

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population differentiation, and analysis of molecular variance, the present study upholds the distinctiveness of the four currently recognized management units and further supports recognition of discrete central eastern, southern (southeastern and southwestern), and central western Florida management units. Further subdivision may be warranted, but more intensive genetic sampling is required. In addition, tools such as telemetry and mark-recapture are needed to complement genetic data and overcome limitations of genetic markers in resolving loggerhead turtle rookery connectivity in the southeastern USA.

Introduction

Defining population boundaries for highly vagile marine species often presents challenges given the lack of apparent barriers to movement across sometimes vast spatial scales. For some such species, natal homing behavior to specific reproductive sites dictates population boundaries. Natal homing to breeding sites often occurs as part of a complex life history involving ontogenetic or seasonal migrations by individuals that may encompass entire ocean basins, where individuals from distinct breeding populations mix (anadromous salmonids, reviewed in Allendorf and Waples 1995; many cetacean species, reviewed in Hoelzel 1998; marine turtles, reviewed in Bowen and Karl 2007). Lohmann et al. (2008b) proposed that both breeding salmon and marine turtles locate natal regions via a biphasic navigation process first involving magnetic cues to direct long distance ocean migration to the general vicinity of the natal area. Salmon then use local olfactory cues in choosing their target spawning rivers (Wisby and Hasler 1954); however, the local cues driving fine-scale nesting beach selection by marine turtles are less well understood (Lohmann et al. 2008b). As such, the precise scale of natal philopatry remains unresolved for many marine turtle species and may vary across nesting populations within species depending on local biotic and abiotic conditions. The presence of long stretches of suitable nesting habitat along continental coastlines further complicates assessments of population structure for marine turtles. Nonetheless, given that migratory reproductive behavior contributes significantly to patterns of population structure for these species, properly defining the scale of natal homing behavior is critical to ensuring that demographically discrete populations receive adequate recognition and protection.

Loggerhead sea turtles occur globally in warm temperate and tropical waters, though nesting effort is typically focused on warm temperate beaches (Bolten 2003). Loggerhead turtles nesting in the western North Atlantic have a complex life history marked by extensive developmental migrations and seasonal migrations. Genetic analyses and size frequency data have provided strong evidence that loggerhead turtles originating from western North Atlantic beaches spend their early years as pelagic foragers in the eastern Atlantic (Bolten et al. 1998). Broad-scale natal homing by neritic juveniles is supported by mixed stock analysis of several aggregations foraging along the continental shelf of the eastern United States (Bowen et al. 2004). Upon reaching sexual maturity, females migrate to their natal regions to nest (Bowen et al. 1993; Bowen et al. 1994; Bowen et al. 2005). Defining the spatial scale of this natal neighborhood is an important consideration for delimiting population boundaries, particularly across continuous nesting habitats.

The southeastern United States of America (USA) loggerhead turtle nesting aggregation is one of two globally significant nesting populations, the other being Masirah and other islands along the coast of Oman in the Arabian Sea (Dodd 1988; Baldwin et al. 2003). Loggerhead turtle nesting densities vary considerably over the southeastern USA coastline; approximately 69% of the loggerhead turtle nesting in Florida takes place on 411 km of the 1,300 km of surveyed beaches (Witherington et al. 2009). Annual mean nest numbers on Florida's index nesting beaches declined by approximately 44% from 1998 through 2006 (Witherington et al. 2009), prompting concern that the largest nesting population in the Atlantic may be in decline.

In the USA, management and protection of loggerhead turtles is jointly the responsibility of National Oceanographic and Atmospheric Administration's National Marine Fisheries Service (NMFS) and the United States Fish and Wildlife Service (USFWS). Defining the boundaries of nesting populations for management and conservation purposes is a critical element of the recently updated Recovery Plan for the Northwest Atlantic Population of the Loggerhead Sea Turtle (*Caretta caretta*) (hereafter Recovery Plan, NMFS and USFWS 2008).

Numerous concepts have been proposed to identify and classify intra-specific units for conservation or management purposes, and many of these incorporate genetic data (reviewed in Fraser and Bernatchez 2001). Management units, as defined by Moritz (1994), have formed the basis of characterizing loggerhead turtle population structure in the Atlantic basin (Encalada et al. 1998; Bowen et al. 2005). Management units "represent populations connected by such low gene flow that they are functionally independent" and are "recognized as populations with significant divergence of allele frequencies at nuclear or mitochondrial loci" (Moritz 1994). In the case of marine turtle populations, rookeries are demographically distinct entities based on female natal philopatry, irrespective of the level of nuclear gene flow (Avise 1995; Bowen et al. 2005). Thus, significant divergence of mitochondrial haplotype frequencies between rookeries suggests demographic partitioning, which

qualifies each rookery as a distinct management unit. For the purposes of the Recovery Plan, the Atlantic Loggerhead Sea Turtle Recovery Team chose to designate intra-specific conservation units known as recovery units. "Recovery units are subunits of the listed species that are geographically or otherwise identifiable and essential to the recovery of the species. Recovery units are individually necessary to conserve genetic robustness, demographic robustness, important life history stages, or some other feature necessary for long-term sustainability of the species" (NMFS and USFWS 2008). Genetic data have been used as the basis for recovery unit designations where such data are available (NMFS and USFWS 2008). We will use "management unit" to describe demographically and genetically distinct nesting populations in the spirit of Moritz (1994) and "recovery unit" only in the context of agency designations outlined above.

Genetic structure among rookeries comprising the southeastern USA loggerhead turtle nesting aggregation has received considerable attention. Restriction fragment length polymorphism analyses of mitochondrial DNA provided strong support for regional natal homing by loggerhead turtles and established the presence of at least two distinct populations nesting in the USA (Bowen et al. 1993; Bowen et al. 1994). Based on significant differences in frequencies of sequence-defined haplotypes and geographic considerations, Encalada et al. (1998) proposed a minimum of three demographically independent nesting populations in the southeastern USA corresponding to beaches from (1) North Carolina through northeast Florida, (2) central and southern peninsular Florida, and (3) northwest Florida. Pearce (2001) analyzed mitochondrial haplotype frequencies and allele frequencies at five microsatellite loci of the original and additional southeastern USA samples. Mitochondrial control region analysis supported previous management unit groupings and added the Dry Tortugas rookery as a distinct management unit (Pearce 2001). Structure inferred from nuclear markers was much weaker than structure inferred from mitochondrial markers, presumably due to weaker natal philopatry in some males or male-mediated gene flow facilitated by turtles from different rookeries mixing along migration routes or on foraging grounds (Pearce 2001; Bowen et al. 2005). Male-mediated gene flow does not detract from the classification of rookeries as independent populations given the fact that female natal site fidelity defines reproductive population boundaries, irrespective of male behavior (Bowen et al. 2005).

Whereas geographic structure among rookeries has been clearly demonstrated in several marine turtle species using mitochondrial DNA tools (reviewed in Bowen and Karl 2007), it is uncertain whether temporal variation in mitochondrial haplotype frequencies at rookeries may also occur. Undetected temporal variation in haplotype frequencies at rookeries could affect the interpretation of spatial structuring among rookeries as well as the integrity of estimates of rookery contributions to juvenile foraging aggregations. Explicit tests of interannual variation in haplotype frequencies have been conducted at a few marine turtle rookeries, and none have detected any statistically significant temporal structuring. Hatase et al. (2002) did not detect significant haplotype frequency variation between two sampling years at four Japanese loggerhead turtle rookeries. The pooled sample was dominated by a single haplotype (Haplotype $B = 89\%$, Hatase et al. 2002), potentially limiting the power to detect any temporal differences. Tests for intraseasonal and interannual variation in haplotype frequencies among green turtles nesting at Tortuguero, Costa Rica also failed to detect any significant temporal structuring (Bjorndal et al. 2005). However, the authors cautioned that the results should be tempered by the recognition that the tests likely had low statistical power given the high frequency of the common haplotype (CM-A3 $> 90\%$, Bjorndal et al. 2005). Similarly, no significant interannual variation was found at the Mona Island hawksbill turtle rookery sampled in 1993, 2003, 2004, and 2005 (Velez-Zuazo et al. 2008). Whether haplotype frequencies are stable at relatively low-density rookeries is unclear, and temporal variation may have important implications for spatial structuring and management unit designations for the southeastern United States loggerhead turtle nesting aggregation given the wide range of nesting densities at different rookeries.

Despite increased resolution with each previous investigation, questions of management interest remain regarding genetic structure among rookeries along the southeastern USA coast. The Recovery Plan currently recognizes four recovery units nesting in the southeastern United States roughly concordant with previous genetic analyses: (1) the northern recovery unit corresponding to beaches from Virginia through the Georgia/Florida border, (2) the peninsular Florida recovery unit, corresponding to all eastern Florida beaches and those in central and southwestern Florida, (3) the Dry Tortugas in the Gulf of Mexico off the southwest coast of Florida, and (4) the northern Gulf of Mexico recovery unit, corresponding to beaches in northwestern Florida through the Texas/Mexico border (NMFS and USFWS 2008). It is uncertain whether these recovery units adequately reflect the level of genetic differentiation present among rookeries within the southeastern USA nesting aggregation given low power to detect frequency differences based on small sample sizes for some rookeries. Given that local threats to females concentrated in the vicinity of their nesting beaches will have pinpoint impact on the corresponding nesting population (Bowen et al. 2005), it is critical to recognize genetic structuring and define management

units at appropriate spatial scales. An important unresolved question is determining whether a precise boundary exists between the northern management unit and the remaining Florida rookeries. Encalada et al. (1998) anticipated the boundary would occur between Cape Canaveral and Jacksonville based on an established biogeographic discontinuity and the sharp decline in loggerhead turtle nesting density north of Canaveral. Initial analysis of samples obtained from Volusia County suggested that this nesting population represented a distinct management unit (Francisco et al. 1999). However, pairwise Volusia County and Melbourne population comparisons based on a larger Melbourne sample size were not significantly different, prompting Pearce (2001) to include Volusia County within the South Florida management unit.

We re-assessed population genetic structure among rookeries in the southeastern USA loggerhead turtle nesting aggregation by sequencing of a portion of the mitochondrial control region of 834 samples collected during the 2002–2008 nesting and hatching seasons to: (1) test for interannual variation in haplotype frequencies at individual rookeries, (2) determine the number of management units comprising the southeastern US nesting aggregation and identify potential boundaries, and (3) compare the recovery unit groupings designated in the Recovery Plan with the structure suggested by haplotype frequency and demographic data.

Methods

Field methods

Samples from 834 individual loggerhead turtles or nests were collected from 19 different southeastern USA beach locations over the 2002–2008 nesting seasons (Table 1). Sample sites were chosen to represent the extent of loggerhead turtle nesting in the USA where nesting densities were sufficient to provide adequate sample sizes (Figs. 1 and 2). Sites typically included the highest density nesting beaches within each respective region. Each rookery is represented by either samples obtained directly from nesting females or by nest contents obtained during post-emergence nest evaluations. Samples from nesting females were collected from the shoulder region using 6-mm biopsy punches following oviposition and during the nest covering and camouflaging process. Precautions were taken to ensure that each nesting female was represented in each annual dataset only once, either via tagging to prevent duplicate sampling, or by using microsatellite genotyping that would allow recognition of individual turtles (Shamblin et al. 2007, 15 loci, microsatellite data not shown). Nest samples were comprised of tissue from dead hatchlings or hatched eggshells collected during post-emergence nest evaluations, and each nest was represented by a single sample. Sampled clutches were laid June 15 through June

Table 1 Sample site and collection data for samples collected as part of the present study

Site code	Sample site	Sample size	Year	Sample type
BHI	Bald Head Island, Brunswick County, North Carolina	15	2006	Female[a]
CAP	Cape Island, Charleston County, South Carolina	53	2006	Female
WAS	Wassaw Island, Chatham County, Georgia	42	2005, 2006	Female
AML	Amelia Island, Nassau County, Florida	20	2006, 2008	Nest[b]
SJC	St. Johns County, Florida	37	2007, 2008	Nest
FLG	Flagler County, Florida	55	2007, 2008	Nest
VOL	Northern Volusia County, Florida	90	2006–2008	Nest
NSB	New Smyrna Beach, Volusia County, Florida	46	2006, 2008	Nest
CAN	Canaveral National Seashore, Volusia County, Florida	58	2006	Female
MEL	Melbourne Beach, Brevard County, Florida	106	2006	Female
JUN	Juno Beach, Palm Beach County, Florida	49	2006	Female
FTL	Ft. Lauderdale, Broward County, Florida	48	2006	Nest
SBR	Hollywood and John U. Lloyd State Park, Broward County, Florida	21	2006	Nest
MID	Virginia Key and Cape Florida State Park, Miami-Dade County, Florida	22	2006	Nest
KEY	Keewaydin Island, Collier County, Florida	40	2006	Female
CSK	Casey Key, Sarasota County, Florida	57	2006	Female
SGI	St. George Island, Franklin County, Florida	13	2006	Nest
CSB	Cape San Blas, Gulf County, Florida	47	2002–2005	Female
STJ	St. Joseph Peninsula State Park, Gulf County, Florida	15	2006	Nest

[a] Female samples were collected as biopsy punches

[b] Nest samples were dead hatchling tissue or hatched egg shells

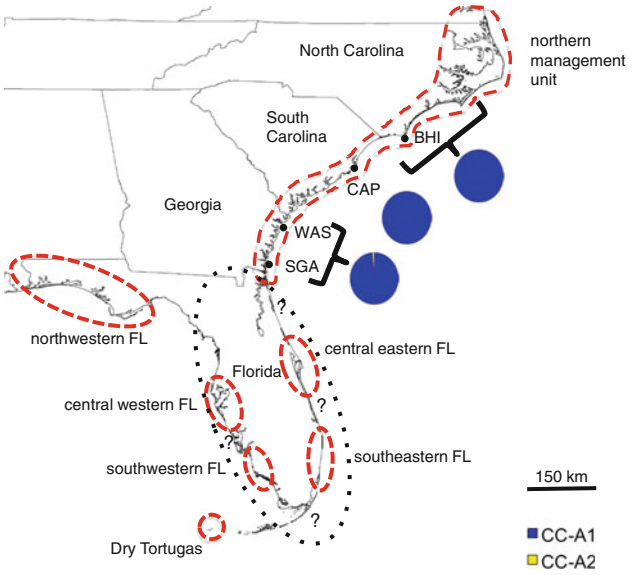

Fig. 1 Sample locations and haplotype distributions for northern sampled rookeries of southeastern USA loggerhead turtles. Regional rookery groupings discussed in the text are outlined in *dashed lines*. The currently recognized peninsular Florida recovery unit is outlined by a *dotted line*. See Fig 2 for Florida sample sites and haplotype frequency pie charts

24, 2006; June 15 through June 24, 2007; and June 17 through June 26, 2008. A 10-day sampling window was chosen to maximize sample sizes while minimizing the probability of re-sampling females. The average inter-nesting interval for southeastern USA loggerhead turtles is approximately 14 days, with females rarely re-nesting at fewer than 11 days (reviewed in Dodd 1988). Samples were stored in 95% ethanol prior to DNA extraction.

Laboratory methods

Genomic DNA was extracted using the DNeasy blood and tissue kit (QIAGEN) following standard protocols. Polymerase chain reaction (PCR) amplifications of a 380 bp portion of the mitochondrial control region were carried out using primers TCR5 and TCR6 (Norman et al. 1994). Universal M13 primer sequences were added to the 5′ end of each PCR primer to facilitate sequencing. PCR reactions were carried out in 10 µl volumes containing 10 mM Tris, pH 8.4; 50 mM KCl, 1.0 µM of each primer, 1.5 mM $MgCl_2$, 0.5 mM dNTPs, 0.5 unit of *Taq* DNA Polymerase, and approximately 25–75 ng of genomic DNA. PCR cycling parameters were as follows: 95°C for 3 min; 30 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 30 s; and a final extension of 72°C for 10 min. PCR products were purified by adding 2 µl of ExoSAP-IT® (USB Corporation) to 7 µl of PCR amplicon and incubated according to manufacturer's instructions. The mtDNA amplicons were sequenced in both directions using ABI BigDye v3.1 (PE Applied Biosystems) and an ABI 3730*xl* DNA Analyzer. Negative controls were included in each batch of PCR amplifications and sequencing reactions to detect contamination.

Data analysis

Sequences were aligned, edited, and compared to previously described haplotypes using the program Sequencher 4.2 (Gene Codes Corporation). Sequences were assigned haplotype designations after nomenclature published on the Archie Carr Center for Sea Turtle Research (ACCSTR) website (http://accstr.ufl.edu/ccmtdna.html). Samples producing novel or ambiguous sequences were subjected to a second round of DNA extraction, PCR amplification, and sequencing for verification. Novel haplotypes were deposited with Genbank and ACCSTR. Haplotype frequency data from Encalada et al. (1998); Francisco et al. (1999); Pearce (2001), and Bowen et al. (2005) were included in analyses to test for temporal variation and to fill in the geographic gap at Dry Tortugas for the present study (Supplemental Table 1).

Haplotype diversity (h), nucleotide diversity (π), pairwise F_{ST} comparisons, pairwise exact tests of population differentiation, and analysis of molecular variance (AMOVA) were conducted using the software Arlequin version 3.1 (Excoffier et al. 2005). Haplotype diversity was estimated based on Nei (1987). Nucleotide diversity was calculated assuming the model of Tamura and Nei (1993). Significance values for AMOVA were obtained from 10,000 permutations. Exact tests of population differentiation were conducted with 100,000 permutations and 10,000 dememorization steps after the method of Raymond and Rousset (1995). These statistics were used to test for temporal as well as spatial structure. For the purposes of temporal tests, BHI samples were compared with previously collected samples from Bald Head Island, Cape Lookout, Topsail Beach, Camp Lejeune, and Caswell Beach (NC, Encalada et al. 1998); WAS samples were compared with previously collected samples from Little Cumberland and Cumberland islands, Georgia (SGA, Encalada et al. 1998); CSK was compared with samples previously collected more broadly in Sarasota County (SAR, Encalada et al. 1998; Pearce 2001); and SBR was compared with samples previously collected near the Port Everglades inlet (PEV, Pearce 2001). All interannual samples for each site that were not significantly different were pooled for spatial analyses. Because nest samples represent unknown turtles, combining sample sets across more then two consecutive years may have resulted in individual nesting females being represented in the dataset more than once.

Following pairwise F_{ST} comparisons and exact tests of population differentiation, all proximal sample sites that were not significantly different were pooled for further

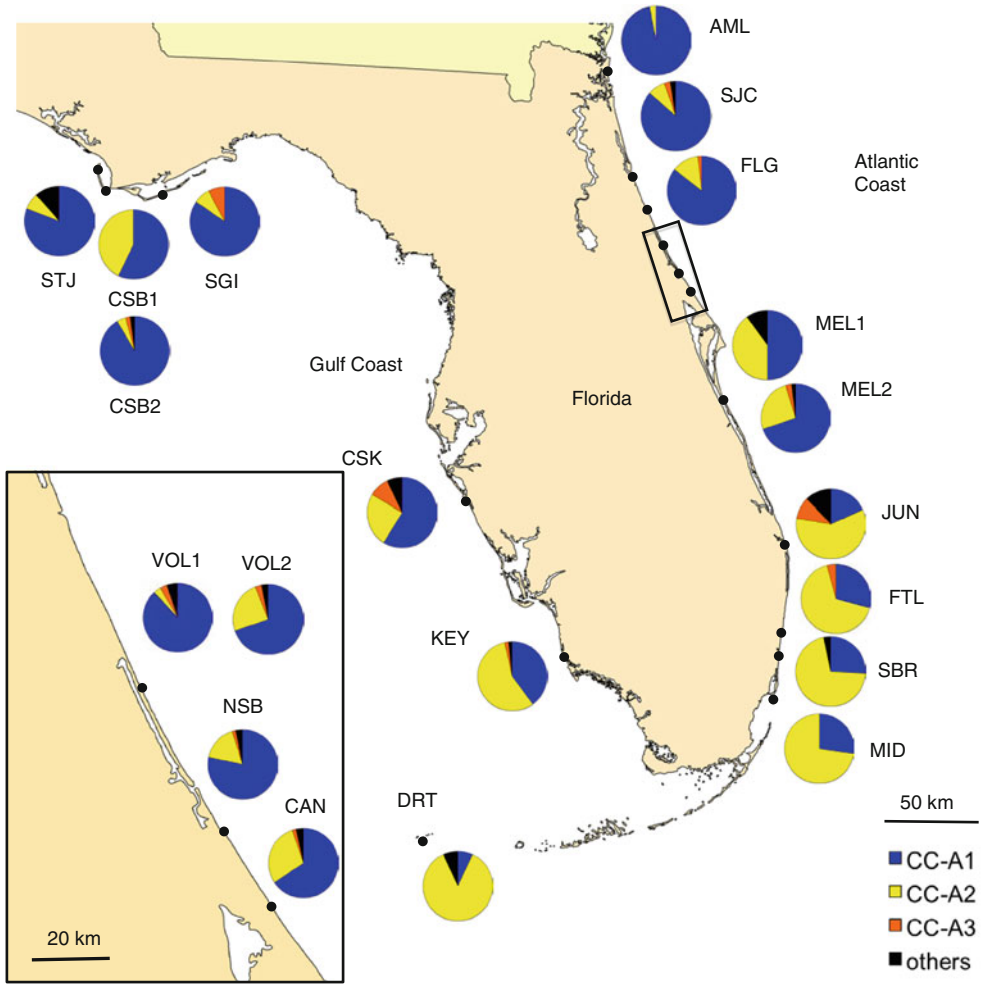

Fig. 2 Sample locations and partial mitochondrial control region haplotype frequencies for Florida loggerhead turtle rookeries based on combined haplotype frequency data from the present study and previous studies. Site abbreviations are explained in Table 1. Northeastern Florida data are *highlighted* in the inset map

analyses. In the case of ambiguous pairwise comparisons, several a priori sample-clustering iterations were performed and examined using pairwise tests and AMOVA. Optimal rookery clusters were chosen by maximizing F_{CT} (genetic variation occurring among management units) and minimizing F_{SC} (genetic variation occurring among sampled rookeries within defined management units) in an AMOVA framework. Significance of the final round of pairwise F_{ST} comparisons and exact tests of population differentiation were adjusted using sequential Bonferroni correction with a table-wide α of 0.05 (Rice 1989).

Results

Haplotype and nucleotide diversity

Sequence analysis of newly collected samples identified thirty polymorphic positions, corresponding to 22 transitions and eight indels (Table 2). Position 358 contained both an indel and a transition. The variable positions resolved nine haplotypes, eight of which have previously been described from loggerhead turtles nesting in Florida (Bowen et al. 2005). The new haplotype contained an A to G transition at position 119 and has been designated CC-A43 (Genbank accession number EF396287). All 12 haplotypes in the pooled dataset belonged to two phylogenetically distinct haplogroups (Fig. 3) as previously described by Encalada et al. (1998).

Haplotypes CC-A1 and CC-A2 accounted for approximately 94% of all individuals sampled, but these haplotypes were not randomly distributed (Supplemental Table 1). Haplotype CC-A9, previously described from Quintana Roo, Mexico, and the Dry Tortugas, was detected for the first time on mainland Florida Gulf coast nesting areas. Haplotype CC-A14, previously described from peninsular Florida beaches, was detected among northwest Florida samples. Haplotypes CC-A5, CC-A11, and CC-A13, each represented

Table 2 Variable positions of a 380 base pair fragment of the mitochondrial control region observed in loggerhead turtles nesting in the southeastern USA

Haplotype	Variable positions																																
	32	35	37	51	53	63	78	96	104	119	161	162	188	210	230	244	246	259	294	312	314	315	317	327	355	356	357	358	359	360	363
CC-A2	C	A	C	C	–	G	G	A	G	A	T	A	G	T	T	A	T	G	G	A	G	C	G	T	G	C	A	A	G	T	A
CC-A3	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CC-A5[a]	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	–
CC-A7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.
CC-A9	.	.	.	T	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CC-A10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.
CC-A13[a]	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CC-A20	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CC-A43	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CC-A1	T	G	T	T	T	A	.	.	A	.	C	G	.	C	C	G	C	A	–	.	A	T	A	C	–	–	–	–	–	–	.
CC-A11[a]	T	G	T	T	T	A	.	.	A	.	C	.	.	C	C	G	C	A	–	.	A	T	A	C	–	–	–	–	–	–	.
CC-A14	T	G	T	T	T	A	A	.	A	.	C	.	.	C	C		C	A	–	.	A	T	A	C	–	–	–	–	–	–	.

Haplotype designations are based on Archie Carr Center for Sea Turtle Research nomenclature. '–' indicates a deletion

[a] Previously described haplotypes from Florida rookeries not detected in the present study

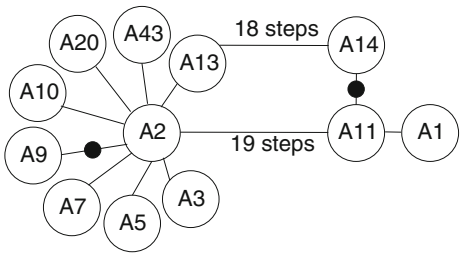

Fig. 3 Haplotype network depicting the two phylogenetically distinct haplogroups previously described by Encalada et al. (1998). *Filled circles* indicate hypothetical haplotypes

by single nesting females from Florida nesting beaches in previous analysis (Bowen et al. 2005), were not found in the present study. Haplotype diversity ranged from 0 to 0.615 (Supplemental Table 1), and there was a strong latitudinal component to the haplotype frequency distribution with the northernmost and southernmost beaches exhibiting the lowest haplotype diversity.

Interannual variation

Annual samples from BHI and CAP were fixed for haplotype CC-A1 and therefore exhibited no temporal variation between sampling years. Comparisons among annual samples at 12 sites using AMOVA suggested differentiation among sample years at the VOL and CSB rookeries (Supplemental Table 2). Pairwise F_{ST} values suggested that the previously collected CSB sample set (Pearce 2001) was differentiated from the 2002 ($F_{ST} = 0.33237$, $P = 0.02257$) and 2004 ($F_{ST} = 0.40552$, $P = 0.04930$) samples, but none of the remaining comparisons were significant. Exact tests of population differentiation indicated a significant difference only between the original CSB sample and 2002 ($P = 0.02182$). Pairwise F_{ST} comparisons among VOL annual samples yielded significant differences for 1998 and 2006 ($F_{ST} = 0.11984$, $P = 0.01673$) and 1998 and 2008 comparisons ($F_{ST} = 0.08205$, $P = 0.02297$). These annual classes were also significantly different as measured by exact tests of population differentiation (1998 and 2006, $P = 0.01445$; 1998 and 2008, $P = 0.02034$). Additionally, an exact test of population differentiation indicated a significant difference between the 2006 and 2007 VOL samples ($P = 0.02793$). Despite lack of a signal of differentiation with AMOVA at MEL, pairwise F_{ST} comparisons and exact tests of population differentiation indicated a significant difference between the 1996 and 2006 samples ($F_{ST} = 0.04768$, $P = 0.03287$; exact test $P = 0.01751$). None of the remaining pairwise comparisons or exact tests of population differentiation within sites were significant. The pooled WAS and SGA samples were labeled as GA. The pooled SAR and CSK samples were labeled as CSK, and the pooled PEV and SBR samples were designated SBR in spatial analyses.

All undifferentiated temporal samples were pooled for spatial population comparisons. The original CSB sample (Pearce 2001) was treated as a discrete unit (CSB1) with the remaining samples pooled (CSB2). The 2006 and 2008 VOL samples were pooled (VOL1), as were the 1998 and

2007 samples (VOL2). Pooling of MEL annual samples was ambiguous as haplotype frequencies of the oldest sample (Encalada et al. 1998) were not different from those of 1996 or 2006. Because this sample was so small ($n = 6$) relative to nesting effort (>450 nests/km; NMFS and USFWS 2008) and compared with the other year samples, it was excluded from spatial analysis. The 1996 and 2006 MEL samples were treated as discrete sample units for spatial analysis (MEL1 and MEL2, respectively).

Population structure

With all sample sites treated discretely, including two temporal samples each from VOL, MEL, and CSB, there were 23 sample units. Among the 253 pairwise comparisons, 170 of the pairwise F_{ST} comparisons and 166 of the exact tests of population differentiation were significant without correction for multiple tests (Table 3). Most non-significant comparisons were between adjacent sample sites within regions, between sites at similar latitude across the axis of the Florida peninsula, or involved a site with small sample size ($n < 20$). Haplotype frequencies produced a slightly skewed mirror image across the axis of the Florida peninsula with rookeries paired across northwest and northeast Florida, central western and central eastern Florida, and southwestern and southeastern Florida having similar and not significantly different haplotype frequencies, in these respective pairings (Table 3, Fig. 2). Results from pairwise F_{ST} comparisons and exact tests of population differentiation were generally consistent with seven regional groupings: (1) North Carolina through Georgia, (2) northeastern Florida, (3) central eastern Florida, (4) southeastern and southwestern Florida, (5) Dry Tortugas, (6) central western Florida, and (7) northwestern Florida.

Some proximal inter-regional comparisons produced ambiguous results with all sample sites treated discretely. Haplotype frequencies of CSB1 and SGI from northwest Florida were not significantly different from those of CSK in central western Florida in pairwise F_{ST} comparisons. None of the haplotype frequencies of the small sample units from northwest Florida (SGI, CSB1, and STJ) were significantly different from those of CSK in central western Florida with respect to exact tests of population differentiation. Additionally, frequencies at MID in southeastern Florida were not significantly different from frequencies at DRT in the Gulf of Mexico with an exact test. To address whether these ambiguities were related to small sample sizes, iterations of pairwise comparisons were performed with alternative a priori sample site clustering to determine the most appropriate regional groupings for final comparisons. Comparisons for combined SGI/CSB2 versus CSK and combined SGI/CSK versus CSB2 were both significant, but the former comparison yielded the stronger signal of differentiation (pairwise $F_{ST} = 0.12756$, $P < 0.00001$; exact test $P = 0.00063$, compared with pairwise $F_{ST} = 0.10946$, $P = 0.00098$; exact test $P = 0.00664$). Combined STJ/CSB2 versus CSK yielded a stronger signal of differentiation (exact test $P = 0.00017$) than did combined STJ/CSK versus CSB2 (exact test $P = 0.02103$). Combined CSB1/CSK versus CSB2 yielded a stronger signal of differentiation than did combined CSB1/CSB2 versus CSK ($F_{ST} = 0.13670$, $P = 0.00098$; exact test $P < 0.00001$, compared with FST = 0.09814, $P < 0.00001$; exact test $P = 0.00735$). However, as CSB1 was clearly an outlier relative to more recent sample sets from CSB and other rookeries in the region, CSB1 was pooled with all other northwest Florida samples. The SBR versus combined MID/DRT test was not significantly different (exact test $P = 0.15578$), whereas combined SBR/MID haplotype frequencies were significantly different from frequencies at DRT (exact test $P = 0.00442$).

Regional affiliation of the northeast Florida sample units was not clear (Table 3). Haplotype frequencies at AML were not different from those of any sample units north of VOL. Haplotype frequencies of SJC, FLG, and VOL1 were significantly different from those of the northern rookeries and CAN, MEL1, and MEL2 to the south. Haplotype frequencies of VOL2 and NSB were significantly different from those of AML and rookeries north, but not from those of SJC or FLG to the north or CAN and MEL2 to the south. Therefore, there were no clear boundaries as haplotype frequencies transitioned clinally. To resolve the most appropriate rookery clustering, several iterations of AMOVA were performed for two cases: (1) recognition of a distinct northeastern Florida management unit and (2) absorption of this region into northern and central eastern Florida management units. Clustering of remaining rookeries was consistent with results from pairwise tests and held constant across all AMOVA iterations: northern sites (NC, CAP, GA); central eastern Florida (CAN, MEL); southern Florida (JUN, FTL, SBR, MID, and KEY); the Dry Tortugas (DRT); central western Florida (CSK); and northwestern Florida (SGI, CSB, and STJ). A total of 16 rookery-clustering scenarios were considered (Supplemental Table 3).

With no northeastern Florida management unit recognized, the optimal clustering was achieved by placing AML, SJC, FLG, and VOL1 within the northern management unit and placing VOL2 and NSB within the central eastern Florida group (scenario NEFL 5, Supplemental Table 4). With recognition of a northeastern Florida management unit, the optimal clustering was produced by grouping SJC, FLG, and VOL1 samples into a northeastern Florida management unit while AML was grouped with the northern management unit and VOL2 and NSB were grouped with CAN and MEL (scenario NEFL 15, Supplemental Table 4). In both these cases, VOL is split into

Table 3 Pairwise F_{ST} values for discrete rookery sample comparisons (above the diagonal) and P values of exact tests of population differentiation (below the diagonal)

	NC	CAP	GA	AML	SJC	FLG	VOL1	VOL2	NSB	CAN	MEL1	MEL2	JUN
NC		0.000	−0.009	0.009	**0.081**	**0.098**	**0.046**	**0.201**	**0.136**	**0.255**	**0.407**	**0.185**	**0.608**
CAP	0.9990		−0.002	0.029	**0.120**	**0.134**	**0.069**	**0.250**	**0.177**	**0.314**	**0.488**	**0.220**	**0.675**
GA	0.9990	1.0000		−0.010	**0.089**	**0.109**	**0.052**	**0.238**	**0.159**	**0.302**	**0.481**	**0.210**	**0.675**
AML	0.4473	0.3048	0.4745		0.016	0.035	0.006	**0.136**	**0.073**	**0.182**	**0.317**	**0.131**	**0.537**
SJC	0.0147	0.0031	0.0124	0.6174		−0.017	−0.016	0.048	0.002	**0.083**	**0.199**	**0.052**	**0.437**
FLG	0.0117	0.0012	0.0022	0.2533	0.6427		0.006	0.035	−0.006	**0.067**	**0.187**	**0.038**	**0.437**
VOL1	0.0420	0.0022	0.0167	0.8286	0.9261	0.1386		**0.089**	**0.031**	**0.130**	**0.262**	**0.090**	**0.497**
VOL2	<0.0001	<0.0001	<0.0001	0.0162	0.1203	0.2470	0.0008		0.001	−0.010	0.044	−0.011	**0.263**
NSB	0.0010	<0.0001	0.0002	0.1512	0.5205	0.8228	0.0332	0.7695		0.021	**0.114**	0.006	**0.359**
CAN	<0.0001	<0.0001	<0.0001	0.0031	0.0357	0.0403	0.0006	0.7812	0.3852		0.013	−0.009	**0.215**
MEL1	<0.0001	<0.0001	<0.0001	0.0004	0.0007	0.0006	<0.0001	0.0484	0.0035	0.4102		**0.045**	**0.091**
MEL2	<0.0001	<0.0001	<0.0001	0.0089	0.0893	0.1446	0.0001	0.5723	0.2587	0.7459	0.0257		**0.270**
JUN	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0065	<0.0001	
FTL	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	0.0160	0.0006	0.2410
SBR	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0000	0.0276	<0.0001	0.3013
MID	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	0.0033	0.1536	0.0005	0.6941
DRT	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0012
KEY	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	0.0008	<0.0001	0.0058	0.2299	0.0005	0.0303
CSK	<0.0001	<0.0001	<0.0001	0.0015	0.0615	0.0152	0.0004	0.1951	0.0432	0.2506	0.0423	0.0971	<0.0001
SGI	0.0557	0.0213	0.0486	0.1979	0.8246	0.5718	0.6252	0.4508	0.5709	0.2486	0.0762	0.3370	0.0003
CSB1	0.0020	0.0004	0.0008	0.0148	0.1098	0.1850	0.0348	0.5760	0.3819	0.7650	1.0000	0.5584	0.5431
CSB2	0.1289	0.0193	0.0622	1.0000	0.8193	0.3461	0.7359	0.0098	0.1104	0.0014	<0.0001	0.0038	<0.0001
STJ	0.0049	0.0012	0.0020	0.2673	0.8146	0.1339	0.4607	0.0857	0.3673	0.0747	0.0074	0.0253	<0.0001

	FTL	SBR	MID	DRT	KEY	CSK	SGI	CSB1	CSB2	STJ
NC	**0.649**	**0.739**	**0.791**	**0.846**	**0.527**	**0.225**	0.181	**0.713**	0.038	**0.130**
CAP	**0.713**	**0.801**	**0.849**	**0.878**	**0.596**	**0.269**	0.273	**0.804**	**0.062**	**0.191**
GA	**0.709**	**0.791**	**0.833**	**0.870**	**0.592**	**0.263**	0.180	**0.738**	0.036	**0.156**
AML	**0.575**	**0.656**	**0.699**	**0.800**	**0.450**	**0.170**	0.035	**0.500**	−0.009	0.048
SJC	**0.463**	**0.524**	**0.545**	**0.710**	**0.340**	**0.085**	−0.047	0.210	−0.013	−0.021
FLG	**0.452**	**0.512**	**0.529**	**0.692**	**0.327**	**0.079**	−0.037	0.177	0.004	−0.012
VOL1	**0.522**	**0.583**	**0.604**	**0.738**	**0.403**	**0.123**	−0.035	0.294	−0.012	−0.008
VOL2	**0.259**	**0.301**	**0.302**	**0.514**	**0.151**	0.003	0.014	−0.025	**0.096**	0.024
NSB	**0.365**	**0.415**	**0.423**	**0.614**	**0.246**	**0.037**	−0.024	0.065	0.037	−0.012
CAN	**0.207**	**0.246**	**0.245**	**0.468**	**0.106**	−0.002	0.046	−0.057	**0.140**	0.050
MEL1	**0.081**	**0.107**	**0.103**	**0.329**	0.013	0.017	0.144	−0.083	**0.273**	**0.144**
MEL2	**0.259**	**0.300**	**0.300**	**0.498**	**0.152**	0.006	0.021	−0.025	**0.097**	0.030
JUN	0.002	0.005	0.003	**0.092**	0.030	**0.182**	**0.373**	0.070	**0.505**	**0.378**
FTL		−0.021	−0.028	**0.099**	0.003	**0.186**	**0.410**	0.055	**0.535**	**0.406**
SBR	0.5061		−0.038	**0.068**	0.018	**0.220**	**0.470**	**0.095**	**0.604**	**0.459**
MID	1.0000	1.0000		**0.068**	0.012	**0.218**	**0.489**	0.094	**0.632**	**0.473**
DRT	0.0015	0.0218	0.1045		**0.195**	**0.416**	**0.697**	**0.411**	**0.759**	**0.670**
KEY	0.4796	0.2452	0.6398	<0.0001		**0.101**	**0.287**	−0.037	**0.411**	**0.287**
CSK	0.0000	0.0000	0.0028	<0.0001	0.0012		**0.042**	−0.042	**0.133**	**0.055**
SGI	0.0001	0.0004	0.0006	<0.0001	0.0028	0.6460		0.134	−0.028	−0.042
CSB1	0.3962	0.3234	0.1926	0.0089	0.5655	0.8236	0.1651		**0.342**	0.125
CSB2	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0019	0.4767	0.0526		0.006
STJ	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	0.0544	0.9155	0.2483	0.2773	

Significant pairwise F_{ST} comparisons (alpha = 0.05, no correction for multiple tests) are indicated in bold

Table 4 AMOVA results for rookery-clustering scenarios for the southeastern USA loggerhead turtle nesting aggregation

	F_{CT}	F_{CT} P	AMU (%)	F_{SC}	F_{SC} P	AR/WMU (%)
Scenario 1	0.24224	0.00248	24.22	0.18863	<0.00001	14.29
Scenario 2	0.33606	<0.00001	33.61	0.01647	0.01089	1.09
Scenario 3	0.32512	<0.00001	32.51	0.01137	0.05604	0.77
Scenario 4	0.33467	<0.00001	33.47	0.01363	0.01465	0.91
Scenario 5	0.32444	<0.00001	32.44	0.00787	0.09386	0.53

AMU is the proportion of genetic variation partitioned among management units. AR/WMU is the total proportion of genetic variance partitioned among rookeries within management units

two groups, further complicating boundary placement. Optimal clustering (based on minimizing F_{SC}) when both temporal VOL samples are considered jointly included a boundary at the Flagler-Volusia County line in the case that northeastern Florida was not recognized as a discrete management unit (scenario NEFL 4), and inclusion of VOL and NSB as part of a recognized northeastern Florida management unit (scenario NEFL 13, Supplemental Table 4).

Given the optimized boundaries for northeastern Florida considering separate treatment of the temporal VOL samples, a final round of AMOVA iterations was performed to test for optimal rookery clustering for the southeastern USA nesting aggregation. A total of five scenarios were considered given genetic evidence and inferences of rookery connectivity based on available demographic data and loggerhead turtle life history traits.

Scenario 1: Recognition of four management units: northern (Virginia through the Georgia-Florida border), peninsular Florida, Dry Tortugas, and northern Gulf (northwest Florida and westward). These are the currently recognized recovery units designated in the Recovery Plan (NMFS and USFWS 2008), and this scenario was considered a control.

Scenario 2: Recognition of six management units: northern, central eastern Florida, southern Florida (southeastern and southwestern), Dry Tortugas, central western Florida, and northwestern Florida.

Scenario 3: Recognition of seven management units: northern, northeastern Florida, central eastern Florida, southern Florida (southeastern and southwestern), Dry Tortugas, central western Florida, and northwestern Florida.

Scenario 4: Recognition of seven management units: northern, central eastern Florida, southeastern Florida, southwestern Florida, Dry Tortugas, central western Florida, and northwestern Florida.

Scenario 5: Recognition of eight management units: northern, northeastern Florida, central eastern Florida, southeastern Florida, southwestern Florida, Dry Tortugas, central western Florida, and northwestern Florida.

There was strong genetic structure among the discrete sample locations ($F_{ST} = 0.30325$, $P < 0.00001$) as well as among the management units tested in the five potential management scenarios (Table 4). AMOVA results indicated that a significant proportion (14.29%, $F_{SC} = 0.18863$, $P < 0.00001$; Table 4) of the overall genetic diversity of the southeastern USA nesting aggregation was partitioned among sampled rookeries within recovery units as they are currently recognized in the Recovery Plan. Although F_{SC} was reduced and F_{CT} was increased for all four remaining management schemes relative to the current Recovery Plan groupings, there was no clear best management scheme given the goal of maximizing F_{CT} and minimizing F_{SC}. Maximal F_{CT} was achieved with management scenario 2, recognition of central eastern, southern, and central western Florida management units from the current peninsular Florida recovery unit (Table 4). Minimal F_{SC} was achieved with management scenario 5, recognition of northeastern, central eastern, southeastern, southwestern, and central western Florida management units from the current peninsular Florida recovery unit (Table 4). A final round of pairwise F_{ST} comparisons and exact tests of population differentiation provided further support for recognition of the discrete management units outlined in scenario 2, with the only non-significant comparison being that of central western and central eastern Florida across the axis of the Florida peninsula (Supplemental Table 5). Southwestern Florida was not significantly different from the combined southeastern Florida rookeries in pairwise F_{ST} comparisons or exact tests of population differentiation (Supplemental Tables 7 and 8). Northeastern Florida was significantly different from proximal rookery clusters (Supplemental Tables 6 and 8).

Discussion

Population structure

The present study identified a pattern of haplotype frequency transitions that is generally consistent with earlier

analyses that detected decreasing frequencies of haplotype CC-A1 and increasing frequencies of CC-A2 from north to south (Encalada et al. 1998; Bowen et al. 2005). However, the haplotype frequency patterns observed in the present study suggest an alternative interpretation to that of continuous, clinal variation in CC-A1 and CC-A2 along the Atlantic coast of Florida. Although there is an apparent cline across northeastern Florida rookeries, CAN and MEL2, separated by approximately 90 beach kilometers along the central coast of eastern Florida, had nearly identical and not significantly different haplotype frequencies. Similarly, the southeastern Florida sites, spanning roughly 125 km (JUN through MID), had quite similar and not significantly different haplotype frequencies. Yet, the frequencies of CC-A1 and CC-A2 are essentially inverted between MEL and JUN, which are separated by approximately 135 km, a distance comparable to that spanning the southeastern Florida sites. The lack of a standard yardstick of geographic isolation that might predict genetic differentiation is echoed in the structure among loggerhead turtle rookeries in the Mediterranean basin. For instance, the sampled Greek rookeries of Zakynthos, Kyparissia, and Lakoninkos, each separated from the others by 100 km or more, all shared nearly identical frequencies of haplotypes CC-A2 and CC-A6 (Encalada et al. 1998; Carreras et al. 2007). Yet the eastern Turkey and northern Cyprus rookeries, separated by approximately 100 km, had significantly different haplotype frequencies owing to the presence of CC-A3 at high frequency at the former and the absence of CC-A3 at the latter (Laurent et al. 1998; Carreras et al. 2007). Another similarity between southeastern USA and Mediterraean loggerhead turtle nesting aggregations is the inference of a cline in the frequencies of CC-A2 and CC-A3 along the Turkish coast (Schroth et al. 1996; Carreras et al. 2007) that may mirror the observed cline in northeastern Florida. The broad nature of the apparent cline across northeastern Florida may have arisen out of the disparity in nesting densities between the northern management unit and the central eastern Florida rookeries (NMFS and USFWS 2008). Even a small proportion of females straying northward from central eastern Florida beaches would have a significant impact on haplotype frequencies given the nearly complete lack of CC-A2 individuals among northern rookeries.

The haplotype frequency transition patterns observed along the Atlantic coast of Florida suggests that rather than displaying broad clinal variation over the entire region, haplotype frequencies may be reasonably stable over 100 km. Such a pattern may result from female natal homing at sufficiently fine scales to maintain the frequency divergence between central and southern regions of Florida. The probability that a female strays to a non-natal site may not simply be a function of distance. Nesting females may be honing in on specific bathymetric (Mortimer 1982; Provancha and Ehrhart 1987), or other physical or chemical cues (Lohmann et al. 2008a) that could give rise to observed nesting density distribution patterns. Spatial analysis of 17 years of nesting density distribution data from the Florida Index Nesting Beach Survey program has revealed remarkable conservation of fine-scale nesting density patterns across nesting seasons (Witherington et al. 2009).

The strong divergence between central and southern Florida rookeries may reflect independent colonization of these areas. Encalada et al. (1998) hypothesized that an equatorial lineage (precursor to CC-A1, formerly haplotype A) may have colonized more northerly latitudes (into the Caribbean) prior to ultimately colonizing both the western and eastern coasts of Florida. Bowen et al. (1994) hypothesized that the CC-A2 lineage may have invaded the western Atlantic via southern Africa. Haplotype CC-A2 is the dominant haplotype in the Quintana Roo, Mexico loggerhead turtle rookery (55% Encalada et al. 1998), as well as the most frequent haplotype among analyzed Cuban rookeries (Ruiz-Urquiola et al. 2010), so colonization may have proceeded from either of these rookeries to southern Florida. One possible scenario is that the current nesting density peaks in Brevard (represented by MEL) and northern Palm Beach (represented by JUN) counties (NMFS and USFWS 2008) represent sites that were initially colonized independently (perhaps originally by CC-A1 and CC-A2 lineages, respectively) and that the intervening beaches were colonized via diffusive natal dispersal from these core areas. Another possibility is that the region was initially colonized by the CC-A1 lineage and that the CC-A2 lineage represents a more recent colonization event. Given thermal constraints on incubation, the rookeries of northwestern and northeastern Florida and northward along the eastern coast of the USA most likely arose via recent colonization events since the Wisconsin glaciation (Encalada et al. 1998). Encalada et al. (1998) predicted that more recently colonized (more northerly) nesting areas would harbor decreasing haplotype diversity as haplotypes were sorted through a series of colonization bottlenecks. Whereas this is consistent with observations for the rookeries in northwestern Florida and northeastern Florida through North Carolina, the pattern did not hold for the southern sampled rookeries in the present study. The highest haplotype diversity was generally recorded at rookeries of intermediate latitude (CSK, JUN, and MEL) rather than those in southernmost Florida, suggesting the possibility that the more southern sites may have been colonized recently. Another possibility is that these high-density nesting beaches (relative to each respective region) have higher haplotype diversity by virtue of specific physical attributes that might attract nesting females

carrying rare haplotypes that have strayed from other rookeries in the western Atlantic. While it is clear that at least two independent colonizations of the southeastern USA from external refugia occurred, it is uncertain whether the Gulf and Atlantic coasts of Florida were independently colonized from refugia or whether founders for novel rookeries on one coast may have originated from the other. Poor resolution of the mitochondrial marker does not permit unequivocal determination of the colonization pathways for the various rookeries comprising the nesting effort in the southeastern USA and requires more extensive screening of the mitochondrial genome for informative variation.

A striking feature of the haplotype frequency distribution is the slightly skewed mirror image pattern produced by comparable haplotype frequencies occurring at roughly similar latitudes across the Florida peninsula. One possible explanation for the overall pattern is error in natal homing that would compel females to nest on beaches with magnetic signatures similar to their natal beaches but on opposing coastlines across the Florida peninsula. Neonate marine turtles may imprint on the geomagnetic signature of their natal site and use this positional information to home to natal regions for nesting (Lohmann et al. 2008b). Marine turtles are sensitive to both magnetic inclination and intensity (Lohmann et al. 2007); however, navigation utilizing a bicoordinate map may not be required to locate beaches along continental coastlines. The coastline itself may serve as a fixed coordinate; therefore, turtles in search of natal regions would only need to follow the coastline to an appropriate inclination or intensity angle (Lohmann et al. 2008b). Tag returns demonstrate that some proportion of central eastern Florida nesting loggerhead turtles enter the Gulf of Mexico to forage (Meylan et al. 1983). Similarly, satellite telemetry indicated that six of twenty-eight females nesting in Sarasota County on the Gulf coast left the Gulf of Mexico following nesting to forage in the Bahamas (Girard et al. 2009). It is conceivable that a small proportion of females hatched on one coast of Florida but foraging off the other might inadvertently travel along the closest coastline and nest at a site with a similar one-dimensional magnetic signature as their natal area, but on the opposing coast across the axis of the Florida peninsula.

Nesting dispersal by individual females among rookeries as measured through flipper-tagging studies may provide an alternative means of characterizing the magnitude and spatial scale of female gene flow among rookeries. For instance, extensive supplemental tagging of nesting Australian green turtles during the 1998–1999 nesting season revealed 8.3% interseasonal dispersal among southern Great Barrier Reef rookeries and 6% interseasonal dispersal among northern Great Barrier Reef rookeries, whereas no dispersal between southern and northern Great Barrier Reef rookeries was detected (Dethmers et al. 2006). The tagging observations were concordant with mtDNA analysis, suggesting that rookeries within each region were not genetically differentiated, but that the two regions represented distinct management units (Dethmers et al. 2006). Unfortunately, MEL currently hosts the only loggerhead turtle tagging project along the eastern coast of Florida; therefore, contemporary data documenting west coast and east coast Florida nesting dispersal are scarce. Of thousands of loggerhead turtles tagged at CSK and at MEL since the mid-1980s, only seven have been recorded nesting at both of these sites (Mote Marine Laboratory and University of Central Florida Marine Turtle Research Group, unpubl. data). If effective, this level of migration is theoretically sufficient to prevent genetic differentiation of these rookeries (e.g. Slatkin 1987).

A limitation of nesting beach flipper-tagging studies for rookery connectivity inference is that such studies measure nest site fidelity, the relative placement of nests by an individual female after she has been tagged while nesting, also known as site fixity or site tenacity (Carr and Carr 1972), rather than explicitly measuring natal philopatry (where the female nests relative to where she herself hatched). Nesting dispersal between distant rookeries represents natal dispersal by default, as it is illogical that a turtle could have hatched in two different regions. However, it is also conceivable that females exhibiting high site tenacity at a particular rookery could be nesting at a non-natal site (high nest site fidelity but low natal site fidelity). This type of natal dispersal would not be detectable with the tagging methodologies currently employed in the southeastern USA. Therefore, testing the hypothesis of inter-coastal natal dispersal within Florida in the absence of nesting dispersal will require a means of directly linking nesting females to their natal beaches.

Sample sizes and sampling error

Small sample sizes and resulting sampling error likely contributed to underestimation, and in a few cases overestimation, of population differentiation. Despite complete sharing of haplotype CC-A1 between CAP ($n = 73$) and NC ($n = 43$), pairwise exact tests of population differentiation between these sites and all others yielded 19 and 17 significant comparisons, respectively. Sampling error by virtue of overestimation of the frequency of rare haplotypes in a particular rookery based on a sample may also lead to differing conclusions regarding genetic divergence. For instance, the 10-day nest sample at STJ yielded individuals carrying three rare haplotypes absent among the much larger sample of nesting females from CSB (on the same peninsula, <20 km away) obtained through saturation sampling over a period of 4 years. Larger sample sizes,

particularly from sites with low nesting densities, will be required from many areas to make robust inferences regarding the possibility of additional management units within the southeastern United States nesting aggregation.

Temporal variation in haplotype frequencies

It is unclear whether the apparent differentiation detected among year classes at CSB and MEL truly represents temporal variation or could have arisen through sampling error. The sample size of CSB1 was small ($n = 7$), and sampling methodology was unclear. Haplotype frequencies of CSB1 were significantly different from 2002 and 2004, while none of the remaining annual samples differed from one another, suggesting that CSB1 was an outlier that may have arisen through sampling error. MEL1 represents only a portion of turtles sampled (40 samples sequenced of 150 samples collected for a multiple paternity study, [Moore and Ball 2002]), so the difference between MEL1 and MEL2 may also be attributable to sampling error. Because of the high nesting densities at MEL (>450 nests/km; NMFS and USFWS 2008), neither the 1996 nor the 2006 sample set represent strong sampling effort relative to nesting effort. The differentiation among annual VOL samples, however, does appear to truly reflect temporal variation given that sampling effort was high relative to nesting effort (>70% of clutches laid during each sampling period) and sampling methodologies were consistent among years.

Lack of temporal variation at most sample sites is not surprising given the short duration between sampling periods and the estimated loggerhead turtle generation length of approximately 50 years (NMFS and USFWS 2008). Tag recoveries suggest that individual females are capable of nesting over a period of at least 25 years (NMFS and USFWS 2008). Thus, any divergence in haplotype frequency via genetic drift would be expected to occur gradually as neophyte females are absorbed into the nesting population, slowly replacing senescent females. Bjorndal and Bolten (2008) argued that aggregates of females nesting at a rookery each year are probably well mixed due to individual females switching between remigration intervals of two, three, or more years (e.g. Carr et al. 1978), likely maintaining genetic homogeneity among years.

If the apparent temporal variation observed at VOL is real, there are several alternative hypotheses worth considering. One possibility is that this variation is interannual and could be driven by differential aggregate mixing based on divergent foraging habitat use and differing mean remigration intervals for each foraging aggregation. Given the energetic costs of undertaking reproductive migrations and producing several clutches of eggs over the course of a nesting season, ecological conditions on the foraging grounds have been postulated to affect variability in remigration intervals (Carr and Carr 1970); Tröeng and Chaloupka (2007) hypothesized that the shorter observed population average remigration interval for Tortuguero green turtles relative to that of many other green turtle rookeries could be attributable, at least in part, to greater forage availability, better forage quality, and shorter distance between the nesting beach and the main foraging ground. Satellite telemetry and tag return data suggest that northern management unit loggerhead turtle females forage primarily along the continental shelf of the eastern United States, with a relatively small proportion of females moving south of the Cape Canaveral area to forage in the northern Caribbean or Gulf of Mexico (Bell and Richardson 1978; Plotkin and Spotila 2002; Williams and Frick 2008). Loggerhead turtles nesting in central eastern and western Florida typically forage in the Gulf of Mexico or in the northern Caribbean region (Meylan et al. 1983; Dodd and Byles 2003; Foley et al. 2008; Girard et al. 2009; Turtle Expert Working Group 2009), and only one satellite-tagged female has been recorded foraging north of the Cape Canaveral area (Dodd and Byles 2003).

Another possibility is that temporal variation exists within a nesting season. The initiation of nesting by central Florida and northern management unit females could be sufficiently staggered to produce cyclical changes in haplotype frequencies depending on the precise placement of the sampling window within the nesting season. Beyond different usage patterns of spatially discrete neritic habitats suggested by tag return and satellite telemetry data, analyses of stable isotopes and epibiota suggested that loggerhead turtles nesting along the eastern coast of Florida may be utilizing both oceanic and neritic foraging habitats (Reich et al. 2010). Although observed latitudinal trends in mtDNA haplotypes and stable isotope patterns were independent (Reich et al. 2010), the possibility remains that divergent foraging strategies or use of different foraging habitats could be driving sufficiently staggered nesting phenology for representatives of each group so as to cause temporal variation of haplotype frequencies on the nesting beach. Further research is warranted to determine whether haplotype frequency variations occur across individual nesting seasons at the northeastern Florida rookeries.

We concur with Bjorndal and Bolten (2008) that temporal variation should be considered in population structure analyses of rookeries as well as mixed stock analyses of foraging aggregations. Apparent temporal variation in the VOL rookery clearly had a significant impact on the interpretation of spatial genetic structure. The 1998 and 2007 samples would have lead to grouping of this rookery with those in central eastern Florida, whereas the 2006 and 2008 samples indicated a much closer affiliation with the northern management unit. Overall, these data suggest that a

geographic transition zone occurs across northeastern Florida between the population nesting north of Florida and the population nesting in central eastern Florida. The apparent temporally transitional nature of haplotype frequencies at this rookery would have gone undetected without sampling over multiple years. Temporal variation of genetic diversity over short frames (e.g. within a nesting season or less than a generation) may not occur as a rule at most rookeries, but should be considered particularly when rookeries may be suspected of being geographically transitional.

Defining management units

Defining management unit boundaries is inherently difficult when habitat is relatively homogenous and obvious barriers to movement are absent, such as the case of several 100 km of essentially continuous coastline that provides suitable nesting habitat for loggerhead turtles. In cases where nesting habitats are discrete (e.g., Dry Tortugas) or are separated from other nesting areas by over 100 km of unsuitable nesting habitat (e.g., northwest Florida beaches relative to central western Florida), management unit assessments may be straightforward if proximal rookeries have significantly different haplotype frequencies. However, boundaries along continuous nesting habitat must be artificially imposed in the sense that some proportion of females will distribute nesting effort on both sides of designated boundaries. Despite this complication, ignoring the genetic structure among peninsular Florida nesting areas could lead to inadequate protection of demographically distinct rookeries as well as misinterpretation of nesting trends at finer spatial scales.

Although genetic studies have provided a reasonable first approximation for management unit assignments (Bowen et al. 1993; Encalada et al. 1998; Pearce 2001; Bowen et al. 2005, present study), some inherent limitations of haplotype frequency data bear consideration. Provided sampling has been conducted in such a way as to maximize sample sizes and minimize sampling error, a significant difference in haplotype frequencies implies some level of demographic independence (Avise 1995). However, lack of significant genetic differences does not necessarily confer contemporary demographic connectivity (Taylor and Dizon 1996). Demographic partitioning despite non-significance of haplotype frequency comparisons is possible due to lack of resolution of the genetic markers, shared evolutionary history, and potentially insufficient time for genetic drift to occur. Comparative evidence suggests that marine turtle mitochondrial DNA evolves more slowly than that of most other vertebrates, possibly attributable to long generation time and low metabolic rate (Avise et al. 1992). Therefore, nesting populations may be demographically isolated despite a lack of any detectable genetic differentiation. Distinguishing between recent shared evolutionary heritage in the absence of genetic drift, low levels of contemporary genetic connectivity sufficient to prevent genetic divergence, and contemporary demographic connectivity among rookeries is critical for management on ecological time scales.

Ultimately, marine turtle rookeries flourish or perish based on recruitment of nesting females to a particular rookery (Bowen et al. 2005). Female nesting at non-natal sites is critical for colonization of novel nesting areas over evolutionary time scales, but natal dispersal of small numbers of females among distant established rookeries may be demographically irrelevant over ecological time scales. The level of exchange required to prevent genetic differentiation is many orders of magnitude lower than that required to sustain a population ecologically and demographically (Avise 1992). Whereas a few migrants per generation may be sufficient to maintain genetic homogeneity (Slatkin 1993), demographic independence of two populations may be maintained if less than 10% of individuals disperse between the populations (Hastings 1993). Thus, management unit inferences should be drawn in the context of life history characteristics and available demographic data rather than relying strictly on the statistical significance of population differentiation tests.

Recovery unit recommendations

The present study upholds the distinctiveness of the four currently recognized recovery units: northern, peninsular Florida, Dry Tortugas, and northern Gulf of Mexico. We concur with the argument that the northern Gulf coast nesting population should be treated as a separate recovery unit on the basis of geographic isolation and apparent genetic distinction from the proximal Gulf coast rookeries in central western and southwestern Florida (Encalada et al. 1998).

Sampling effort has not been spatially or temporally adequate to fully resolve the number or boundaries of recovery units within the southeastern USA loggerhead turtle nesting aggregation. However, the present study does suggest more structure among peninsular Florida rookeries than is reflected in the current Recovery Plan designations. Although the lack of data from the rookeries between MEL and JUN in the present analysis limits inferences about the nature of haplotype frequency transitions along the entire length of the Atlantic coast of Florida, similarity of haplotype frequencies within each sampled region and strong divergence of haplotype frequencies between them suggest some level of demographic partitioning. Brevard County in the central portion of the eastern coast of Florida and Palm Beach County in southeastern Florida host the two significant peaks in nesting density of the southeastern USA nesting aggregation with a relative trough of nesting

densities between them (NMFS and USFWS 2008; Witherington et al. 2009). Given the nesting density distribution data and the significant genetic differentiation between the central and southern portions of the eastern coast, we recommend recognition of the central eastern Florida rookery as a distinct recovery unit. Similar genetic divergence occurs along the Gulf coast between KEY in southwestern Florida and CSK in central western Florida and suggests that recognition of a separate central western Florida recovery unit is also warranted.

It is unclear whether the lack of genetic divergence between turtles nesting on the southernmost eastern and western coasts of the Florida peninsula reflects contemporary demographic connectivity, contemporary genetic connectivity, or may result from historical colonization signature. The discontinuity of suitable nesting habitat around the tip of the Florida peninsula (e.g. Davis and Whiting 1977), the scale of distinct management units inferred from the present study in other regions of Florida, and limited observed nesting dispersal between coasts suggest that each coast likely hosts demographically distinct rookeries. Though there is little genetic support for recognition of discrete southwestern and southeastern recovery units given the lack of significant differences of haplotype frequencies at KEY and the southeastern Florida rookeries, the conservative approach may be designation of finer scale recovery units unless or until evidence of sufficient effective movement between them is established. Further studies should address the demographic rookery connectivity between these regions.

The northeastern Florida rookeries present a challenge for recovery planning given the lack of a clear boundary between the northern and proposed central eastern Florida recovery units because of intermediate haplotype frequencies. Pairwise F_{ST} comparisons, exact tests, and AMOVA results support the recognition of a discrete northeastern Florida recovery unit. However, the transitional nature of the haplotype frequencies of northeastern Florida rookeries both spatially and temporally in the case of VOL suggests that rather than representing a discrete nesting population, these rookeries represent a transition zone comprised of nesting females from both the northern and proposed central eastern Florida recovery units. Given the large disparity between nesting densities at Georgia rookeries and rookeries in central eastern Florida (10–20 nests/km versus 300 + nests/km, respectively; (NMFS and USFWS 2008), even a small proportion of central eastern Florida straying northward into northeastern Florida would produce intermediate frequencies of CC-A2 relative to the rookeries to the north and south. Optimal boundaries for a discrete northeastern Florida recovery unit were between AML and SJC to the north and between VOL and NSB to the south when VOL temporal samples were treated discretely. Therefore, the Ponce Inlet may serve as an appropriate northern boundary for the central Florida recovery unit given either recognition of a northeastern Florida recovery unit or absorption of these northeastern Florida rookeries into the northern recovery unit. Under either scenario, AML should be treated as part of the northern recovery unit based on AMOVA results. Further research should focus on the demographic independence of the northeastern Florida rookeries relative to those in Georgia and central eastern Florida.

The genetic data support recognition of a minimum of six distinct recovery units: northern, central eastern Florida, southern Florida (southeastern and southwestern), Dry Tortugas, central western Florida, and northern Gulf of Mexico. The demographic discreteness of northeastern and southwestern Florida rookeries is unclear and warrants further research. More extensive genetic sampling is required to fill geographic gaps in the present study and to better describe the nature of haplotype frequency transitions along continuous coastlines. Further demographic partitioning likely occurs, and additional tools, such as satellite or GPS telemetry and mark-recapture, are required to overcome the limitations of the mitochondrial sequence data used in the present study in generating more robust data on the scale of female natal philopatry, female nest site fidelity, and connectivity among rookeries.

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References

Allendorf FW, Waples RS (1995) Conservation and genetics of salmonid fishes. In: Avise JC, Hamrick JL (eds) Conservation genetics. Chapman and Hall, New York, pp 238–280

Avise JC (1992) Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos 63:62–76

Avise JC (1995) Mitochondrial DNA polymorphism and a connection between genetics and demography of relevance to conservation. Conserv Biol 9:686–690

Avise JC, Bowen BW, Lamb T, Meylan AB, Bermingham E (1992) Mitochondrial DNA evolution at a turtle's pace: evidence for low genetic variability and reduced microevolutionary rate in the testudines. Mol Biol Evol 9:457–473

Baldwin R, Hughes GR, Price RIT (2003) Loggerhead turtles in the Indian Ocean. In: Bolten AB, Witherington BE (eds) Loggerhead sea turtles. Smithsonian Institution Press, Washington, DC, pp 218–232

Bell R, Richardson JI (1978) An analysis of tag recoveries from loggerhead sea turtles (*Caretta caretta*) nesting on Little Cumberland Island, Georgia. Fla Mar Res Publ 33:1–66

Bjorndal K, Bolten A (2008) Annual variation in source contributions to a mixed stock: implications for quantifying connectivity. Mol Ecol 17:2185–2193

Bjorndal K, Bolten A, Troeng S (2005) Population structure and genetic diversity in green turtles nesting at Tortuguero, Costa Rica, based on mitochondrial DNA control region sequences. Mar Biol 147:1449–1457

Bolten AB (2003) The loggerhead sea turtle- so excellent a fishe. In: Bolten AB, Witherington BE (eds) Loggerhead sea turtles. Smithsonian Institution Press, Washington, pp 1–3

Bolten AB, Bjorndal KA, Martins HR, Dellinger T, Biscoito MJ, Encalada SE, Bowen BW (1998) Transatlantic developmental migrations of loggerhead sea turtles demonstrated by mtDNA sequence analysis. Ecol Appl 8:1–7

Bowen BW, Karl SA (2007) Population genetics and phylogeography of sea turtles. Mol Ecol 16:4886–4907

Bowen B, Avise JC, Richardson JI, Meylan AB, Margaritoulis D, Hopkins-Murphy SR (1993) Population structure of loggerhead turtles (*Caretta caretta*) in the northwestern Atlantic Ocean and Mediterranean Sea. Conserv Biol 7:834–844

Bowen BW, Kamezaki N, Limpus CJ, Hughes GR, Meylan AB, Avise JC (1994) Global phylogeography of the loggerhead turte (*Caretta caretta*) as indicated by mitochondrial DNA haplotypes. Evolution 48:1820–1828

Bowen BW, Bass AL, Chow S-M, Bostrom M, Bjorndal KA, Bolten AB, Okuyama T, Bolker BM, Epperly S, La Casella E, Shaver D, Dodd M, Hopkins-Murphy SR, Musick JA, Swingle M, Rankin-Baransky K, Teas W, Witzell WN, Dutton PH (2004) Natal homing in juvenile loggerhead turtles (*Caretta caretta*). Mol Ecol 13:3797–3808

Bowen BW, Bass AL, Soares L, Toonen RJ (2005) Conservation implications of complex population structure: lessons from the loggerhead turtle (*Caretta caretta*). Mol Ecol 14:2389–2402

Carr A, Carr MH (1970) Modulated reproductive periodicity in *Chelonia*. Ecology 51:335–337

Carr A, Carr M (1972) Site fixity in the Caribbean green turtle. Ecology 53:425–429

Carr A, Carr M, Meylan A (1978) The ecology and migrations of sea turtles. No. 7. The West Caribbean green turtle colony. Bull Am Mus Nat Hist 162:1–46

Carreras C, Pascual M, Cardona L, Aguilar A, Margaritoulis D, Rees AF, Turkozan O, Levy Y, Gasith A, Aureggi M, Khalil M (2007) The genetic structure of the loggerhead sea turtle (*Caretta caretta*) in the Mediterranean as revealed by nuclear and mitochondrial DNA and its conservation implications. Conserv Genet 8:1572–9737

Davis GE, Whiting MC (1977) Loggerhead sea turtle nesting in Everglades National Park, Florida, USA. Herpetologica 33:18–28

Dethmers KEM, Broderick D, Moritz C, FitzSimmons N, Limpus C, Lavery S, Whiting S, Guinea M, Prince RIT, Kennet R (2006) The genetic structure of Australasian green turtles (*Chelonia mydas*): exploring the geographical scale of genetic exchange. Mol Ecol 15:3931–3946

Dodd C, Byles R (2003) Post-nesting movements and behavior of loggerhead sea turtles (*Caretta caretta*) departing from east-central Florida nesting beaches. Chelonian Conserv Biol 4:530–536

Dodd CK Jr (1988) Synopsis of the biological data on the loggerhead sea turtle *Caretta caretta* (Linnaeus 1758), 88(14)

Encalada SE, Bjorndal KA, Bolten AB, Zurita JC, Schroeder B, Possardt E, Sears CJ, Bowen BW (1998) Population structure of loggerhead turtle (*Caretta caretta*) nesting colonies in the Atlantic and Mediterranean as inferred from mitochondrial DNA control region sequences. Mar Biol 130:567–575

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50

Foley A, Schroeder BA, MacPherson S (2008) Post-nesting migrations and resident areas of Florida loggerheads. In: Kalb H, Rohde A, Gayheart K, Shanker K (eds) Proceedings of the Twenty-fifth annual symposium on sea turtle biology and conservation. NOAA Technical Memorandum NMFS-SEFSC-582

Francisco AM, Bass AL, Bowen BW (1999) Genetic characterization of loggerhead turtles (*Caretta caretta*) nesting in Volusia County

Fraser D, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Mol Ecol 10:2741–2752

Girard C, Tucker AD, Calmettes B (2009) Post-nesting migrations of loggerhead sea turtles in the Gulf of Mexico: dispersal in highly dynamic conditions. Mar Biol 156:1827–1839

Hastings A (1993) Complex interactions between dispersal and dynamics: lessons from coupled logistic equations. Ecology 74:1362–1372

Hatase H, Kinoshita M, Bando T, Kamezaki N, Sato K, Matsuzawa Y, Goto K, Omuta K, Nakashima Y, Takeshita H, Sakamoto W (2002) Population structure of loggerhead turtles, *Caretta caretta*, nesting in Japan: bottlenescks on the Pacific population. Mar Biol 141:299–305

Hoelzel AR (1998) Genetic structure of cetacean populations in sympatry, parapatry, and mixed assemblages: implications for conservation policy. J Hered 89:451–458

Laurent L, Casale P, Bradai MN, Godley BJ, Gerosa G, Broderick AC, Schroth W, Schierwater B, Levy AM, Freggi D, El-Mawla EMA, Hadoud DA, Gomati HE, Domingo M, Hadjichristophorou M, Kornaraky L, Demirayak F, Gautier CH (1998) Molecular resolution of marine turtle stock composition in fishery bycatch: a case study in the Mediterranean. Mol Ecol 7:1529–1542

Lohmann K, Lohmann C, Putman N (2007) Magnetic maps in animals: nature's GPS. J Exp Biol 211:3697–3705

Lohmann K, Luschi P, Hays G (2008a) Goal navigation and island-finding in sea turtles. J Exp Mar Biol Ecol 356:83–95

Lohmann K, Putman N, Lohmann C (2008b) Geomagnetic imprinting: a unifying hypothesis for long-distance natal homing in salmon and sea turtles. Proc Natl Acad Sci USA 105:19096–19101

Meylan AB, Bjorndal KA, Turner BJ (1983) Sea turtles nesting at Melbourne Beach, Florida, II. Post-nesting movements of Caretta caretta. Biol Conserv 26:79–90

Moore MK, Ball RM Jr (2002) Multiple paternity in loggerhead sea turtles (*Caretta caretta*) nests on Melbourne Beach, Florida: a microsatellite analysis. Mol Ecol 11:281–288

Moritz C (1994) Defining evolutionary significant units for conservation. Trends Ecol Evol 9:373–374

Mortimer JA (1982) Factors influencing beach selection by nesting sea turtles. In: Bjorndal KA (ed) Biology and conservation of sea turtles. Smithsonian Institution Press, Washington, pp 45–52

National Marine Fisheries Service and US Fish and Wildlife Service (2008) Recovery plan for the Northwest atlantic population of the loggerhead sea turtle (*Caretta caretta*), Second Revision. National Marine Fisheries Service, Silver Spring, MD

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Norman JA, Moritz C, Limpus CJ (1994) Mitochondrial DNA control region polymorphisms: genetic markers for ecological studies of marine turtles. Mol Ecol 3:363–373

Pearce AF (2001) Contrasting population structure of the loggerhead turtle (*Caretta caretta*) using mitochondrial and nuclear DNA markers. Masters Thesis. University of Florida. Gainesville, FL

Plotkin PT, Spotila JR (2002) Post-nesting migrations of loggerhead turtles *Caretta caretta* from Georgia, USA: conservation implications for a genetically distinct subpopulation. Oryx 36:396–399

Provancha JA, Ehrhart LM (1987) Sea turtle nesting trends at Kennedy space center and Cape Canaveral air force stattion, Florida, and relationships with factors influencing nest site selection. In: Witzell WN (ed) Ecology of East Florida sea turtles. NOAA Technical Report NMFS 53, Miami, FL

Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49:1280–1283

Reich K, Bjorndal K, Frick M, Witherington B, Johnson C, Bolten A (2010) Polymodal foraging in adult female loggerheads (*Caretta caretta*) Marine Biology 157:113–121

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225

Ruiz-Urquiola A, Vega-Polanco M, Riverón-Gíro F, Abreu-Grobois FA, Solano-Abadía J, Pérez-Bermúdez E, Frías-Soler R, Azanza-Ricardo J, Díaz-Fernández R, Ibarra-Martín M, Espinosa-López G (2010) Genetic structure of loggerhead populations in the greater Caribbean and Atlantic western shore based on mitochondrial DNA sequences, with an emphasis on the rookeries from southwestern Cuba. In: Dean K, López-Castro M (compilers) Proceedings of the Twenty-eighth Annual Symposium on Sea Turtle Biology and Conservation. NOAA Technical Memorandum NOAA NMFS-SEFSC-602

Schroth W, Streit B, Schierwater B (1996) Evolutionary handcap for turtles. Nature 384:521–522

Shamblin BM, Faircloth BC, Dodd M, Wood-Jones A, Castleberry SB, Carroll JP, Nairn CJ (2007) Tetranucleotide microsatellites from the loggerhead sea turtle (*Caretta caretta*). Mol Ecol Notes 7:784–787

Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science 236:787–792

Slatkin M (1993) Isolation by distance in equilibrium and nonequilibrium populations. Evolution 47:264–279

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512–526

Taylor B, Dizon AE (1996) The need to estimate power to link genetics and demography for conservation. Conserv Biol 10:661–664

Tröeng S, Chaloupka M (2007) Variation in adult annual survival probability and remigration intervals of sea turtles. Mar Biol 151:1721–1730

Turtle Expert Working Group (2009) An assessment of the loggerhead turtle population in the western North Atlantic Ocean. NOAA Technical Memorandum NMFS-SEFSC-575

Velez-Zuazo X, Ramos WD, van Dam RP, Diez CE, Abreu-Grobois A, McMillan O (2008) Diespersal, recruiment and migratory behaviour in a hawksbill sea turtle aggregation. Mol Ecol 17:839–853

Williams KL, Frick MG (2008) Tag returns from loggerhead turtles from Wassaw Island, Georgia. Southeast Nat 7:165–172

Wisby WJ, Hasler AD (1954) Effect of olfactory occlusion on migrating silver salmon (*O. kisutch*). J Fish Res Board Canada 11:472–478

Witherington B, Kubilis P, Brost B, Meylan A (2009) Decreasing annual nest counts in a globally important loggerhead sea turtle population. Ecol Appl 19:30–54